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(54) GH61 GLYCOSIDE HYDROLASE PROTEIN VARIANTS AND COFACTORS THAT **ENHANCE GH61 ACTIVITY**

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ABSTRACT (57)

The present invention provides various GH61 protein variants comprising various amino acid substitutions. The GH61 protein variants have an improved ability to synergize with cellulase enzymes, thereby increasing the yield of fermentable sugars obtained by saccharification of biomass. In some embodiments, sugars obtained from saccharification are fermented to produce numerous end-products, including but not limited to alcohol.

Figure 1.

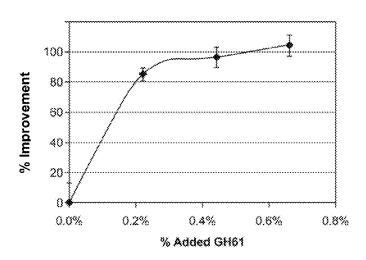


Figure 2.

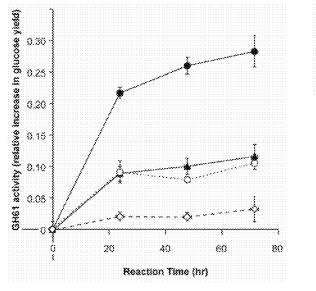
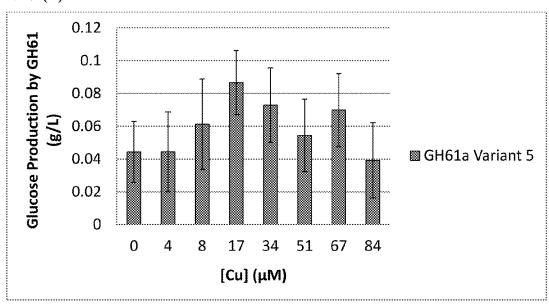




Figure 3.

Panel (A)



Panel B

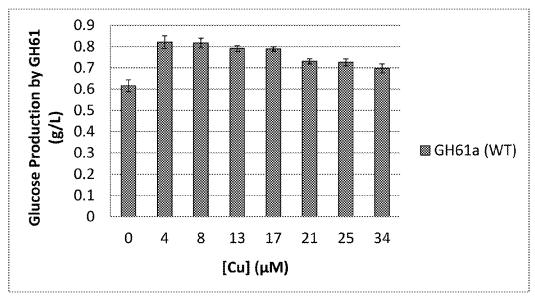
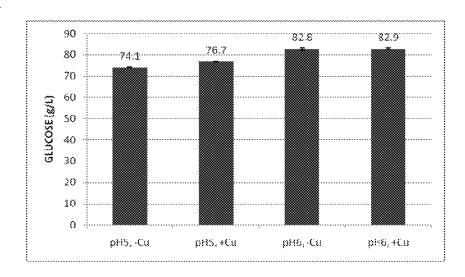


Figure 4.

Panel A



Panel B

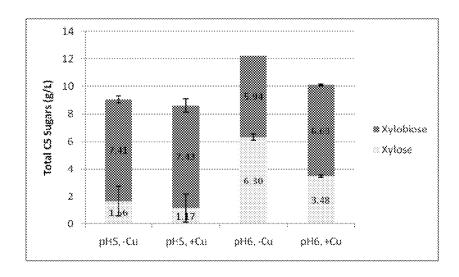
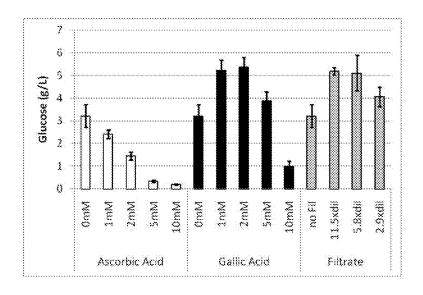


Figure 5.

Panel A



Panel B

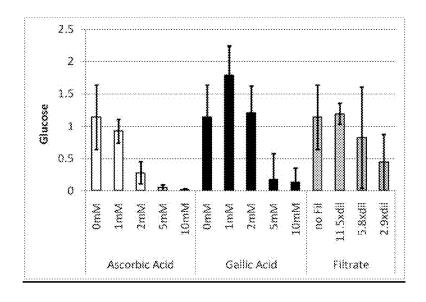
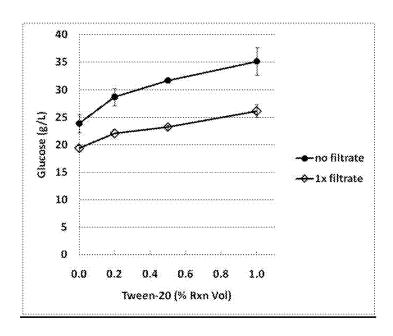
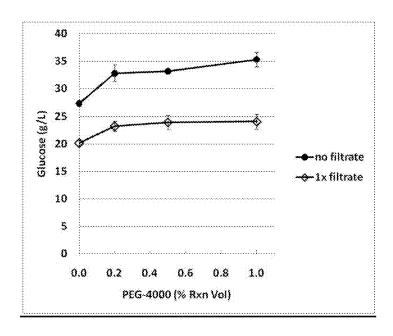


Figure 6.

Panel A



Panel B



GH61 GLYCOSIDE HYDROLASE PROTEIN VARIANTS AND COFACTORS THAT ENHANCE GH61 ACTIVITY

[0001] The present application is a Continuation of copending U.S. patent application Ser. No. 14/496,979, filed Sep. 25, 2014, which is a Divisional of U.S. patent application Ser. No. 13/592,024, filed Aug. 22, 2012, now U.S. Pat. No. 8,951,758, which claims priority to previously filed U.S. patent application Ser. No. 13/215,193, filed Aug. 22, 2011, now U.S. Pat. No. 8,298,795, which claims priority to U.S. Prov. Appln. Ser. No. 61/526,224, filed Aug. 22, 2011, and U.S. Prov. Appln. Ser. No. 61/601,997, filed Feb. 22, 2012, all of which are hereby incorporated in their entireties for all purposes.

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED AS AN ASCII TEXT FILE

[0002] The Sequence Listing written in file CX35-101US2A_ST25.TXT, created on Aug. 20, 2012, 416,766 bytes, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference.

FIELD OF THE INVENTION

[0003] The invention relates generally to the field of glycolytic enzymes and their use, and to the field of directed enzyme evolution or modification. More specifically, the present invention provides GH61 protein variants, and methods for the use of such protein variants in production of fermentable sugars and ethanol from cellulosic biomass.

BACKGROUND

[0004] Cellulosic biomass is a significant renewable resource for the generation of fermentable sugars. These sugars can be used as substrates for fermentation and other metabolic processes to produce biofuels, chemical compounds and other commercially valuable end-products.

[0005] The conversion of cellulosic biomass to fermentable sugars may begin with chemical, mechanical, enzymatic or other pretreatments to increase the susceptibility of cellulose to hydrolysis. Such pretreatment may be followed by the enzymatic conversion of cellulose to cellobiose, cello-oligosaccharides, glucose, and other sugars and sugar polymers, using enzymes that break down cellulose. These enzymes are collectively referred to as "cellulases" and include endoglucanases, beta-glucosidases and cellobiohydrolases.

SUMMARY OF THE INVENTION

[0006] The invention provides numerous variants of GH61 proteins. In some embodiments, these variants comprise amino acid substitutions as set forth herein. In some embodiments, these variants exhibit an improved ability to synergize with cellulase enzymes, thereby increasing the yield of fermentable sugars obtained by saccharification of cellulose-containing biomass. Sugars obtained from saccharification can be fermented to produce alcohol and other end-products. Thus, the GH61 variant proteins of this invention have important commercial applicability in the production of biofuels and other end-products. In some embodiments, the present invention provides GH61 variant proteins

comprising an amino acid sequence that is substantially identical (for example, at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical) to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity as defined below. In some embodiments, the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2 or a fragment of SEQ ID NO:2. In some embodiments, the GH61 is at least 95% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity. In some embodiments, the GH61 variant proteins have increased thermoactivity compared with the GH61 wild-type protein of SEQ ID NO:2. In some further embodiments, the GH61 variant proteins have increased thermostability compared with the GH61 wild-type protein of SEQ ID NO:2.

[0007] In some embodiments, the present invention provides GH61 variants comprising substitution(s) in at least one of the positions as indicated herein. In some embodiments, the substitution(s) provide GH61 variants that have increased activity as compared to wild-type GH61. In some embodiments, the GH61 variants comprise at least one substitution selected from those listed in Table 1 and/or Table 2 in any combination, wherein the positions are numbered with reference to SEQ ID NO:2.

[0008] In some further embodiments, the GH61 variants provided herein comprise the any one or more of the mutations listed in Table 1 and/or Table 2 in any combination. It is not intended that the present invention be limited to the specific substitutions. Any two, three, four, or more than four substitutions find use in any combination that improves GH61 activity. Non-limiting illustrations of effective combinations are provided herein.

[0009] In some embodiments, a substitution or combination of substitutions in the amino acid sequence as provided herein results in the variant protein having increased GH61 activity in a saccharification reaction. In some embodiments, crystalline cellulose undergoes saccharification by cellulase enzymes that are contained in culture broth from M. thermophila cells. When measured in this manner, a GH61 variant protein of this invention causes increase in yield of fermentable sugars (e.g., glucose) to a degree that is about 1.5-fold, about 2-fold, about 3-fold, about 5-fold, about 8-fold, about 10-fold or more compared with the parental GH61 sequence (SEQ ID NO:2) or biologically active fragment, compared with a reference protein comprising SEQ ID NO:2 or the fragment, without any substitutions. It is not intended that the present invention be limited to the production of any particular fermentable sugar(s). It is also not intended that the present invention be limited to any specific level of improvement in the yield of fermentable sugar using at least one of the variants provided herein.

[0010] This invention also provides GH61 protein variants that are more resistant to the presence of enzyme inhibitors that may be present in commercial sources of biomass, or be generated as a result of pretreatment of the biomass substrate.

[0011] In some embodiments, the present invention provides GH61 variant proteins comprising amino acid sequences that are at least about at least about 60%, at least about 65%, at least about 70%, 75%, at least 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least

about 98%, at least about 99%, or at least about 100% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2 or the fragment.

[0012] In some embodiments, the present invention provides GH61 variant proteins comprising amino acid sequences that are at least about at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2 or the fragment, and wherein the substitution(s) in the amino acid sequence result in the variant protein having increased GH61 activity in a reaction where crystalline cellulose undergoes saccharification by cellulase enzymes that are contained in culture broth from M. thermophila cells, compared with a reference protein comprising SEQ ID NO:2 or the fragment, without any substitutions.

[0013] In some embodiments, the present invention provides GH61 variant proteins comprising amino acid sequences that are at least about 60%, at least about 65%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2 or the fragment, and wherein the polynucleotide encoding the GH61 variant protein comprises at least one mutation and/or mutation set selected from those listed in Table 1 and/or Table 2 in any combination, wherein the nucleotide positions of the substitutions are determined by alignment with SEQ ID NO:1.

[0014] In some embodiments, the present invention provides enzyme compositions comprising at least one GH61 variant of the present invention and/or at least one wild-type GH61 protein. In some embodiments, the present invention provides enzyme compositions comprising at least one GH61 variant protein of this invention is combined with one or more cellulase enzyme(s), including but not limited to endoglucanases (EG), beta-glucosidases (BGL), cellobiohydrolases (e.g., CBH1 and/or CBH2), and/or at least one wild-type GH61 protein. In some embodiments, the enzyme compositions further comprise one or more enzymes selected from cellulases, hemicellulases, xylanases, amylases, glucoamylases, proteases, esterases xylosidases, and lipases.

[0015] The invention also includes polynucleotides encoding GH61 variant proteins, recombinant cells expressing such polynucleotides and optionally one or more cellulase enzymes, and methods for increasing yield of fermentable sugars in a saccharification reaction by conducting the reaction in the presence of at least one GH61 protein of this invention.

[0016] In some embodiments, the present invention provides at least one polynucleotide comprising at least one

nucleic acid sequence encoding at least one GH61 variant protein; at least one polynucleotide that hybridizes under stringent hybridization conditions to at least one polynucleotide encoding at least one GH61 variant protein; and/or at least one polynucleotide that hybridizes under stringent hybridization conditions to the complement of at least one polynucleotide encoding at least one polypeptide comprising at least one GH61 variant protein.

[0017] The present invention also provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one GH61 protein, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to SEQ ID NO:2, wherein the amino acid sequence comprises at least one substitution and/or substitution set provided herein; (b) a polynucleotide that hybridizes under stringent hybridization conditions to at least a fragment of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein; and/or (c) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of at least a fragment of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein.

[0018] The present invention further provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one GH61 protein, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, wherein the amino acid sequence comprises at least one substitution and/or substitution set provided herein; (b) a polynucleotide that hybridizes under stringent hybridization conditions to a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein; and/or (c) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein. In some embodiments of the nucleic acid constructs, the polynucleotide sequence is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEO ID NO:1, and wherein the polynucleotide sequence comprises at least one mutation and/or at least one mutation set provided herein. Exemplary

are those shown in Table 1 and Table 2, which may be incorporated into the polynucleotide in any combination.

[0019] In some embodiments, the present invention provides polynucleotides and nucleic acid constructs comprising polynucleotides encoding at least one GH61 variant and/or wild-type protein (e.g., any of SEQ ID NOS:2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 41, 42, 44, 45, 47, 48, 50, 51, 53, 54, 56, 57, 59, 60, 62, 64, 65, 67, 68, 70, 71, 73, 74, 76, 77, 79, 80, 82, 83, 85, 86, 88, 89, 91, 93, 95, 96, 98, 99, 101, 102, 104, 105, 107, 108), operably linked to promoters. In some embodiments, the promoters are heterologous promoters. In some embodiments, the present invention provides expression constructs comprising polynucleotides and/or nucleic acid constructs that comprise polynucleotides encoding at least one GH61 variant and/or wild-type protein. In some embodiments, the expression constructs comprise at least one nucleic acid sequence operably linked to at least one additional regulatory sequence.

[0020] The present invention also provides recombinant host cells that express at least one polynucleotide sequence encoding at least one GH61 variant protein. In some embodiments, the host cell also expresses at least one polynucleotide sequence encoding at least one GH61 wildtype protein. In some embodiments, the expressed GH61 variant and/or wild-type protein is secreted from the host cell. In some embodiments, the host cell also produces at least one cellulase enzyme selected from endoglucanases (EG), beta-glucosidases (BGL), cellobiohydrolases (e.g., CBH1 and/or CBH2), xylanases, xylosidases, etc. In some embodiments, the host cell is a yeast, while in some other embodiments, the host cell is a filamentous fungal cell. In some further embodiments, the filamentous fungal cell is a Myceliophthora, a Thielavia, a Trichoderma, or an Aspergillus cell. In some embodiments, the filamentous fungal cell is Myceliophthora thermophila. In some additional embodiments, the host cell also produces at least one additional enzyme (e.g., esterase, protease, amylase, laccase, etc.).

[0021] In some additional embodiments, the present invention provides methods for producing at least one end-product from at least one cellulosic substrate. The substrate is contacted with at least one GH61 variant protein of the invention, and one or more cellulase enzymes. The fermentable sugars that are produced as a result are contacted with a microorganism in a fermentation to produce an end-product (e.g., an alcohol such as ethanol). The fermentation may be simultaneous with the saccharification, or may occur subsequently. It is not intended that the fermentation end-product be limited to any specific composition, as various end-products may be obtained from the fermentation reaction, including but not limited to alcohols.

[0022] The present invention also provides methods for producing fermentable sugars from cellulosic substrates, comprising contacting at the cellulosic substrate with at least one enzyme composition provided herein, under culture conditions whereby fermentable sugars are produced. In some embodiments the enzyme composition comprises a plurality of enzymes selected from at least one GH61 variant, at least one wild-type GH61, at least one endoglucanase (EG), at least one beta-glucosidase (BGL), at least one cellobiohydrolase (e.g., CBH1 and/or CBH2), at least one xylanase, at least one xylosidase, and/or at least one esterase. In some embodiments, the CBH1 is CBH1a. In further embodiments, the CBH2 is CHB2b. In some embodi-

ments, the methods further comprise the step of pretreating the cellulosic substrate prior to the contacting step. In some embodiments, the enzyme composition is added concurrently with the pretreating step.

[0023] In some embodiments, the cellulosic substrate comprises wheat grass, wheat straw, barley straw, sorghum, rice grass, sugarcane, sugarcane straw, bagasse, switchgrass, corn stover, corn fiber, grains, or a combination thereof. In further embodiments, the fermentable sugars comprise glucose and/or xylose. In some embodiments, the methods further comprise the step of recovering the fermentable sugars. In some embodiments, the methods further comprise the step of contacting the fermentable sugars with a microorganism under conditions such that the microorganism produces at least one fermentation end product. In further embodiments, the fermentation end product is selected from alcohols, fatty alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, amino acids, 1,3-propanediol, ethylene, glycerol, butadiene, and/or beta-lactams. In some still further embodiments, the fermentation end product is an alcohol selected from ethanol and butanol. In some embodiments, the alcohol is ethanol. It is not intended that the fermentation end-product be limited to any specific composition(s), as various end-products can be produced using the present invention.

[0024] The present invention also provides methods for producing an end product from a cellulosic substrate, comprising: contacting the cellulosic substrate with at any enzyme composition provided herein, under conditions whereby fermentable sugars are produced from the substrate; and contacting the fermentable sugars with a microorganism in a fermentation to produce an end-product. In some embodiments, the methods comprise simultaneous saccharification and fermentation reactions (SSF). In some alternative embodiments, the methods comprise saccharification of the cellulosic substrate and fermentation in separate reactions (SHF). In some additional embodiments, the methods comprise production of at least one enzyme simultaneously with hydrolysis and/or fermentation (e.g., "consolidated bioprocessing").

[0025] The present invention also provides methods for producing a fermentation end product from a cellulosic substrate, comprising obtaining fermentable sugars produced according to any method provided herein, and contacting the fermentable sugars with a microorganism in a fermentation to produce a fermentation end product. In some embodiments, the fermentation end product is selected from alcohols, fatty alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, citric acid, malic acid, fumaric acid, succinic acid, amino acids, 1,3-propanediol, ethylene, glycerol, butadiene, and/or beta-lactams. In some embodiments, the fermentation end product is at least one alcohol selected from ethanol and butanol. In further embodiments, the alcohol is ethanol. In some still further embodiments, the microorganism is a yeast. In some embodiments, the methods further comprise the step of recovering the fermentation end product. It is not intended that the fermentation end-product be limited to any specific composition(s), as various end-products can be produced using the present invention. It is also not intended that the present invention be limited to any particular microorganism. It is further not intended that the present invention be limited to any particular yeast, as any suitable yeast finds use in the present invention.

[0026] The present invention also provides for use of at least one GH61 variant protein as provided herein to produce at least one fermentation end product. The present invention also provides for use of at least one GH61 variant protein provided herein to produce at least one fermentation end product selected from alcohols, fatty alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, citric acid, malic acid, fumaric acid, succinic acid, amino acids, 1,3-propanediol, ethylene, glycerol, butadiene, and/or beta-lactams. In some embodiments, the fermentation end product is an alcohol selected from ethanol and butanol. In some embodiments, the alcohol is ethanol. It is not intended that the fermentation end-product be limited to any specific composition(s), as various end-products can be produced using the present invention.

[0027] A further embodiment of the invention is a composition comprising a GH61 protein, one or more cellulase enzymes, a cellulosic substrate, and an effective concentration of Cu++ and/or gallic acid, as further described and illustrated below. The GH61 protein may be any GH61 protein disclosed herein, such as a protein comprising an amino acid sequence at least about 60%, at least about 65%. at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO:2, or a fragment thereof with GH61 activity. In some embodiments, the GH61 protein is a variant protein comprising all or part of SEQ ID NO:2 having GH61 activity, wherein the variant comprises one or more of the amino acid substitutions provided herein. In some embodiments, the cellulase enzyme(s) are selected from endoglucanases (EG), beta-glucosidases (BGL), cellobiohydrolases (e.g., CBH1 and/or CBH2), xylanases, xylosidases, etc. In some embodiments, the presence of Cu++, gallic acid, or both enhances activity of the GH61 protein, thereby increasing the rate of glucose production or reducing the amount of GH61 protein needed to supply GH61 activity in a saccharification reaction.

[0028] In another embodiment, the present invention provides methods for producing fermentable sugars from cellulosic substrate(s), in which a composition comprising at least one GH61, at least one cofactor, at least one additional cellulase enzyme, and at least one cellulosic substrate is cultured or maintained under conditions whereby fermentable sugars are produced from the substrate(s). The fermentable sugars can then be contacted with a microorganism under conditions such that the microorganism produces at least one fermentation end product, such as ethanol. A further embodiment of the invention is use of Cu++ to increase production of fermentable sugars from a saccharification reaction where cellulase activity is enhanced in the presence of a protein or protein variant with GH61 activity. [0029] The present invention provides GH61 variant proteins comprising amino acid sequences that are at least about 75%, at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2 or the fragment. In some embodiments, the GH61 variant proteins comprise an amino acid sequence that is at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2 or the fragment. In some embodiments, the GH61 variant proteins are at least 95% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity. In some embodiments, the GH61 variant proteins have increased thermoactivity, thermostability, and/ or activity, as compared to the GH61 wild-type protein of SEQ ID NO:2. In some further embodiments, the GH61 variant proteins comprise at least one substitution(s) at one or more of the following amino acid positions: 20, 35, 42, 44, 45, 68, 87, 97, 103, 104, 127, 131, 132, 133, 134, 137, 139, 142, 143, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 190, 191, 192, 192, 205, 212, 215, 218, 232, 236, 239, 244, 246, 258, 270, 273, 317, 322, 323, 328, 330, and/or 341, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some embodiments, the GH61 variant proteins comprise at least one substitution(s) at one or more of the following amino acid positions: H20, N35, W42, Q44, P45, F68, T87, V97, P103, E104, S127, W131, F132, K133, I134. A137, Y139, A142, A143, I162, P163, S164, D165, L166, K167, A168, G169, N170, Y171, V172, L173, R174, H175, E176, I177, 1178, A179, L180, H181, Q190, A191, Y192, Y192, S205, A212, S215, K218, S232, T236, G239, A244, A246, T258, G270, P273, N317, P322, T323, G328, S330, and/or C341, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some further embodiments, the GH61 variant proteins comprise at least one substitution(s) at one or more of the following amino acid positions: H20, N35, W42, E104, I134, S164, K167, A168, V172, I177, A179, and/or A191, wherein the amino acid positions are numbered with reference to SEO ID NO:2. In some additional embodiments, the GH61 variant proteins comprise at least two amino acid substitutions. In still some further embodiments, the GH61 variant proteins comprise at least one substitution set selected from: N35/E104/A168; W42/E104/K167; N35/W42/V97/A191; W42/E104; E104/ K167; W42/A191; N35/W42/A191; V97/A191; and N35/ E104/A191, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some embodiments, the GH61 variant proteins comprise at least one amino acid substitution comprising one or more of the following substitutions numbered with reference to SEQ ID NO:2: H20C/ D, N35G, W42P, Q44V, P45T, F68Y, T87P, V97Q, P103E/ H, E104C/D/H/Q, S127T, W131X, F132X, K133X, 134X, A137P, Y139L, A142W, A143P, I162X, P163X, S164X, D165X, L166X, K167A/X, A168P/X, G169X, N170X, Y171A/R, V172X, L173X, R174X, H175X, E176X, I177X, I178X, A179X, L180M/W, H181X, Q190E/H, A191N/T, Y192H, Y192Q, S205N, A212P, S215W, K218T, S232A, T236P, G239D, A244D, A246T, T258I, G270S, P273S, N317K, P322L, T323P, G328A, 5330R, and/or C341R,

wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some additional embodiments, the GH61 variant proteins comprise one or more of the following substitutions: N35G, W42P, V97Q, E104H, K167A, A168P, and/or A191N, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some embodiments, the GH61 variant proteins comprise one or more of the following substitution sets: N35G/E104H/A168P; W42P/E104H/K167A; N35G/W42P/V97O/A191N; W42P/ E104H; E104H/K167A; W42P/A191N; N35G/W42P/ A191N; V97Q/A191N; and/or N35G/E104H/A191N, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some additional embodiments, the GH61 variant proteins comprise the substitutions N35G/ E104H/A168P, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some further embodiments, the GH61 variant proteins comprise the sequence set forth in any of SEQ ID NOS:4, 6, and/or 8. In some additional further embodiments, the GH61 variant proteins are encoded by at least one polynucleotide sequence set forth in SEQ ID NOS:3, 5, and/or 7. In some embodiments, the GH61 variant proteins comprise at least one substitution(s) at one or more of the following amino acid positions: 24, 28, 32, 34, 35, 40, 44, 45, 46, 49, 51, 54, 55, 56, 58, 64, 66, 67, 69, 70, 71, 78, 80, 82, 83, 88, 93, 95, 101, 104, 116, 118, 128, 130, 136, 137, 141, 142, 144, 145, 150, 155, 161, 164, 168, 184, 187, 199, 203, 205, 212, 218, 219, 230, 231, 232, 233, 234, 236, 237, 245, 253, 263, 266, 267, 268, 269, 270, 271, 280, 281, 282, 290, 295, 297, 303, 305, 310, 317, 320, 324, 326, 327, 329, 330, 332, 333, 336, 337, and/or 339, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some further embodiments, the GH61 variant proteins, comprise at least one substitution(s) at one or more of the following amino acid positions: S24, V28, Y32, R34, N35, T40, Q44, P45, N46, T49, 151, T54, A55, A56, Q58, E64, N66, S67, G69, T70, P71, S78, T80, G82, G83, V88, K93, N95, E101, E104, A116, N118, S128, R130, G136, A137, K141, A142, G144, R145, A150, G155, Q161, S164, A168, Q184, N187, R199, G203, S205, A212, K218, A219, V230, S231, S232, P233, D234, T236, V237, G245, S253, A263, P266, G267, G268, G269, G270, A271, A280, T281, S282, R290, S295, A297, P303, G305, K310, N317, T320, V324, A326, P327, S329, S330, S332, V333, E336, W337, and/or 5339, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some further embodiments, the GH61 variant proteins comprise a plurality of amino acid substitutions as set forth herein. In some embodiments, the GH61 variant proteins comprise at least one substitution set selected from: N35/T40/E104/A168/P327; N35/P45/E104/A168/N317; N35/E104/A168/N317; N35/E104/A168/N317/S329; N35/ E104/A137/A168/S232; N35/E104/A168/N317/T320; N35/ E104/A168/D234; N35/T40/E104/A142/A168; N35/E104/ R145/A168; N35/T40/S78N88/E104/S128K/A168/D234; N35/E104/A168/S330; N35/E104/A168/G203/P266; N35/ E104/A168/D234; N35/E104/A168/S330; N35/E104/A168/ W337; R34/N35/E104/R145/A168; Y32/N35/E64/E104/ A168; V28/N35/P45/E104/A168; N35/E104/G144/A168/ V333; N35/N66/E104/A168; N35/E104/A168/P327; N35/ E104/A168/G203; N35/E104/A168/S339; N35/P45/N46/ E104/A150/A168; N35/E104/A168/S231; N35/T40/E104/ A168/D234/P327; N35/E104/A168/S231; N35/E104/A168/ N317; N35/E104/A168/S330; N35/E104/A168/S329; N35/ E104/A168/P327; N35/P45/E104/A168; N35/E104/A116/

A168; N35/T40/E104/A168N230/P327; N35/E104/A168/ S332; N35/E104/A168/G203; N35/E104/R145/A168/S329; N35/T40/T49/E104/A168/D234; /P327; N35/A56/E104/ A168; N35/E104/Q161/A168; N35/E104/A168/S332; N35/ P45/T49/E104/A168/N317/T320; N35/E104/A168/V237; N35/E104/A168/E336; N35/E104/A168/P233; N35/E104/ R130/A168; N35/E104/A168/P327; N35/E104/A168/N317; N35/Q44/E104/A168; N35/E104/A168/A326; N35/E104/ A168/N317; N35/T40/E104/S128/A168; N35/T80/E104/ A168/P303; N35/E104/A116/A168; N35/E104/A168/S231/ S295; N35/T40/E101/E104/A168/P327; N35/P45/E104/ A168/A219/S232; N35/N46/E104/A168; N35/E104/A168/ A326; N35/E104/A168/G203/T281; N35/E104/A168/E336; N35/T40/E104/S128/A142/A168; N35/E104/N118/A168; N35/E104/G155/A168; S24/N35/E104/A168/V237/P303; N35/E104/Q161/A168; N35/Q44/S67/E104/A168; V28/ N35/E104/A168; N35/E104/A168/O184; N35/T54/E104/ A168; N35/N66/E104/A168; N35/E64/E104/A168; N35/ E104/S164/A168/A271; N35/N66/E104/A168; N35/G83/ E104/A168; N35/E104/K141/A168; N35/E104/A168/ N317/T320; N35/E104/R130/A168; N35/E104/R145/A168; N35/T70/E104/A168; N35/E104/R130/A168; N35/E104/ A168/Q184; N35/E104/A168/S329; N35/T49/E104/A168; Y32/N35/E104/A168; N35/E104/A168/S330; N35/Q58/ E104/A168; Y32/N35/P71/E104/A168; N35/E104/A168/ S330; N35/T80/E104/A168; N35/G82/E104/A168; N35/ E104/A168/S295; N35/N66/E104/A168; N35/T54/E104/ A168; N35/P45/E104/A168; N35/E104/S128/A168; N35/ N66/N95/E104/S164/A168; /G267; N35/T54/E104/A168; N35/P45/E104/K141/A168; N35/E104/A168/S332; N35/ E104/A168/A297; N35/E104/K141/R145/A168; N35/Q44/ E104/A168/S231; N35/T40/T49/S78/E104/A142; /A168; N35/E104/S164/A168/S295; N35/E104/A168/N317; N35/ P45/E104/A168; N35/G82/E104/A168; N35/N46/E104/ A168/G203/A263; N35/Q58/E104/A168; N35/G69/E104/ A168; N35/S67/E104/A168; N35/E104/A168/R199; N35/ E104/A168/G203/G268/G269/G270; N35/E104/A168/ V324; N35/E104/A168/P266; N35/E104/A168/G245; N35/ N66/E104/A168; S24/N35/Q44/T80/E104/A168; N35/ E104/A168/T236; N35/E104/A168/K310; N35/E104/R130/ A168; N35/N66/S78/E104/A168/S253; N35/N66/E104/ S164/A168/S282; N35/E104/A142/A168; N35/E104/R145/ A168; N35/E104/A168/S231; N35/E104/A168/Q184; N35/ E104/A168/K218; N35/E104/A168/P233; N35/T49/E104/ A168/Q184; N35/T40/E104/A168/P327; N35/T54/E104/ A168; N35/N66/E104/S164/A168/S231/S253; N35/E104/ A168/G203; N35/T49/E104/A168; N35/E104/A168/P266/ G267; N35/Q44/N66/E104/A168; N35/S67/E104/A168; N35/E104/A137/A168; N35/T49/E104/S128/A168; N35/ T49/E104/A168/K218/N317; N35/I51/E104/A168; N35/ E104/A168/A326; N35/P45/E104/A168/T320; N35/N66/ E104/A168; N35/E104/A168N237/P303; N35/P45/E104/ A168/K218/N317; N35/T80/E104/A168; N35/A55/E104/ A168; N35/E104/K141/A168/P266; N35/E104/A168/S330; N35/N66/E104/A168/R290; N35/E104/N118/A168; N35/ E104/A168/A212; N35/K93/E104/R130/A168; N35/E104/ A168/G267; N35/P45/T49/E104/A168/N317; N35/E104/ A168/V230; N35/E104/A168/S329; N35/P45/E104/A168/ A219; N35/S78/E104/S164/A168; N35/E104/A168/S205; V28/N35/N46/Q58/E104/A168; N35/E104/A168/Q184; N35/E104/A142/A168; N35/E104/A168/E336; N35/E104/ A168/A280; N35/E104/A168/A219; N35/E104/A168/ P303/G305; R34/N35/E104/A168/A280; N35/E104/A168/ N187; N35/E104/G136/A168; N35/E104/A168/Q184; N35/

T49/E104/A168/N317: N35/T40/T49/S78/E104/A168: R34/N35/K93/E104/R130/R145/A168/R199/K218/A280; N35/T40/E104/A142/A168; and N35/N66/E104/A168, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some further embodiments, the GH61 variant proteins comprise at least one amino acid substitution comprising one or more of the following substitutions numbered with reference to SEO ID NO:2: S24O; V28H; Y32S; R34E; N35G; T40A/G/L/S; O44K; P45D/E/ K/R/S; N46E/R; T49A/Q/R/Y; I51A; T54G/M/S/W; A55G; A56S; Q58H/P; E64L/S; N66A/D/G/L/M/Q/RN; S67G/H/ T; G69T; T70A; P71A; S78C/D; T80H/L/V; G82A/S; G83R; V88I; K93N/T; N95E; E101T; E104H; A116Q/S; N118E/S; S128K/L/N; R130E/G/H/K/Y; G136H; A137M/ S; K141A/N/P/R; A142D/G/L; G144S; R145H/L/N/Q/T; A150Y; G155N; Q161E/R; S164E; A168P; Q184E/H/L/N/ R; N187D; R199E; G203E/V/Y; S205T; A212M; K218L/T; A219R/T; V230I/Q; S231A/H/K/I; S232E; P233F/T; D234E/M/N; T236E; V237I; G245A; S253D/T; A263V; P266S; G267D/V; G268A; G269A; G270A; A271T; A280D/T; T281A; S282D; R290K; S295D/L/T; A297T; P303T; G305D; K310I; N317D/H/I/M/Q/R; T320A; V324M; A326C/Q/V; P327F/K/L/M; S329H/I/Q/T/Y; S330A/H/I/TN; S332C/F/R; V333Q; E336L/R/S; W337R; and/or S339W, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some embodiments, the GH61 variant proteins comprise a plurality of substitutions and/or substitution sets as provided therein. In some additional embodiments, the GH61 variant proteins comprise one or more of the following substitution sets: N35G/T40A/E104H/A168P/P327M; N35G/P45D/E104H/ A168P/N317R; N35G/E104H/A168P/N317R; E104H/A168P/N317D/S329Y; N35G/E104H/A137S/ N35G/E104H/A168P/N317R/T320A; A168P/S232E; N35G/E104H/A168P/D234E; N35G/T40S/E104H/A142G/ A168P; N35G/E104H/R145L/A168P; N35G/T40S/S78C/ V88I/E104H/S128K/A168P/D234M; N35G/E104H/ A168P/S330V: N35G/E104H/A168P/G203E/P266S: N35G/E104H/A168P/D234N; N35G/E104H/A168P/ N35G/E104H/A168P/W337R; R34E/N35G/ E104H/R145T/A168P; Y32S/N35G/E64S/E104H/A168P; V28H/N35G/P45K/E104H/A168P; N35G/E104H/G144S/ A168P/V333O: N35G/N66O/E104H/A168P; E104H/A168P/P327K; N35G/E104H/A168P/G203E: N35G/E104H/A168P/S339W; N35G/P45K/N46E/E104H/ A150Y/A168P; N35G/E104H/A168P/S231K; N35G/T40A/ N35G/E104H/A168P/ E104H/A168P/D234E/P327M; N35G/E104H/A168P/N317M: N35G/E104H/ A168P/S330Y; N35G/E104H/A168P/S329I; N35G/E104H/ A168P/P327F; N35G/P45D/E104H/A168P; N35G/E104H/ A116S/A168P; N35G/T40A/E104H/A168P/V230I/P327M; N35G/E104H/A168P/S332R; N35G/E104H/A168P/ G203V; N35G/E104H/R145N/A168P/S329H; N35G/T40S/ T49R/E104H/A168P/D234E; /P327M; N35G/A56S/ N35G/E104H/Q161R/A168P; E104H/A168P; N35G/ E104H/A168P/S332F; N35G/P45R/T49A/E104H/A168P/ N35G/E104H/A168P/V237I; N317R/T320A: N35G/ E104H/A168P/E336S; N35G/E104H/A168P/P233T; N35G/E104H/R130H/A168P; N35G/E104H/A168P/ P327L; N35G/E104H/A168P/N317I; N35G/Q44K/E104H/ A168P; N35G/E104H/A168P/A326V; N35G/E104H/ A168P/N317H; N35G/T40L/E104H/S128K/A168P; N35G/ T80V/E104H/A168P/P303T; N35G/E104H/A116Q/A168P; N35G/E104H/A168P/S231A/S295L; N35G/T40S/E101T/ E104H/A168P/P327M: N35G/P45K/E104H/A168P/ A219R/S232E; N35G/N46R/E104H/A168P; N35G/E104H/ A168P/A326Q; N35G/E104H/A168P/G203E/T281A; N35G/E104H/A168P/E336R; N35G/T40S/E104H/S128K/ A142G/A168P; N35G/E104H/N118S/A168P; E104H/G155N/A168P; S24Q/N35G/E104H/A168P/V237I/ P303T; N35G/E104H/O161E/A168P; N35G/O44K/S67T/ E104H/A168P; V28H/N35G/E104H/A168P; N35G/E104H/ A168P/Q184L; N35G/T54G/E104H/A168P; N35G/N66M/ E104H/A168P; N35G/E64L/E104H/A168P; N35G/E104H/ S164E/A168P/A271T; N35G/N66A/E104H/A168P; N35G/ G83R/E104H/A168P; N35G/E104H/K141A/A168P; N35G/E104H/A168P/N317Q/T320A; N35G/E104H/ N35G/E104H/R145Q/A168P; R130G/A168P; T70A/E104H/A168P; N35G/E104H/R130K/A168P; N35G/ E104H/A168P/Q184E; N35G/E104H/A168P/S329T; N35G/T49A/E104H/A168P; Y32S/N35G/E104H/A168P; N35G/E104H/A168P/S330I; N35G/Q58H/E104H/A168P; Y32S/N35G/P71A/E104H/A168P; N35G/E104H/A168P/ S330T; N35G/T80V/E104H/A168P; N35G/G82A/E104H/ A168P; N35G/E104H/A168P/S295T; N35G/N66G/E104H/ A168P; N35G/T54S/E104H/A168P; N35G/P45S/E104H/ A168P; N35G/E104H/S128L/A168P; N35G/N66D/N95E/ E104H/S164E/A168P; /G267D; N35G/T54W/E104H/ A168P; N35G/P45E/E104H/K141R/A168P; N35G/E104H/ A168P/S332C; N35G/E104H/A168P/A297T; E104H/K141P/R145Q/A168P; N35G/Q44K/E104H/ A168P/S231T; N35G/T40G/T49R/S78C/E104H/A142G; N35G/E104H/S164E/A168P/S295D; E104H/A168P/N317Q; N35G/P45R/E104H/A168P; N35G/ G82S/E104H/A168P; N35G/N46R/E104H/A168P/G203E/ A263V; N35G/Q58P/E104H/A168P; N35G/G69T/E104H/ A168P; N35G/S67G/E104H/A168P; N35G/E104H/A168P/ R199E: N35G/E104H/A168P/G203E/G268A/G269A/ G270A; N35G/E104H/A168P/V324M; N35G/E104H/ A168P/P266S; N35G/E104H/A168P/G245A; N35G/N66R/ E104H/A168P; S24Q/N35G/Q44K/T80H/E104H/A168P; N35G/E104H/A168P/T236E; N35G/E104H/A168P/K310I; N35G/E104H/R130Y/A168P; N35G/N66D/S78D/E104H/ A168P/S253D; N35G/N66D/E104H/S164E/A168P/S282D; N35G/E104H/A142L/A168P; N35G/E104H/R145H/ N35G/E104H/A168P/S231T; N35G/E104H/ A168P; A168P/O184R: N35G/E104H/A168P/K218L; N35G/ E104H/A168P/P233F; N35G/T49A/E104H/A168P/O184H; N35G/T40S/E104H/A168P/P327M; N35G/T54M/E104H/ A168P; N35G/N66D/E104H/S164E/A168P/S231T/S253T; N35G/E104H/A168P/G203Y; N35G/T49Q/E104H/A168P; N35G/E104H/A168P/P266S/G267V; N35G/Q44K/N66V/ E104H/A168P; N35G/S67H/E104H/A168P; N35G/E104H/ A137M/A168P; N35G/T49A/E104H/S128N/A168P; N35G/T49R/E104H/A168P/K218L/N317Q; N35G/I51A/ E104H/A168P; N35G/E104H/A168P/A326C; N35G/P45R/ E104H/A168P/T320A; N35G/N66L/E104H/A168P; N35G/ E104H/A168P/V237I/P303T; N35G/P45R/E104H/A168P/ K218L/N317Q; N35G/T80L/E104H/A168P; N35G/A55G/ N35G/E104H/K141N/A168P/P266S; E104H/A168P; N35G/E104H/A168P/S330A; N35G/N66D/E104H/A168P/ R290K: N35G/E104H/N118E/A168P; N35G/E104H/ N35G/K93N/E104H/R130Y/A168P; A168P/A212M; N35G/E104H/A168P/G267D; N35G/P45R/T49Y/E104H/ A168P/N317D; N35G/E104H/A168P/V230Q; N35G/ E104H/A168P/S329Q; N35G/P45K/E104H/A168P/ A219R; N35G/S78D/E104H/S164E/A168P; N35G/E104H/ A168P/S205T; N35G/E104H/A168P/Q184H; V28H/N35G/

N46E/Q58H/E104H/A168P; N35G/E104H/A142D/A168P; N35G/E104H/A168P/E336L; N35G/E104H/A168P/ A280T: N35G/E104H/A168P/A219T; N35G/E104H/ A168P/P303T/G305D; R34E/N35G/E104H/A168P/A280T; N35G/E104H/A168P/N187D; N35G/E104H/G136H/ N35G/E104H/A168P/Q184N; N35G/T49Y/ E104H/A168P/N317R; N35G/T40A/T49Q/S78C/E104H/ A168P; R34E/N35G/K93T/E104H/R130E/R145T/A168P/ R199E/K218T/A280D; N35G/T40L/E104H/A142G/ A168P; and/or N35G/N66G/E104H/A168P, wherein the amino acid positions are numbered with reference to SEQ ID NO:2. In some further embodiments, the GH61 variant proteins comprise a plurality of substitutions as provided herein. In some additional embodiments, the GH61 variant proteins comprise polypeptide sequences that are at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to any of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9, and/or a biologically active fragment of any of SEQ ID NOS: 2, 3, 5, 6, 8, and/or 9, wherein the fragment has GH61 activity. In still some additional embodiments, the GH61 variant proteins comprise polypeptide sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9, and/or a biologically active fragment of any of SEQ ID NOS: 2, 3, 5, 6, 8, and/or 9, wherein the fragment has GH61 activity.

[0030] The present invention also provides GH61 variant proteins comprising amino acid sequences that are at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9, or a fragment of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NOS:2, 3, 5, 6, 8, and/or 9 or the fragment, and wherein the substitution(s) in the amino acid sequences result in the variant proteins having increased GH61 activity in a reaction where crystalline cellulose undergoes saccharification by cellulase enzymes that are contained in culture broth from M. thermophila cells, compared with a reference protein comprising SEQ ID NO:2, 3, 5, 6, 8, and/or 9 or the fragment, without any substitutions. In some embodiments, the GH61 variant proteins comprise amino acid sequences that are at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9, or a fragment of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NOS:2, 3, 5, 6, 8, and/or 9 or the fragment, and wherein the substitution(s) in the amino acid sequences result in the variant proteins having increased GH61 activity in a reaction where crystalline cellulose undergoes saccharification by cellulase enzymes that are contained in culture broth from M. thermophila cells, compared with a reference protein comprising SEQ ID NO:2, 3, 5, 6, 8, and/or 9 or the fragment, without any substitutions. In some further embodiments, the present invention provides GH61 variant proteins encoded by polynucleotides, wherein the proteins comprise amino acid sequences that are at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9 or a fragment of SEQ ID NO:2, 3, 5, 6, 8, and/or 9 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2, 3, 5, 6, 8, and/or 9 or the fragment, and wherein the polynucleotide encoding the GH61 variant protein comprises at least one mutation and/or mutation set selected from t60c/c573g, t60c/c573g/g1026a, c573g, t60c/c291a/c573g, t60c/c291a, t60c/c876t, a312g, t60c, t379a/c380g/g381c, c300t, t204c/ t379a/c380g/g381c/c385t, g1026a, c246t, c597g, c72t, c732g/c843t/c882t, c909t, c912g, g921a, c792t, g972t, g921a, t379a/c380g/g381c/c454a/c456a/c732t/c843t/c849t, c520a/c522g, t60c/c573g; t60c/c288t/c573g; t60c/c198t/ c573g; and/or t60c/g399a/c573g; wherein the nucleotide positions are numbered with reference to SEO ID NO:1. In still some further embodiments, the present invention provides GH61 variant proteins encoded by polynucleotides, wherein the proteins comprise amino acid sequences that are at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of SEQ ID NOS:2, 3, 5, 6, 8, and/or 9 or a fragment of SEQ ID NO:2, 3, 5, 6, 8, and/or 9 having GH61 activity, wherein the amino acid sequence of the variant protein has one or more amino acid substitutions with respect to SEQ ID NO:2, 3, 5, 6, 8, and/or 9 or the fragment, and wherein the polynucleotide encoding the GH61 variant protein comprises at least one mutation and/or mutation set selected from t60c/c573g, t60c/c573g/g1026a, c573g, t60c/ c291a/c573g, t60c/c291a, t60c/c876t, a312g, t60c, t379a/ c380g/g381c, c300t, t204c/t379a/c380g/g381c/c385t, g1026a, c246t, c597g, c72t, c732g/c843t/c882t, c909t, c912g, g921a, c792t, g972t, g921a, t379a/c380g/g381c/ c454a/c456a/c732t/c843t/c849t, c520a/c522g, t60c/c573g; t60c/c288t/c573g; t60c/c198t/c573g; and/or t60c/g399a/ c573g; wherein the nucleotide positions are numbered with reference to SEQ ID NO:1.

[0031] The present invention also provides polynucleotides comprising a nucleic acid sequences encoding the GH61 variant proteins provided herein, as well as polynucleotides that hybridize under stringent hybridization conditions to at least one polynucleotide and/or a complement of at least one polynucleotide encoding GH61 variant proteins provided herein. In some embodiments, the present invention provides polynucleotide sequences encoding GH61 variant proteins, wherein the polynucleotide sequences are at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any of SEQ ID NOS:1, 4, 7, and/or 10, or at least one polynucleotide that hybridizes under stringent hybridization conditions to at least one polynucleotide and/or complement of any of SEQ ID NOS:1, 4, 7, and/or 10. In some additional embodiments, the present invention provides polynucleotide sequences encoding GH61 variant proteins, wherein the polynucleotide sequences are at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any of SEQ ID NOS:1, 4, 7, and/or 10, or at least one polynucleotides that hybridizes under stringent hybridization conditions to at least one polynucleotide and/or complement of any of SEQ ID NOS: 1, 4, 7, and/or 10.

[0032] The present invention also provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one GH61 protein, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to SEQ ID NO:2, 3, 5, 6, 8, and/or 9, wherein the amino acid sequence comprises at least one substitution and/or substitution set provided herein; (b) a polynucleotide that hybridizes under stringent hybridization conditions to at least a fragment of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 3, 5, 6, 8, and/or 9, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein; and/or (c) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of at least a fragment of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 3, 5, 6, 8, and/or 9, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein. In some embodiments, the recombinant nucleic acid constructs comprise at least one polynucleotide sequence encoding at least one GH61 protein, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:2, wherein the amino acid sequence comprises at least one substitution and/or substitution set provided herein; (b) a polynucleotide that hybridizes under stringent hybridization conditions to a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein; and/or (c) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, and wherein the amino acid sequence comprises at least one substitution and/or at least one substitution set provided herein. In some additional embodiments, the recombinant nucleic acid constructs comprise at least one polynucleotide sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any of SEQ ID NOS:1, 4, 7, and/or 10, and wherein the polynucleotide sequence comprises at least one mutation and/or at least one mutation set provided herein. In some further additional embodiments, the recombinant nucleic acid constructs comprise polynucleotide sequences that are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any of SEQ ID NOS:1, 4, 7, and/or 10, and wherein the polynucleotide sequence comprises at least one mutation and/or at least one mutation set provided herein. In some embodiments, the polynucleotides and/or nucleic acid constructs provided herein comprise at least one polynucleotide sequence comprising at least one mutation or mutation set selected from t60c/c573g, t60c/c573g/g1026a, c573g, t60c/c291a/c573g, t60c/c291a, t60c/c876t, a312g, t60c, t379a/c380g/g381c, c300t, t204c/t379a/c380g/g381c/ c385t, g1026a, c246t, c597g, c72t, c732g/c843t/c882t, c909t, c912g, g921a, c792t, g972t, g921a, t379a/c380g/ g381c/c454a/c456a/c732t/c843t/c849t, c520a/c522g; t60c/ c573g; t60c/c288t/c573g; t60c/c198t/c573g; and/or t60c/ g399a/c573g. In some additional embodiments, the polynucleotide and/or nucleic acid construct comprise at least one nucleic acid sequence operably linked to a promoter. In some additional embodiments, the promoter is a heterologous promoter. In some further embodiments, the nucleic acid constructs further encode at least one enzyme in addition to the GH61 variant protein. In some embodiments, the nucleic acid constructs comprise at least one additional enzyme is selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases. In some further embodiments, at least one additional enzyme is selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), Type 2 cellobiohydrolases (CBH2), xylanases, and xylosidases.

[0033] The present invention also provides expression constructs comprising at least one polynucleotide or nucleic acid construct as provided herein. In some expression construct embodiments, the nucleic acid construct and/or the polynucleotide is operably linked to a promoter. In some embodiments, the promoter is heterologous. In some further embodiments of the expression constructs provided herein, the nucleic acid sequence is operably linked to at least one additional regulatory sequence.

[0034] The present invention also provides host cells that express at least one polynucleotide sequence encoding at least one GH61 variant protein provided herein. In some embodiments, the host cells produce at least one GH61 variant protein provided herein. In some additional embodiments, at least one GH61 variant protein is secreted from the host cells. In some further embodiments, the host cells further produce at least one enzyme selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases. In some additional embodiments, the host cell further produces at least one enzyme selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), and Type 2 cellobiohydrolases (CBH2). In some embodiments, the host cell is a yeast or filamentous fungal cell. In some embodiments, the filamentous fungal cell is a Myceliophthora, a Chrysosporium a Thielavia, a Trichoderma, or an Aspergillus cell. In some further embodiments, the filamentous fungal cell is Myceliophthora thermophila. In some additional embodiments, the host cell is a yeast cell. In some further additional embodiments, the host cell is Saccharomyces. In some further embodiments, the host cells further comprise at least one polynucleotide, polynucleotide construct, and/or expression construct as provided herein.

[0035] The present invention also provides methods of producing at least one GH61 variant protein comprising culturing the host cell set forth herein under conditions such that the host cell produces at least one GH61 variant proteins as provided herein. In some embodiments of the methods, the host cell further produces at least one additional enzyme selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases. In some embodiments of the methods, the host cell further produces at least one EG, at least one BGL, at least one CBH1, at least one CBH2, and/or at least one wild-type GH61 enzyme. In some further embodiments of the methods, the conditions comprise culturing at about pH 5, while in some alternative embodiments of the methods, the conditions comprise culturing at about pH 6.7. In some embodiments of the methods, the filamentous fungal cell is a Myceliophthora, a Chrysosporium, a Thielavia, a Trichoderma, or an Aspergillus cell. In some further embodiments of the methods, the filamentous fungal cell is a Myceliophthora thermophila. In some additional embodiments of the methods, the host cell is a yeast cell. In some further additional embodiments of the methods, the host cell is Saccharomyces.

[0036] The present invention also provides enzyme compositions comprising at least one GH61 variant protein as provided herein. In some embodiments, the enzyme compositions further comprise one or more enzymes selected from wild-type GH61 enzymes, endoglucanases (EG), betaglucosidases (BGL), Type 1 cellobiohydrolases (CBH1), and/or Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases. In some additional embodiments, the enzyme compositions further comprise at least two additional enzymes selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), and/or Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases. In some embodiments, the enzyme compositions are produced by the host cells provided herein. In some additional embodiments, the enzyme compositions further comprise a microorganism. In some further embodiments, the microorganism comprises M. thermophila. In some embodiments, the enzyme compositions further comprise at least one adjunct composition. In some additional embodiments, the enzyme compositions comprise at least one adjunct composition selected from divalent metal cations, reductants, surfactants, buffers, culture media, and enzyme stabilizing systems. In some further embodiments, the enzyme compositions comprise adjunct composition comprising copper and/or gallic acid. In some additional embodiments, the enzyme compositions find use in saccharification reactions.

[0037] The present invention also provides compositions comprising at least one GH61 protein, one or more cellulase enzymes, a cellulosic substrate, and Cu++, wherein the GH61 protein is at least about 70%, about 75%, about 80%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to any of SEO ID NOS:2, 5, 6, 8, 9, 11, and/or 12, and/or a biologically fragment thereof with GH61 activity. In some embodiments, the present invention provides compositions comprising at least one GH61 protein, one or more cellulase enzymes, a cellulosic substrate, and Cu⁺⁺, wherein the GH61 protein is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:2, 5, 6, 8, 9, 11, and/or 12, and/or a biologically fragment thereof with GH61 activity. In some embodiments, the concentration of Cu++ is at least about 4 µM. In some embodiments, the concentration of Cu⁺⁺ is between about 1 μ M and about 100 μ M, between about 4 μ M and about 100 μM, or between about 5 μM and about 100 μM.

[0038] The present invention also provides compositions comprising at least one GH61 protein, one or more cellulase enzymes, a cellulosic substrate, and gallic acid, wherein the GH61 protein is at least about 70%, about 80%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to any of SEQ ID NO:2, 5, 6, 8, 9, 11, and/or 12, and/or a biologically fragment thereof with GH61 activity. The present invention also provides compositions comprising at least one GH61 protein, one or more cellulase enzymes, a cellulosic substrate, and gallic acid, wherein the GH61 protein is at least 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of SEQ ID NO:2, 5, 6, 8, 9, 11, and/or 12, and/or a biologically fragment thereof with GH61 activity. In some embodiments, the concentration of gallic acid in the compositions is at least about 0.1 mM. In some embodiments, the compositions comprise gallic acid at a concentration between about 1 mM and about 5 mM. In some embodiments, the concentration of gallic acid in the composition is at least 0.1 mM. In some embodiments, the compositions comprise gallic acid at a concentration between 1 mM and 5 mM. In some embodiments, the compositions comprise at least one GH61 protein comprising SEQ ID NO:2, 5, 6, 8, 9, 11, and/or 12, and/or a biologically active fragment thereof with GH61 activity. In some embodiments, the compositions comprise at least one GH61 variant protein as provided herein. In some embodiments, the compositions comprise at least one cellulase enzyme selected from endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), and/or Type 2 cellobiohydrolases (CBH2). In some embodiments, the compositions comprise at least one BGL, CBH1, and CBH2. In some additional embodiments, the compositions further comprise at least one additional enzyme. In some further embodiments, at least one additional enzyme is selected from hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases. In still some further embodiments of the compositions, the cellulosic substrate is selected from wheat grass, wheat

straw, barley straw, sorghum, rice grass, sugarcane straw, bagasse, switchgrass, corn stover, corn fiber, grains, or any combination thereof.

[0039] The present invention also provides methods for producing fermentable sugars from a cellulosic substrate, comprising contacting the cellulosic substrate with at least one enzyme composition as provided herein under conditions whereby fermentable sugars are produced. In some embodiments, the methods further comprise pretreating the cellulosic substrate prior to the contacting. In some additional embodiments of the methods, the enzyme composition is added concurrently with pretreating. In some further embodiments of the methods, the cellulosic substrate comprises wheat grass, wheat straw, barley straw, sorghum, rice grass, sugarcane, sugarcane straw, bagasse, switchgrass, corn stover, corn fiber, grains, or any combination thereof. In some additional embodiments of the methods, the fermentable sugars comprise glucose and/or xylose. In some embodiments, the methods further comprise recovering the fermentable sugars. In some embodiments of the methods, the conditions comprise using continuous, batch, and/or fed-batch culturing conditions. In some further embodiments, the method is a batch process, while in some alternative embodiments, the method is a continuous process, and in some still further embodiments, the method is a fed-batch process. In some embodiments, the methods comprise any combination of batch, continuous, and/or fed-batch processes conducted in any order. In still some further embodiments, the methods are conducted in a reaction volume of at least 10,000 liters, while in some other embodiments, the methods are conducted in a reaction volume of at least 100,000 liters. In some embodiments, the methods further comprise use of at least one adjunct composition. In some embodiments, the adjunct composition is selected from at least one divalent metal cation, gallic acid, and/or at least one surfactant. In some embodiments, the divalent metal cation comprises copper and/or gallic acid. In some additional embodiments, the surfactant is selected from TWEEN®-20 non-ionic detergent and polyethylene glycol. In some further embodiments, the methods are conducted at about pH 5.0, while in some alternative embodiments, the methods are conducted at about pH 6.0. In some additional embodiments, the pH is in the range of about 4.5 to about 7. In some embodiments, the methods further comprise contacting the fermentable sugars with a microorganism under conditions such that the microorganism produces at least one fermentation end product. In some embodiments, the fermentation end product is selected from alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, amino acids, 1,3-propanediol, ethylene, glycerol, fatty alcohols, butadiene, and beta-lactams. In some further embodiments, the fermentation product is an alcohol selected from ethanol and butanol. In some still further embodiments, the alcohol is ethanol.

[0040] The present invention also provides methods for increasing production of fermentable sugars from a saccharification reaction comprising combining at least one cellulase substrate, one or more cellulase enzymes, and at least one GH61 protein wherein the protein is at least about 70%, about 75%, about 80%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to SEQ ID

NO:2, and an adjunct composition in a saccharification reaction, wherein the adjunct composition comprises Cu⁺⁺ at a concentration of at least about 4 µM and/or gallic acid at a concentration of at least about 0.5 mM. The present invention also provides methods for increasing production of fermentable sugars from a saccharification reaction comprising combining at least one cellulase substrate, one or more cellulase enzymes, and at least one GH61 protein wherein the protein is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:2, and an adjunct composition in a saccharification reaction, wherein the adjunct composition comprises Cu⁺⁺ at a concentration of at least about 4 µM and/or gallic acid at a concentration of at least about 0.5 mM. In some embodiments, at least one GH61 protein comprises SEQ ID NO:2, 5, 6, 8, 9, 11, and/or a biologically active fragment thereof. In some embodiments of the methods, the GH61 protein is at least one GH61 protein variant as provided herein. In some embodiments, the methods further comprise use of at least one surfactant selected from TWEEN®-20 non-ionic detergent and polyethylene glycol. In some additional embodiments, the methods are conducted at about pH 5.0, while in some other embodiments, the methods are conducted at about pH 6.0.

[0041] The present invention also provides methods of producing at least one end product from at least one cellulosic substrate, comprising: a) providing at least one cellulosic substrate and at least one enzyme composition as provided herein; b) contacting the cellulosic substrate with the enzyme composition under conditions whereby fermentable sugars are produced from the cellulosic substrate in a saccharification reaction; and c) contacting the fermentable sugars with a microorganism under fermentation conditions such that at least one end product is produced. In some embodiments, the method comprises simultaneous saccharification and fermentation reactions (SSF), while in some alternative embodiments of the methods, saccharification of the cellulosic substrate and fermentation are conducted in separate reactions (SHF). In some additional embodiments, the methods comprise production of at least one enzyme simultaneously with hydrolysis and/or fermentation (e.g., "consolidated bioprocessing"; CBP). In some embodiments, the enzyme composition is produced simultaneously with the saccharification and fermentation reactions. In some additional embodiments at least one enzyme of said composition is produced simultaneously with the saccharification and fermentation reactions. In some embodiments, in which at least one enzyme and/or the enzyme composition is produced simultaneously with the saccharification and fermentation reactions, the methods are conducted in a single reaction vessel. In some embodiments, the methods further comprise use of at least one adjunct composition in the saccharification reaction. In some embodiments of the methods, at least one adjunct composition is selected from at least one divalent metal cation, gallic acid, and/or at least one surfactant. In some further embodiments of the methods, the divalent metal cation comprises copper. In some further embodiments of the methods, the adjunct composition comprises gallic acid. In some additional embodiments of the methods, the surfactant is selected from TWEEN®-20 nonionic detergent and polyethylene glycol. In some embodiments, the method is conducted at about pH 5.0. In some embodiments, the method is conducted at about pH 6.0. In some further embodiments, the method is conducted at a pH

in the range of about 4.5 to about 7.0. In some embodiments, the methods further comprise recovering at least one end product. In some embodiments of the methods the end product comprises at least one fermentation end product. In some further embodiments of the methods, the fermentation end product is selected from alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propanediol, ethylene, glycerol, fatty alcohols, butadiene, and beta-lactams. In some embodiments of the methods, the fermentation end product is at least one alcohol selected from ethanol and butanol. In some embodiments of the methods, the alcohol is ethanol. In some additional embodiments of the methods, the microorganism is a yeast. In some further embodiments, the yeast is Saccharomyces. In some further additional embodiments, the methods further comprise recovering at least one fermentation end product.

[0042] The present invention also provides for use of at least one GH61 variant protein provided herein to produce at least one fermentation end product. In some embodiments, at least one GH61 variant protein provided herein is used to produce at least one fermentation end product selected from alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, citric acid, malic acid, fumaric acid, succinic acid, amino acids, 1,3-propanediol, ethylene, glycerol, butadiene, fatty alcohols, and beta-lactams. In some embodiments, the fermentation end product is at least one alcohol selected from ethanol and butanol. In some further embodiments, the alcohol is ethanol.

[0043] Additional embodiments of the invention are apparent from the present description.

DESCRIPTION OF THE DRAWINGS

[0044] FIG. 1 provides results of an experiment using recombinantly produced GH61a protein having the sequence shown in SEQ ID NO:2. The protein was tested for its ability to promote the activity of cellulases present in culture broth of *M. thermophila*. The graph shows the improvement in the yield of the fermentable sugar glucose that is attained by adding GH61 to the reaction.

[0045] FIG. 2 shows specific GH61 activity observed in a reaction where a wheat straw substrate was hydrolyzed by cellulase enzymes CBH1, CBH2, and beta-glucosidase. The results show that GH61a Variants 5 and 9 have a 2.0 to 2.9 fold improvement over the parental GH61 sequence (SEQ ID NO:2); and Variant 1 has a 3.0 to 3.9 fold improvement.

[0046] FIG. 3 shows the increase in glucose production in the presence of GH61 protein when Cu⁺⁺ is included the reaction. In this Figure, n=4; and mean±SD. Panel A shows the increase with a GH61 variant protein "Variant 5," while Panel B shows the increase with the wild-type GH61a protein (SEQ ID NO:2).

[0047] FIG. 4 shows activity of GH61a pre-incubated with 0 or 50 μ M CuSO₄, copper(II) ion at either saccharification pH 5.0 or pH 6.0. Panel A shows glucose production, while Panel B shows the total production of C5 sugars.

[0048] FIG. 5 shows activity of *M. thermophila*-produced GH61a Variant 1 on cellulosic substrates. Panel A shows the results on AVICEL® PH microcrystalline cellulose, and Panel B shows the results on phosphoric acid swollen cellulose (PASC), in the presence of ascorbic acid, gallic acid and pretreatment filtrate.

[0049] FIG. 6 provides results showing the effects of surfactants on saccharification. Panel A shows enzymatic hydrolysis activity of a cellulase mixture in the presence of TWEEN®-20, while Panel B shows the enzymatic hydrolysis activity of a cellulase mixture in the presence of PEG-4000.

DETAILED DESCRIPTION OF THE INVENTION

[0050] As described herein, the present invention provides GH61 proteins of the filamentous fungus *Myceliophthora thermophila* that have been genetically modified. These GH61 protein variants exhibit improved activity and other benefits, as compared to wild-type GH61 proteins.

[0051] Before modification, the GH61 protein having the sequence shown in SEQ ID NO:2 improves the yield of fermentable sugars produced from a cellulosic substrate through the activity of cellulase enzymes (e.g., endoglucanase, beta-glucosidase (BGL), cellobiohydrolase, and combinations of such enzymes; See, FIG. 1). The GH61 variant proteins of this invention have certain amino acid substitutions in relation to SEQ ID NO:2, either alone or in various combinations. GH61 variant proteins that have gone through one round of optimization, when included in a saccharification assay, improve the yield of fermentable sugars in such reactions by at least about 2-fold, about 3-fold, or more, in relation to the improvement in yield when wild-type GH61a (SEQ ID NO:2) is used instead. (See, FIG. 2). After multiple rounds of optimization, the GH61 activity can be improved by a further 1.5-fold, 2-fold, 3-fold or more.

[0052] The GH61 variant proteins of the present invention have important industrial applicability in the processing of cellulosic biomass to produce fermentable sugars, which in turn can be fermented or processed to produce commercially important fermentation products (e.g., "fermentation endproducts" or "end-products"), including but not limited to, at least one alcohol, fatty acid, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, amino acid, 1,3-propanediol, ethylene, glycerol, fatty alcohol, butadiene, and/or beta-lactam. In further embodiments, the alcohol is ethanol, butanol, and/or a fatty alcohol. In some embodiments, the fermentation product is ethanol. In some still further embodiments, the fermentation product is a fatty alcohol that is a C8-C20 fatty alcohol. In some embodiments, the fermentation medium comprises at least one product from a saccharification process.

[0053] GH61 proteins, their production and use are generally described in PCT/US11/488700. This application claims priority to U.S. Ser. No. 61/375,788, both of which are incorporated herein by reference in their entirety. Proteins, procedures, and uses described in these applications find use with the GH61 variant proteins of the present invention.

DEFINITIONS

[0054] All patents and publications, including all sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference. Unless otherwise indicated, the practice of the present invention involves conventional techniques commonly used in molecular biology, fermentation, microbiology, and related fields, which are known to those of skill in the art.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. Indeed, it is intended that the present invention not be limited to the particular methodology, protocols, and reagents described herein, as these may vary, depending upon the context in which they are used. The headings provided herein are not limitations of the various aspects or embodiments of the present invention.

[0055] Nonetheless, in order to facilitate understanding of the present invention, a number of terms are defined below. Numeric ranges are inclusive of the numbers defining the range. Thus, every numerical range disclosed herein is intended to encompass every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein. It is also intended that every maximum (or minimum) numerical limitation disclosed herein includes every lower (or higher) numerical limitations were expressly written herein.

[0056] As used herein, the term "comprising" and its cognates are used in their inclusive sense (i.e., equivalent to the term "including" and its corresponding cognates).

[0057] As used herein and in the appended claims, the singular "a", "an" and "the" include the plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "host cell" includes a plurality of such host cells.

[0058] Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. The headings provided herein are not limitations of the various aspects or embodiments of the invention that can be had by reference to the specification as a whole. Accordingly, the terms defined below are more fully defined by reference to the specification as a whole.

[0059] As used herein, the term "produces" refers to the production of proteins (polypeptides) and/or other compounds by cells. It is intended that the term encompass any step involved in the production of polypeptides including, but not limited to, transcription, post-transcriptional modification, translation, and post-translational modification. In some embodiments, the term also encompasses secretion of the polypeptide from a cell.

[0060] As used in this disclosure, the term "GH61 protein" means a protein that has GH61 activity, including GH61 variants and wild-type GH61 enzymes. In some embodiments, the GH61 proteins have been purified from *M. thermophila* cells, while in other embodiments, they are structurally related to the amino acid sequences shown in Tables 1 and 2. The terms also encompasses species and strain homologs and orthologs comprising protein sequences listed in Tables 1 and 2, as well as variants, and fragments of such sequences (produced using any suitable means known in the art), having GH61 activity.

[0061] As used herein, the terms "variant," "GH61 variant," refer to a GH61 polypeptide or polynucleotide encoding a GH61 polypeptide comprising one or more modifications relative to wild-type GH61 or the wild-type polynucleotide encoding GH61 (such as substitutions, inser-

tions, deletions, and/or truncations of one or more amino acid residues or of one or more specific nucleotides or codons in the polypeptide or polynucleotide, respectively), and biologically active fragments thereof. In some embodiments, the variant is derived from a M. thermophila polypeptide and comprises one or more modifications relative to wild-type M. thermophila GH61 or the wild-type polynucleotide encoding wild-type M. thermophila GH61, or a biologically active fragment thereof. In some embodiments, a "GH61 variant protein" ("GH61 variant polypeptide") of the present invention is a protein that is structurally related to a reference protein comprising SEQ ID NO:2 or a fragment of SEQ ID NO:2 that has GH61 activity, but has one or more amino acid substitutions in relation to the reference protein. In some embodiments, the GH61 variant is a GH61a variant (i.e., a variant of GH61a enzyme). In some embodiments, the GH61 variant polypeptide is a "polypeptide of interest." In some additional embodiments, the GH61 variant polypeptide is encoded by a "polynucleotide of interest."

[0062] The terms "improved" or "improved properties," as used in the context of describing the properties of a GH61 variant, refers to a GH61 variant polypeptide that exhibits an improvement in a property or properties as compared to the wild-type GH61 (e.g., SEQ ID NO:2) or a specified reference polypeptide. Improved properties may include, but are not limited to increased protein expression, increased thermoactivity, increased thermostability, increased pH activity, increased stability (e.g., increased pH stability or pH tolerance at various pH levels), increased product specificity, increased specific activity, increased substrate specificity, increased resistance to substrate or end-product inhibition, increased chemical stability, reduced inhibition by glucose, increased resistance to inhibitors (e.g., acetic acid, lectins, tannic acids, and phenolic compounds), and altered pH/temperature profile.

[0063] The term "biologically active fragment," as used herein, refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion and/or internal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the sequence to which it is being compared (e.g., a full-length GH61 variant of the invention) and that retains substantially all of the activity of the full-length polypeptide. A biologically active fragment can comprise about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, at about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% of a full-length GH61 polypeptide.

[0064] A GH61 variant protein of this invention having "increased GH61 activity" has more GH61 activity when that protein is present in a saccharification reaction with a specified substrate and specified cellulase enzyme(s), compared with a saccharification reaction conducted with the same substrate and enzyme(s) under the same conditions in the presence of a reference protein (e.g., including but not limited to wild-type GH61). The increase is determined by measuring the amount of fermentable sugar produced in the reaction in the presence of the GH61 variant protein, in the presence of the reference protein (Positive Control), and in the absence of either protein (Negative Control). The Improvement Over Positive Control (FIOPC) is calculated as ([Glucose production of the GH61 Variant Protein]—

[Glucose production of the Negative Control]]/([Glucose production of the Positive Control]–[Glucose production of the Negative Control]).

[0065] As used herein, "GH61 activity" is the functional activity of a GH61 protein that results in production of more fermentable sugar from a polysaccharide substrate when the GH61 protein is present in a saccharification reaction, compared with a saccharification reaction conducted under the same conditions in the absence of the GH61 protein.

[0066] A GH61 variant protein of this invention having "increased GH61 thermoactivity" has more GH61 activity in a saccharification reaction conducted at an elevated temperature (about 50° C., about 55° C., about 60° C., or higher) with a specified substrate and specified cellulase enzyme(s), compared with a saccharification reaction conducted under the same conditions in the presence of the reference protein (e.g., including but not limited to wild-type GH61).

[0067] GH61 proteins of this invention may be said to "enhance", "promote", or "facilitate" activity of one or more cellulase enzymes during hydrolysis of sugar polymers (e.g., cellulosic and/or lignocellulosic biomass) such that the enzyme(s) produce(s) more product over a particular time period, hydrolysis proceeds more rapidly, or goes further to completion when the GH61 protein is present, compared with a similar reaction mixture in which the GH61 protein is absent. This invention may be practiced by following GH61 activity in an empirical fashion using assay methods provided in this disclosure, without knowing the mechanism of operation of the GH61 variant protein being used. However, it is not intended that the present invention be limited to any particular assay system and/or method, as any suitable method known in the art finds use.

[0068] The terms "transform" or "transformation," as used in reference to a cell, mean a cell has a non-native nucleic acid sequence integrated into its genome and/or as an episome (e.g., plasmid) that is maintained through multiple generations.

[0069] The term "introduced," as used in the context of inserting a nucleic acid sequence into a cell, means that the nucleic acid has been conjugated, transfected, transduced or transformed (collectively "transformed") or otherwise incorporated into the genome of and/or maintained as an episome in the cell. Thus, the term encompasses transformation, transduction, conjugation, transfection, and/or any other suitable method(s) known in the art for inserting nucleic acid sequences into host cells. Any suitable means for the introduction of nucleic acid into host cells find use in the present invention.

[0070] The terms "percent identity," "% identity", "percent identical", and "% identical" are used interchangeably to refer to a comparison of two optimally aligned sequences over a comparison window. The comparison window may include additions or deletions in either sequence to optimize alignment. The percentage of identity is the number of positions that are identical between the sequences, divided by the total number of positions in the comparison window (including positions where one of the sequences has a gap). For example, a protein with an amino acid sequence that matches at 310 positions a sequence of GH61a (which has 323 amino acids in the secreted form), would have 310/323=95.9% identity to the reference. Similarly, a protein variant that has 300 residues (i.e., less than full-length) and matches the reference sequence at 280 positions would have 280/300=93.3% identity. Computer-implemented alignment

algorithms useful in determining the degree of identity are known in the art, including the BLAST and BLAST 2.0 algorithms (See e.g., Altschul et al., J. Mol. Biol., 215: 403-410 [1990]; and Altschul et al., Nucl. Acids Res., 3389-3402 [1977]).

[0071] As used herein, "polynucleotide" refers to a polymer of deoxyribonucleotides or ribonucleotides in either single- or double-stranded form, and complements thereof.

[0072] As used herein, the term "allelic variant" refers to any of two or more (e.g., several) alternative forms of a gene occupying the same chromosomal locus. In some embodiments, allelic variation arises naturally through mutation and results in genetic polymorphism within populations. In some embodiments, gene mutations are silent (i.e., there is no change in the encoded polypeptide), while in some other embodiments the genes encode polypeptides that have altered amino acid sequences. An "allelic variant of a polypeptide" is a polypeptide encoded by an allelic variant of a gene.

[0073] As used herein, "cDNA" refers to a DNA molecule that can be prepared by reverse transcription from a mature, spliced, mRNA molecule obtained from a eukaryotic or prokaryotic cell. cDNA sequences lack 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.

[0074] As used herein, the term "coding sequence" refers to a polynucleotide that directly specifies the amino acid sequence of a polypeptide. The boundaries of the coding sequence are generally determined by an open reading frame, which begins with a start codon (e.g., ATG, GTG, or TTG) and ends with a stop codon (e.g., TAA, TAG, or TGA). In some embodiments, a coding sequence comprises genomic DNA, while in some alternative embodiments, the coding sequence comprises cDNA, synthetic DNA, and/or a combination thereof.

[0075] As used herein, the terms "control sequences" and "regulatory sequences" refer to nucleic acid sequences necessary and/or useful for expression of a polynucleotide encoding a polypeptide. In some embodiments, control sequences are native (i.e., from the same gene) or foreign (i.e., from a different gene) to the polynucleotide encoding the polypeptide. Control sequences include, but are not limited to leaders, polyadenylation sequences, propeptide sequences, promoters, signal peptide sequences, and transcription terminators. In some embodiments, at a minimum, control sequences include a promoter, and transcriptional and translational stop signals. In some embodiments, control sequences are 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 the polypeptide.

[0076] A nucleic acid construct, nucleic acid (e.g., a polynucleotide), polypeptide, or host cell is referred to herein as "recombinant" when it is non-naturally occurring, artificial or engineered. The present invention also provides recombinant nucleic acid constructs comprising at least one GH61 variant polynucleotide sequence that hybridizes under stringent hybridization conditions to the complement of a polynucleotide which encodes a polypeptide comprising the amino acid sequence of any of SEQ ID NOS:2, 3, 5, 6, 8, 9, 11, and/or 12.

[0077] The term "recombinant nucleic acid" has its conventional meaning. A recombinant nucleic acid, or equivalently, "polynucleotide," is one that is inserted into a heterologous location such that it is not associated with nucleotide sequences that normally flank the nucleic acid as it is found in nature (for example, a nucleic acid inserted into a vector or a genome of a heterologous organism). Likewise, a nucleic acid sequence that does not appear in nature, for example a variant of a naturally occurring gene, is recombinant. A cell containing a recombinant nucleic acid, or protein expressed in vitro or in vivo from a recombinant nucleic acid are also "recombinant." Examples of recombinant nucleic acids include a protein-encoding DNA sequence that is (i) operably linked to a heterologous promoter and/or (ii) encodes a fusion polypeptide with a protein sequence and a heterologous signal peptide sequence.

[0078] For purposes of this disclosure, a promoter is "heterologous" to a gene sequence if the promoter is not associated in nature with the gene. A signal peptide is "heterologous" to a protein sequence when the signal peptide sequence is not associated with the protein in nature. In some embodiments, "hybrid promoters" find use. Hybrid promoters are promoters comprising portions of two or more (e.g., several) promoters that are linked together to generate a sequence that is a fusion of the portions of the two or more promoters, which when operably linked to a coding sequence, mediates the transcription of the coding sequence into mRNA.

[0079] In relation to regulatory sequences (e.g., promoters), the term "operably linked" refers to a configuration in which a regulatory sequence is located at a position relative to a polypeptide encoding sequence such that the regulatory sequence influences the expression of the polypeptide. In relation to a signal sequence, the term "operably linked" refers to a configuration in which the signal sequence encodes an amino-terminal signal peptide fused to the polypeptide, such that expression of the gene produces a pre-protein.

[0080] Nucleic acids "hybridize" when they associate, typically in solution. Nucleic acids hybridize due to a variety of well-characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. As used herein, the term "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments, such as Southern and Northern hybridizations, are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen, 1993, "Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes," Part I, Chapter 2 (Elsevier, New York), which is incorporated herein by reference. For polynucleotides of at least 100 nucleotides in length, low to very high stringency conditions are defined as follows: prehybridization and hybridization at 42° C. in 5×SSPE, 0.3% SDS, 200 μg/ml sheared and denatured salmon sperm DNA, and either 25% formamide for 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 polynucleotides of at least 100 nucleotides in length, the carrier material is finally washed three times each for 15 minutes using 2×SSC, 0.2% SDS 50° C. (low

stringency), at 55° C. (medium stringency), at 60° C. (medium-high stringency), at 65° C. (high stringency), or at 70° C. (very high stringency).

[0081] As used herein, a "vector" and "nucleic acid construct" comprise nucleic acid (e.g., DNA) constructs for introducing a DNA sequence into a cell. In some embodiments, the vector is an expression vector that is operably linked to a suitable control sequence capable of effecting the expression in a suitable host of the polypeptide encoded in the DNA sequence. The term "expression vector" refers to a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of the invention, and which is operably linked to additional segments that provide for its transcription (e.g., a promoter, a transcription terminator sequence, enhancers, etc.) and optionally a selectable marker.

[0082] As used herein, the term "isolated" refers to a nucleic acid, polypeptide, or other component that is partially or completely separated from components with which it is normally associated in nature. Thus, the term encompasses a substance in a form or environment that does not occur in nature. Non-limiting examples of isolated substances include, but are not limited to: any non-naturally occurring substance; any substance including, but not limited to, any enzyme, variant, polynucleotide, protein, peptide or cofactor, that is at least partially removed from one or more or all of the naturally occurring constituents with which it is associated in nature; any substance modified by the hand of man relative to that substance found in nature; and/or any substance modified by increasing the amount of the substance relative to other components with which it is naturally associated (e.g., multiple copies of a gene encoding the substance; and/or use of a stronger promoter than the promoter naturally associated with the gene encoding the substance). In some embodiments, a polypeptide of interest is used in industrial applications in the form of a fermentation broth product (i.e., the polypeptide is a component of a fermentation broth) used as a product in industrial applications such as ethanol production. In some embodiments, in addition to the polypeptide of interest (e.g., a GH61 variant polypeptide), the fermentation broth product further comprises ingredients used in the fermentation process (e.g., cells, including the host cells containing the gene encoding the polypeptide of interest and/or the polypeptide of interest), cell debris, biomass, fermentation media, and/or fermentation products. In some embodiments, the fermentation broth is optionally subjected to one or more purification steps (e.g., filtration) to remove or reduce at least one components of a fermentation process. Accordingly, in some embodiments, an isolated substance is present in such a fermentation broth product.

[0083] As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues.

[0084] As used herein, the term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified (e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine) Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, (i.e., an α -carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, such as homoserine, norleu-

cine, methionine sulfoxide, and methionine methyl sulfonium). Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid.

[0085] An "amino acid substitution" in a protein sequence is replacement of a single amino acid within that sequence with another amino acid. Unless indicated otherwise, variant GH61 proteins of this invention have substitutions as specifically indicated. In some embodiments, the variant GH61 proteins of the present invention also have other substitutions and/or alterations at any position in any combination with the substitutions specifically indicated.

100861 Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. [0087] An amino acid or nucleotide base "position" is denoted by a number that sequentially identifies each amino acid (or nucleotide base) in the reference sequence based on its position relative to the N-terminus (or 5'-end). Due to deletions, insertions, truncations, fusions, and the like that must be taken into account when determining an optimal alignment, the amino acid residue number in a test sequence determined by simply counting from the N-terminus will not necessarily be the same as the number of its corresponding position in the reference sequence. For example, in a case where a test sequence has a deletion relative to an aligned reference sequence, there will be no amino acid in the variant that corresponds to a position in the reference sequence at the site of deletion. Where there is an insertion in an aligned reference sequence, that insertion will not correspond to a numbered amino acid position in the reference sequence. In the case of truncations or fusions there can be stretches of amino acids in either the reference or aligned sequence that do not correspond to any amino acid in the corresponding sequence.

[0088] As used herein, the terms "numbered with reference to" or "corresponding to," when used in the context of the numbering of a given amino acid or polynucleotide sequence, refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence.

[0089] As used herein, the term "reference enzyme" refers to an enzyme to which another enzyme of the present invention (e.g., a "test" enzyme) is compared in order to determine the presence of an improved property in the other enzyme being evaluated. In some embodiments, a reference enzyme is a wild-type enzyme (e.g., wild-type GH61). In some embodiments, the reference enzyme is an enzyme with which a test enzyme of the present invention is compared in order to determine the presence of an improved property in the test enzyme being evaluated, including but not limited to improved thermoactivity, improved thermostability, improved activity, and/or improved stability. In some embodiments, a reference enzyme is a wild-type enzyme (e.g., wild-type GH61).

[0090] Amino acid substitutions in a GH61 protein are referred to in this disclosure using the following notation: The single-letter abbreviation for the amino acid being substituted; its position in the reference sequence (e.g., the wild-type "parental sequence" set forth in SEQ ID NO:2); and the single-letter abbreviation for the amino acid that

replaces it. Thus, the following nomenclature is used herein to describe substitutions in a reference sequence relative to a reference sequence or a variant polypeptide or nucleic acid sequence: "R-#-V," where # refers to the position in the reference sequence, R refers to the amino acid (or base) at that position in the reference sequence, and V refers to the amino acid (or base) at that position in the variant sequence. In some embodiments, an amino acid (or base) may be called "X," by which is meant any amino acid (or base). As a non-limiting example, for a variant polypeptide described with reference to a wild-type GH61 polypeptide (e.g., SEQ ID NO:2), "N35G" indicates that in the variant polypeptide, the asparagine at position 35 of the reference sequence is replaced by glycine, with amino acid position being determined by optimal alignment of the variant sequence with SEQ ID NO:2. Similarly, "H20C/D" describes two variants: a variant in which the histidine at position 20 of the reference sequence is replaced by cysteine and a variant in which the serine at position 20 of the reference sequence is replaced by aspartic acid. In the example "W141X" indicates that the tryptophan at position 131 has been replaced with any amino acid.

[0091] As used herein in reference to nucleotide and amino acid sequences, the term "mutation" refers to any change in the sequence, as compared to a reference nucleotide or amino acid sequence, including but not limited to substitutions, deletions, additions, truncations, modifications, etc. Indeed, it is intended that any change in a reference (or "parent" or "starting") nucleotide or amino acid sequence comprises a mutation in the sequence.

[0092] As used herein, the terms "amino acid mutation set", "mutation set" when used in the context of amino acid sequences (e.g., polypeptides) refer to a group of amino acid substitutions, insertions, deletions and/or other modifications to the sequence. In some embodiments, "mutation set" refers to the nucleic acid mutation sets present in some of the GH61 variants provided in Table 1 and Table 2.

[0093] The term "amino acid substitution set," "substitution set," and "combination of amino acid substitutions" refer to a group (i.e., set of combinations) of amino acid substitutions. A substitution set can have about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more amino acid substitutions. In some embodiments, a substitution set refers to the set of amino acid substitutions that is present in any of the variant GH61 enzymes provided herein.

[0094] As used herein, the terms "nucleic acid substitution set" and "substitution set" when used in the context of nucleotide sequences (e.g., polynucleotides) refer to a group of nucleic acid substitutions. In some embodiments, mutation set refers to the nucleic acid substitution sets present in some of the variant GH61 proteins provided in Table 1 and Table 2.

[0095] As used herein, the terms "nucleic acid mutation set" and "mutation set" when used in the context of nucleotide sequences (e.g., polynucleotides) refer to a group of nucleic acid substitutions, insertions, deletions, and/or other modifications to the sequence. In some embodiments, "mutation set" refers to the amino acid mutation sets present in some of the GH61 variants provided in Table 1 and Table 2

[0096] A "cellulase-engineered" cell is a cell comprising at least one, at least two, at least three, or at least four recombinant sequences encoding a cellulase or cellulase variant, and in which expression of the cellulase(s) or

cellulase variant(s) has been modified relative to the wild-type form. Expression of a cellulase is "modified" when a non-naturally occurring cellulase variant is expressed or when a naturally occurring cellulase is over-expressed. One exemplary means to over-express a cellulase is to operably link a strong (optionally constitutive) promoter to the cellulase encoding sequence. Another exemplary way to over-express a cellulase is to increase the copy number of a heterologous, variant, or endogenous cellulase gene. The cellulase-engineered cell may be any suitable fungal cell, including, but not limited to *Myceliophthora*, *Trichoderma*, *Aspergillus*, cells, etc.

[0097] As used herein, the terms "host cell" and "host strain" refer to suitable hosts for expression vectors comprising DNA provided herein. In some embodiments, the host cells are prokaryotic or eukaryotic cells that have been transformed or transfected with vectors constructed using recombinant DNA techniques as known in the art. Transformed hosts are capable of either replicating vectors encoding at least one protein of interest and/or expressing the desired protein of interest. In addition, reference to a cell of a particular strain refers to a parental cell of the strain as well as progeny and genetically modified derivatives. Genetically modified derivatives of a parental cell include progeny cells that contain a modified genome or episomal plasmids that confer for example, antibiotic resistance, improved fermentation, etc. In some embodiments, host cells are genetically modified to have characteristics that improve protein secretion, protein stability or other properties desirable for expression and/or secretion of a protein. For example, knockout of Alp1 function results in a cell that is protease deficient. Knockout of pyr5 function results in a cell with a pyrimidine deficient phenotype. In some embodiments, host cells are modified to delete endogenous cellulase proteinencoding sequences or otherwise eliminate expression of one or more endogenous cellulases. In some embodiments, expression of one or more endogenous cellulases is inhibited to increase production of cellulases of interest. Genetic modification can be achieved by any suitable genetic engineering techniques and/or classical microbiological techniques (e.g., chemical or UV mutagenesis and subsequent selection). Using recombinant technology, nucleic acid molecules can be introduced, deleted, inhibited or modified, in a manner that results in increased yields of GH61 variant(s) within the organism or in the culture. For example, knockout of Alp1 function results in a cell that is protease deficient. Knockout of pyr5 function results in a cell with a pyrimidine deficient phenotype. In some genetic engineering approaches, homologous recombination is used to induce targeted gene modifications by specifically targeting a gene in vivo to suppress expression of the encoded protein. In an alternative approach, siRNA, antisense, and/or ribozyme technology finds use in inhibiting gene expression.

[0098] As used herein, the term "C1" refers to strains of *Myceliophthora thermophila*, including the fungal strain described by Garg (See, Garg, Mycopathol., 30: 3-4 [1966]). As used herein, "*Chrysosporium lucknowense*" includes the strains described in U.S. Pat. Nos. 6,015,707, 5,811,381 and 6,573,086; US Pat. Pub. Nos. 2007/0238155, US 2008/0194005, US 2009/0099079; International Pat. Pub. Nos., WO 2008/073914 and WO 98/15633, all of which are incorporated herein by reference, and include, without limitation, *Chrysosporium lucknowense* Garg 27K, VKM-F 3500 D (Accession No. VKM F-3500-D), C1 strain UV13-6

(Accession No. VKM F-3632 D), C1 strain NG7C-19 (Accession No. VKM F-3633 D), and C1 strain UV18-25 (VKM F-3631 D), all of which have been deposited at the All-Russian Collection of Microorganisms of Russian Academy of Sciences (VKM), Bakhurhina St. 8, Moscow, Russia, 113184, and any derivatives thereof. Although initially described as Chrysosporium lucknowense, C1 may currently be considered a strain of Myceliophthora thermophila. Other C1 strains include cells deposited under accession numbers ATCC 44006, CBS (Centraalbureau voor Schimmelcultures) 122188, CBS 251.72, CBS 143.77, CBS 272.77, CBS122190, CBS122189, and VKM F-3500D. Exemplary C1 derivatives include but are not limited to modified organisms in which one or more endogenous genes or sequences have been deleted or modified and/or one or more heterologous genes or sequences have been introduced. Derivatives include, but are not limited to UV18#100f Δalp1, UV18#100f Δpyr5 Δalp1, UV18#100.f Δalp1 Δpep4 Δalp2, UV18#1001 Δpyr5 Δalp1 Δpep4 Δalp2 and UV18#100.f Δpyr4 Δpyr5 Δalp1 Δpep4 Δalp2, as described in WO2008073914 and WO2010107303, each of which is incorporated herein by reference.

[0099] As used herein, the term "culturing" refers to growing a population of microbial cells under suitable conditions in a liquid, semi-solid, or solid medium.

[0100] In general, "saccharification" refers to the process in which substrates (e.g., cellulosic biomass and/or lignocellulosic biomass) are broken down via the action of cellulases to produce fermentable sugars (e.g. monosaccharides, including but not limited to glucose and/or xylose). In particular, "saccharification" is an enzyme-catalyzed reaction that results in hydrolysis of a complex carbohydrate to produce shorter-chain carbohydrate polymers and/or fermentable sugar(s) that are more suitable for fermentation or further hydrolysis. In some embodiments, the enzymes comprise cellulase enzyme(s) such as endoglucanases, betaglucosidases, cellobiohydrolases (e.g., CBH1 and/or CBH1), a synthetic mixture of any of such enzymes, and/or cellulase enzymes contained in culture broth from an organism that produces cellulase enzymes, such as M. thermophila or recombinant yeast cells. Products of saccharification may include disaccharides, and/or monosaccharides such as glucose or xylose.

[0101] In some embodiments, the fermentable sugars produced by the methods of the present invention are used to produce an alcohol (e.g., including but not limited to ethanol, butanol, etc.). The variant GH61 proteins of the present invention find use in any suitable method to generate alcohols and/or other biofuels from cellulose and/or lignocellulose, and are not limited necessarily to those described herein. Two methods commonly employed are the separate saccharification and fermentation (SHF) method (See, Wilke et al., Biotechnol. Bioengin. 6:155-75 [1976]) or the simultaneous saccharification and fermentation (SSF) method (See e.g., U.S. Pat. Nos. 3,990,944 and 3,990,945). An additional method that finds use with the present invention is consolidated bioprocessing (CBP), which encompasses the combination of the biological steps used in the conversion of lignocellulosic biomass to bioethanol (e.g., production of cellulase(s), hydrolysis of the polysaccharides in the biomass, and fermentation of hexose and pentose sugars) in one reactor (See e.g., Vertes et al., Biomass to Biofuels: Strategies for Global Industries, John Wiley & Sons, Ltd., [2010], Hoboken, N.J., pp. 324-325).

[0102] The SHF method of saccharification comprises the steps of contacting cellulase with a cellulose-containing substrate to enzymatically break down cellulose into fermentable sugars (e.g., monosaccharides such as glucose), contacting the fermentable sugars with an alcohol-producing microorganism to produce alcohol (e.g., ethanol or butanol) and recovering the alcohol. In some embodiments, the method of consolidated bioprocessing (CBP) can be used, in which the cellulase production from the host is simultaneous with saccharification and fermentation either from one host or from a mixed cultivation.

[0103] In addition to SHF methods, a SSF method may be used. In some cases, SSF methods result in a higher efficiency of alcohol production than is afforded by the SHF method (See e.g., Drissen et al., Biocat. Biotransform., 27:27-35 [2009]). One disadvantage of SSF over SHF is that higher temperatures are required for SSF than for SHF. In some embodiments, the present invention provides GH61 polypeptides that have higher thermostability than a wild-type GH61s. Thus, it is contemplated that the present invention will find use in increasing ethanol production in SSF, as well as SHF methods.

[0104] As used herein "fermentable sugars" refers to fermentable sugars (e.g., monosaccharides, disaccharides and short oligosaccharides), including but not limited to glucose, xylose, galactose, arabinose, mannose and sucrose. In general, the term "fermentable sugar" refers to any sugar that a microorganism can utilize or ferment.

[0105] As used herein, the terms "adjunct material," "adjunct composition," and "adjunct compound" refer to any composition suitable for use in the compositions and/or saccharification reactions provided herein, including but not limited to cofactors, surfactants, builders, buffers, enzyme stabilizing systems, chelants, dispersants, colorants, preservatives, antioxidants, solublizing agents, carriers, processing aids, pH control agents, etc. In some embodiments, divalent metal cations are used to supplement saccharification reactions and/or the growth of host cells producing GH61 variant proteins. Any suitable divalent metal cation finds use in the present invention, including but not limited to Cu⁺⁺, Mn⁺⁺, Co⁺⁺, Mg⁺⁺, Ni⁺⁺, Zn⁺⁺, and Ca⁺⁺. In addition, any suitable combination of divalent metal cations finds use in the present invention. Furthermore, divalent metal cations find use from any suitable source.

[0106] In some embodiments, the host cells producing GH61 variant proteins of the present invention are grown under culture conditions comprising about pH 5, while in some other embodiments, the host cells are grown at about pH 6.7. In some embodiments, the host cells cultured at pH 5 provide improved saccharification in the presence of supplemented copper, when saccharification is conducted at about pH 5 or about pH 6.7. In some alternative embodiments, the host cells cultured at about pH 6.7 provide improved saccharification in the absence of supplemented copper when saccharification is conducted at about pH 5 or about pH 6.

[0107] As used herein, the terms "biomass," "biomass substrate," "cellulosic biomass," "cellulosic feedstock," and "cellulosic substrate" refer to any materials that contain cellulose. Biomass can be derived from plants, animals, or microorganisms, and may include, but is not limited to agricultural, industrial, and forestry residues, industrial and municipal wastes, and terrestrial and aquatic crops grown for energy purposes. Examples of cellulosic substrates include,

but are not limited to, wood, wood pulp, paper pulp, corn fiber, corn grain, corn cobs, crop residues such as corn husks, corn stover, grasses, wheat, wheat straw, barley, barley straw, hay, rice, rice straw, switchgrass, waste paper, paper and pulp processing waste, woody or herbaceous plants, fruit or vegetable pulp, corn cobs, distillers grain, grasses, rice hulls, cotton, hemp, flax, sisal, sugar cane bagasse, sorghum, soy, switchgrass, components obtained from milling of grains, trees, branches, roots, leaves, wood chips, sawdust, shrubs and bushes, vegetables, fruits, and flowers and any suitable mixtures thereof. In some embodiments, the cellulosic biomass comprises, but is not limited to cultivated crops (e.g., grasses, including C4 grasses, such as switch grass, cord grass, rye grass, miscanthus, reed canary grass, or any combination thereof), sugar processing residues, for example, but not limited to, bagasse (e.g., sugar cane bagasse, beet pulp [e.g., sugar beet], or a combination thereof), agricultural residues (e.g. soybean stover, corn stover, corn fiber, rice straw, sugar cane straw, rice, rice hulls, barley straw, corn cobs, wheat straw, canola straw, oat straw, oat hulls, corn fiber, hemp, flax, sisal, cotton, or any combination thereof), fruit pulp, vegetable pulp, distillers' grains, forestry biomass (e.g., wood, wood pulp, paper pulp, recycled wood pulp fiber, sawdust, hardwood, such as aspen wood, softwood, or a combination thereof). Furthermore, in some embodiments, the cellulosic biomass comprises cellulosic waste material and/or forestry waste materials, including but not limited to, paper and pulp processing waste, newsprint, cardboard and the like. In some embodiments, the cellulosic biomass comprises one species of fiber, while in some alternative embodiments, the cellulosic biomass comprises a mixture of fibers that originate from different cellulosic biomasses. In some embodiments, the biomass may also comprise transgenic plants that express ligninase and/or cellulase enzymes (US 2008/0104724 A1).

[0108] The terms "lignocellulosic biomass" and "lignocellulosic feedstock" refer to plant biomass that is composed of cellulose and hemicellulose, bound to lignin. The biomass may optionally be pretreated to increase the susceptibility of cellulose to hydrolysis by chemical, physical and biological pretreatments (such as steam explosion, pulping, grinding, acid hydrolysis, solvent exposure, and the like, as well as combinations thereof). Various lignocellulosic feedstocks find use, including those that comprise fresh lignocellulosic feedstock, partially dried lignocellulosic feedstock, fully dried lignocellulosic feedstock, and/or any combination thereof. In some embodiments, lignocellulosic feedstocks comprise cellulose in an amount greater than about 20%, more preferably greater than about 30%, more preferably greater than about 40% (w/w). For example, in some embodiments, the lignocellulosic material comprises from about 20% to about 90% (w/w) cellulose, or any amount therebetween, although in some embodiments, the lignocellulosic material comprises less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, or less than about 5% cellulose (w/w).

[0109] Furthermore, in some embodiments, the lignocellulosic feedstock comprises lignin in an amount greater than about 10%, more typically in an amount greater than about 15% (w/w). In some embodiments, the lignocellulosic feed-

stock comprises small amounts of sucrose, fructose and/or starch. The lignocellulosic feedstock is generally first subjected to size reduction by methods including, but not limited to, milling, grinding, agitation, shredding, compression/expansion, or other types of mechanical action. Size reduction by mechanical action can be performed by any type of equipment adapted for the purpose, for example, but not limited to, hammer mills, tub-grinders, roll presses, refiners and hydrapulpers. In some embodiments, at least 90% by weight of the particles produced from the size reduction have lengths less than between about 1/16 and about 4 in (the measurement may be a volume or a weight average length). In some embodiments, the equipment used to reduce the particle size reduction is a hammer mill or shredder. Subsequent to size reduction, the feedstock is typically slurried in water, as this facilitates pumping of the feedstock. In some embodiments, lignocellulosic feedstocks of particle size less than about 6 inches do not require size reduction.

[0110] As used herein, the term "pretreated lignocellulosic feedstock," refers to lignocellulosic feedstocks that have been subjected to physical and/or chemical processes to make the fiber more accessible and/or receptive to the actions of cellulolytic enzymes, as described above.

[0111] A cellulosic substrate or lignocellulosic substrate is said to be "pretreated" when it has been processed by some physical and/or chemical means to facilitate saccharification. As described further herein, in some embodiments, the biomass substrate is "pretreated," or treated using methods known in the art, such as chemical pretreatment (e.g., ammonia pretreatment, dilute acid pretreatment, dilute alkali pretreatment, or solvent exposure), physical pretreatment (e.g., steam explosion or irradiation), mechanical pretreatment (e.g., grinding or milling) and biological pretreatment (e.g., application of lignin-solubilizing microorganisms) and combinations thereof, to increase the susceptibility of cellulose to hydrolysis. Thus, the term "cellulosic biomass" encompasses any living or dead biological material that contains a polysaccharide substrate, including but not limited to cellulose, starch, other forms of long-chain carbohydrate polymers, and mixtures of such sources. It may or may not be assembled entirely or primarily from glucose or xylose, and may optionally also contain various other pentose or hexose monomers. Xylose is an aldopentose containing five carbon atoms and an aldehyde group. It is the precursor to hemicellulose, and is often a main constituent of biomass. In some embodiments, the substrate is slurried prior to pretreatment. In some embodiments, the consistency of the slurry is between about 2% and about 30% and more typically between about 4% and about 15%. In some embodiments, the slurry is subjected to a water and/or acid soaking operation prior to pretreatment. In some embodiments, the slurry is dewatered using any suitable method to reduce steam and chemical usage prior to pretreatment. Examples of dewatering devices include, but are not limited to pressurized screw presses (See e.g., WO 2010/022511, incorporated herein by reference) pressurized filters and

[0112] In some embodiments, the pretreatment is carried out to hydrolyze hemicellulose, and/or a portion thereof present in the cellulosic substrate to monomeric pentose and hexose sugars (e.g., xylose, arabinose, mannose, galactose, and/or any combination thereof). In some embodiments, the pretreatment is carried out so that nearly complete hydro-

lysis of the hemicellulose and a small amount of conversion of cellulose to glucose occurs. In some embodiments, an acid concentration in the aqueous slurry from about 0.02% (w/w) to about 2% (w/w), or any amount therebetween, is typically used for the treatment of the cellulosic substrate. Any suitable acid finds use in these methods, including but not limited to, hydrochloric acid, nitric acid, and/or sulfuric acid. In some embodiments, the acid used during pretreatment is sulfuric acid. Steam explosion is one method of performing acid pretreatment of biomass substrates (See e.g., U.S. Pat. No. 4,461,648). Another method of pretreating the slurry involves continuous pretreatment (i.e., the cellulosic biomass is pumped though a reactor continuously). This methods are well-known to those skilled in the art (See e.g., U.S. Pat. No. 7,754,457).

[0113] In some embodiments, alkali is used in the pretreatment. In contrast to acid pretreatment, pretreatment with alkali may not hydrolyze the hemicellulose component of the biomass. Rather, the alkali reacts with acidic groups present on the hemicellulose to open up the surface of the substrate. In some embodiments, the addition of alkali alters the crystal structure of the cellulose so that it is more amenable to hydrolysis. Examples of alkali that find use in the pretreatment include, but are not limited to ammonia, ammonium hydroxide, potassium hydroxide, and sodium hydroxide. One method of alkali pretreatment is Ammonia Freeze Explosion, Ammonia Fiber Explosion or Ammonia Fiber Expansion ("AFEX" process; See e.g., U.S. Pat. Nos. 5,171,592; 5,037,663; 4,600,590; 6,106,888; 4,356,196; 5,939,544; 6,176,176; 5,037,663 and 5,171,592). During this process, the cellulosic substrate is contacted with ammonia or ammonium hydroxide in a pressure vessel for a sufficient time to enable the ammonia or ammonium hydroxide to alter the crystal structure of the cellulose fibers. The pressure is then rapidly reduced, which allows the ammonia to flash or boil and explode the cellulose fiber structure. In some embodiments, the flashed ammonia is then recovered using methods known in the art. In some alternative methods, dilute ammonia pretreatment is utilized. The dilute ammonia pretreatment method utilizes more dilute solutions of ammonia or ammonium hydroxide than AFEX (See e.g., WO2009/045651 and US 2007/0031953). This pretreatment process may or may not produce any monosaccharides.

[0114] Additional pretreatment processes for use in the present invention include chemical treatment of the cellulosic substrate with organic solvents, in methods such as those utilizing organic liquids in pretreatment systems (See e.g., U.S. Pat. No. 4,556,430; incorporated herein by reference). These methods have the advantage that the low boiling point liquids easily can be recovered and reused. Other pretreatments, such as the OrganosolvTM process, also use organic liquids (See e.g., U.S. Pat. No. 7,465,791, which is also incorporated herein by reference). Subjecting the substrate to pressurized water may also be a suitable pretreatment method (See e.g., Weil et al., Appl. Biochem. Biotechnol., 68(1-2): 21-40 [1997], which is incorporated herein by reference). In some embodiments, the pretreated cellulosic biomass is processed after pretreatment by any of several steps, such as dilution with water, washing with water, buffering, filtration, or centrifugation, or any combination of these processes, prior to enzymatic hydrolysis, as is familiar to those skilled in the art. The pretreatment produces a pretreated feedstock composition (e.g., a "pretreated feedstock slurry") that contains a soluble component including the sugars resulting from hydrolysis of the hemicellulose, optionally acetic acid and other inhibitors, and solids including unhydrolyzed feedstock and lignin. In some embodiments, the soluble components of the pretreated feedstock composition are separated from the solids to produce a soluble fraction.

[0115] In some embodiments, the soluble fraction, including the sugars released during pretreatment and other soluble components (e.g., inhibitors), is then sent to fermentation. However, in some embodiments in which the hemicellulose is not effectively hydrolyzed during the pretreatment one or more additional steps are included (e.g., a further hydrolysis step(s) and/or enzymatic treatment step(s) and/or further alkali and/or acid treatment) to produce fermentable sugars. In some embodiments, the separation is carried out by washing the pretreated feedstock composition with an aqueous solution to produce a wash stream and a solids stream comprising the unhydrolyzed, pretreated feedstock. Alternatively, the soluble component is separated from the solids by subjecting the pretreated feedstock composition to a solids-liquid separation, using any suitable method (e.g., centrifugation, microfiltration, plate and frame filtration, cross-flow filtration, pressure filtration, vacuum filtration, etc.). Optionally, in some embodiments, a washing step is incorporated into the solids-liquids separation. In some embodiments, the separated solids containing cellulose, then undergo enzymatic hydrolysis with cellulase enzymes in order to convert the cellulose to glucose. In some embodiments, the pretreated feedstock composition is fed into the fermentation process without separation of the solids contained therein. In some embodiments, the unhydrolyzed solids are subjected to enzymatic hydrolysis with cellulase enzymes to convert the cellulose to glucose after the fermentation process. In some embodiments, the pretreated cellulosic feedstock is subjected to enzymatic hydrolysis with cellulase enzymes.

[0116] As used herein, the term "recovered" refers to the harvesting, isolating, collecting, or recovering of protein from a cell and/or culture medium. In the context of saccharification, it is used in reference to the harvesting the fermentable sugars produced during the saccharification reaction from the culture medium and/or cells. In the context of fermentation, it is used in reference to harvesting the fermentation product from the culture medium and/or cells. Thus, a process can be said to comprise "recovering" a product of a reaction (such as a soluble sugar recovered from saccharification) if the process includes separating the product from other components of a reaction mixture subsequent to at least some of the product being generated in the reaction.

[0117] As used herein, the term "slurry" refers to an aqueous solution in which are dispersed one or more solid components, such as a cellulosic substrate.

[0118] "Increasing" yield of a product (such as a fermentable sugar) from a reaction occurs when a particular component present during the reaction (such as a GH61 protein) causes more product to be produced, compared with a reaction conducted under the same conditions with the same substrate and other substituents, but in the absence of the component of interest.

[0119] "Hydrolyzing" cellulose or other polysaccharide occurs when at least some of the glycosidic bonds between

two monosaccharides present in the substrate are hydrolyzed, thereby detaching from each other the two monomers that were previously bonded.

[0120] A reaction is said to be "substantially free" of a particular enzyme if the amount of that enzyme compared with other enzymes that participate in catalyzing the reaction is less than about 2%, about 1%, or about 0.1% (wt/wt).

[0121] "Fractionating" a liquid (e.g., a culture broth) means applying a separation process (e.g., salt precipitation, column chromatography, size exclusion, and filtration) or a combination of such processes to provide a solution in which a desired protein (e.g., GH61 protein, cellulase enzyme, or combination thereof) comprises a greater percentage of total protein in the solution than in the initial liquid product.

GH61 Variant Proteins with Improved Activity

[0122] GH61 variant proteins of the present invention have certain amino acid substitutions in relation to wild-type GH61a protein. In saccharification reactions, wild-type GH61a protein increases the yield of fermentable sugars. An equivalent amount of GH61 variant proteins instead of the wild type increases the yield of fermentable sugars still further. The present invention provides numerous GH61 variants, as indicated herein. Substitutions that have been shown to improve GH61 activity are included in Table 1, below.

TABLE 1

GH61 Variants with Improved Activity				
Var. No.	Amino Acid Changes	Silent Nucleotide Changes		
1	N35G/E104H/A168P (SEQ ID NO: 5)	t60c/c573g		
2	W42P/E104H/K167A	t60c/c573g/g1026a		
3 4	N35G/W42P/V97Q/A191N W42P/E104H	c573g		
5	E104H/K167A	t60c/c291a/c573g		
6	W42P/A191N	t60c/c291a		
7	N35G/W42P/A191N	t60c/c291a		
8	H20D	0000,02010		
9	V97Q/A191N			
10	N35G/E104H/A191N	t60c/c876t		
11	E104H			
	E104Q			
	H20D/E104D/Q190H/Y192H			
	H20D/Q190E/Y192Q	a312g		
	H20D/E104C			
	H20D/P103H/E104C			
	H20D/P103H	a312g		
	N35G/E104H	t60c/c573g		
	H20D/P103H/E104Q/Q190E H20D/P103H/E104C/Y192Q			
	E104D	t60c		
	N35G/W42P	t60c/c573g		
	A137P	1000,0373g		
	H20D/P103H/E104Q			
	P103E/E104D	t60c		
26	N35G/F68Y/A191N	t379a/c380g/g381c		
27	W42P/A168P			
28	H20D/E104C/Q190E/Y192Q			
29	A142W			
30	N35G			
	H20C/Q190E			
32	W42P/A212P/T236P			
33	N35G/W42P/V97Q/K167A/ A168P	t60c/c573g		
34		c573g		
35	S232A	65/3g		
33	OESEL E			

TABLE 1-continued

TABLE 1-continued

	GH61 Variants with Improved Activity		GH61 Variants with Improved Activity			
Var. No.	Amino Acid Changes	Silent Nucleotide Changes	Var. No.	Amino Acid Changes	Silent Nucleotide Changes	
36	W42P/E104H/K167A/A168P/	c573g		H175M		
37	Q190E W42P/A168P/A212P/T236P			A168N A179W		
	N35G/V97Q/K167A			W131K/H175Q	g1026a	
	N35G/V97Q			Y171A	5	
	N35G/A191N			N170H		
	S127T/K167A/A191N			P163R		
	W42P W42P/E104C/K167A/A168P	t60c/c291a/c573g		A168C G169T		
	K167Q	1000/025120575g		R174F		
	W131V		116	W131Y		
	E176C			I134L		
	K167I/P273S W42P/T87P	c300t		I177V K167E		
	W42P/A212P			H175C		
	K133H			W131I		
	D165N			W42P/A143P		
	D165A			I178G	c72t	
	A168D K218T			N170P A179D/N317K	c732g/c843t/c882t/c909t/	
	P45T		120	111/32/1101/11	c912g	
	Q44V			1162V		
	S164W			I178M		
	I177F A191N			V172A K167A/A191N	t60c/c291a	
	I134P			F132A	000,02514	
61	K133F		131	P163E		
	I134D	.co 201 572		F132M		
	N35G/K167A I162R	t60c/c291a/c573g		A179G I177S		
	N35G/K167A	t204c/t379a c380g/g381c/		K167A	g921a	
		c385t	136	K167F		
	D165W/A246T			A168I		
	I162L S164M			A179N I134A	c792t	
	F132D/A244D			K167E	g972t	
	H181Q			R174K	S	
	I177G	g1026a		S164F		
	L166W I162F			V172L A168H		
	I134V			I134T		
	E176Q			K167H		
	H181S			L166A		
	I178A K167A			S164R R174C		
	V172K			A179P		
	I177H			G169R	g1026a	
	I134N			L173M		
	K133Y N35G/Y139L			D165K E176S		
	A168G			F132L		
	T12A/I162G	c246t		F132I/A179I		
	D165E D165M			F132P S164Q		
	I134M			V172Q		
89	A168P		160	W131D		
	I177D			W131Q		
	S164P H175T			A179H I134H/G270S		
	N187K/S330R	c597g		N170G		
94	H175R		165	A168T		
	L166H			A179C		
	I178L L173H			K133N K167L		
	I177T			L180M		
99	N170Y		170	W131F		
	H175S			I134W	g1026a	
	K167T L166R			I178H N170A		
	V172Y			V172H		
	P163S/E176D			A168H/S205N		
105	S164I		176	I134H	g921a	

TABLE 1-continued

GH61 Variants with Improved Activity				
Var. No.	Amino Acid Changes	Silent Nucleotide Changes		
177	S164C			
178 179	S164K I177C			
180	I178Q			
181 182	L180W I177M			
183	R174D			
184	V172M			
185 186	A179M H175Y			
187	I178P			
188	L173A			
189 190	N170E N170F			
191	N35G/A191N/T258I/T323P/G328A/	t379a/c380g/g381c/c454a/		
192	C341R A168R	c456a/c732t/c843t/c849t		
193	D165I			
194	I162M			
195 196	K167V A179S			
197	E176N			
198 199	I134L/P322L P163L			
200	H181D			
201	N170S			
202 203	R174G I177R			
204	K167C			
205 206	L166Q P163I			
207	S164L/L166I			
208	Y171R			
209 210	F132P/Q190E/A191T F132Q			
211	I134C			
212 213	I177A E176R			
214	G169A			
215	G169K			
216 217	H181A I177L			
218	A168G			
219 220	A179R D165T			
221	K167R			
222	L166V			
223 224	N170C I178R			
225	R174H			
226 227	S164H W131R/L166I			
228				
229				
230 231	N170Q I177P			
232	R174N			
233 234	V172K/S215W D165R			
235	G239D	c520a/c522g		
236				
237 238	H181R I134Y			
239	V172F			
240	V172G			

[0123] Positions that were changed in variants with improved GH61 activity listed in Table 1 include 20, 34, 35, 42, 44, 45, 68, 87, 97, 103, 104, 127, 131, 132, 133, 137, 139, 142, 143, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 190,

191, 192, 192, 205, 212, 215, 218, 232, 236, 239, 244, 246, 258, 270, 273, 317, 322, 323, 328, 330, and 341, wherein the amino acid positions are numbered with reference to SEQ ID NO:2.

[0124] Residues that were changed in variants with improved GH61 activity listed in Table 1 include H20, I134, N35, W42, Q44, P45, F68, T87, V97, P103, E104, S127, W131, F132, K133, A137, Y139, A142, A143, I162, P163, S164, D165, L166, K167, A168, G169, N170, Y171, V172, L173, R174, H175, E176, I177, I178, A179, L180, H181, Q190, A191, Y192, Y192, S205, A212, S215, K218, S232, T236, G239, A244, A246, T258, G270, P273, N317, P322, T323, G328, S330, and C341, wherein the amino acid positions are numbered with reference to SEQ ID NO:2.

[0125] Substitutions occurring in variants with improved GH61 activity listed in Table 1 include H20C/D, I134X, N35G, W42P, Q44V, P45T, F68Y, T87P, V97Q, P103E/H, E104C/D/H/Q, S127T, W131X, F132X, K133X, A137P, Y139L, A142W, A143P, I162X, P163X, S164X, D165X, L166X, K167A/X, A168P/X, G169X, N170X, Y171A/R, V172X, L173X, R174X, H175X, E176X, I177X, I178X, A179X, L180M/W, H181X, Q190E/H, A191N/T, Y192H, Y192Q, S205N, A212P, S215W, K218T, S232A, T236P, G239D, A244D, A246T, T258I, G270S, P273S, N317K, P322L, T323P, G328A, 5330R, and C341R, wherein the amino acid positions are numbered with reference to SEQ ID NO:2.

[0126] As shown herein, the changed residues and substitutions of the GH61 variants of this invention may be combined in a manner that produces an effect that is cumulative or synergistic. Cumulative effects occur when adding an additional mutation increases the effect beyond those of the mutations already present. Synergistic effects occur when having two more mutations in a variant produces an effect than is more than the product of the mutations when incorporated by themselves. This invention includes without limitation any and all combinations of any two, three, four, five, six, seven, eight, nine, ten, or more than ten of the mutations listed in this disclosure.

[0127] Useful combinations include but are not limited to the mutations and mutation sets: N35G/E104H/A168P (SEQ ID NO:5); W42P/E104H/K167A; N35G/W42P/V97Q/ A191N; W42P/E104H; E104H/K167A; W42P/A191N; N35G/W42P/A191N: V97O/A191N; N35G/E104H/ A191N; H20D/E104D/Q190H/Y192H; H20D/Q190E/ Y192Q; H20D/E104C; H20D/P103H/E104C; H20D/ H20D/P103H/E104Q/Q190E; P103H: N35G/E104H; H20D/P103H/E104C/Y192Q; N35G/W42P; H20D/P103H/ E104Q; P103E/E104D; N35G/F68Y/A191N; W42P/ A168P; H20D/E104C/Q190E/Y192Q; H20C/Q190E; W42P/A212P/T236P; N35G/W42P/V97Q/K167A/V97Q/ A168P; W42P/E104H/K167A/A168P/Q190E; A168P/A212P/T236P; N35G/V97Q/K167A; N35G/V97Q; N35G/A191N: 5127T/K167A/A191N; W42P/E104C/ K167A/A168P; K1671/P273S; W42P/T87P; W42P/A212P; N35G/K167A; N35G/K167A; D165W/A246T; F132D/ A244D; N35G/Y139L; T12A/I162G; N187K/S330R; P163S/E176D; W131K/H175Q; W42P/A143P; A179D/ N317K; K167A/A191N; F1321/A1791; I134H/G270S; A168H/S205N; N35G/A191N/T258I/T323P/G328A/ C341R; I134L/P322L; S164L/L1661; F132P/Q190E/ A191T; W131R/L1661; I162A/A191T; and V172K/S215W, wherein the amino acid positions are numbered with reference to SEQ ID NO:2.

GH61 Variant Proteins Made with Multiple Rounds of Activity Enhancement

[0128] GH61 variant proteins can be generated that have been further optimized by subjecting to multiple rounds of variation and selection. In some embodiments, additional rounds of optimization increase saccharification reaction yields beyond what is achieved with one round of variation and selection. Substitutions improving GH61 activity are compiled in Table 2 below.

[0129] Table 2 shows GH61a variants derived from the GH61a protein designated "Variant 1" (SEQ ID NO:5) in Table 1 with improved thermoactivity. The second-round variants usually retained the alterations of Variant 1 compared with wild-type GH61a (N35G/E104H/A168P), along with additional modifications.

TABLE 2

		TABLE 2		287 288	l N
	CITA		1	289	N
_	GH6	1 Variants with Improved Activity C	ompared to Variant 1	290	Ņ
	Var.		Cilant Nivelegalida	291	ì
	Var. No.	Amino Acid Changes	Silent Nucleotide Changes		S
_	140.	Animo Acid Changes	Changes	292	N
	241	N35G/T40A/E104H/A168P/	t60c/c573g	293	N
		P327M	2		A
	242	N35G/P45D/E104H/A168P/	t60c/c573g	294	ľ
		N317R		295	ľ
	243	N35G/E104H/A168P/N317R	t60c/c573g	296	ľ
	244	N35G/E104H/A168P/N317L	t60c/c573g		A
	245	N35G/T54H/E104H/A168P	t60c/c573g	297	N
	246	N35G/E104H/A168P/N317D/	t60c/c573g	298	P A
	247	S329Y	460-7-573-	299	N
	247	N35G/E104H/A137S/A168P/ S232E	t60c/c573g	300	N
	248	N35G/E104H/A168P/N317R/	t60c/c573g	301	N
	240	T320A	100c/c3/3g	302	N
	249	N35G/E104H/A168P/D234E	t60c/c573g	303	ì
	250	N35G/T40S/E104H/A142G/	t60c/c573g	304	N
	250	A168P	100 0/0 3/3g	305	N
	251	N35G/T40S/S78C/V88I/	t60c/c573g	306	N
	201	E104H/S128K/A168P/D234M	000 0 700 700	307	N
	252	N35G/E104H/A168P/S330V	t60c/c573g	308	N
	253	N35G/E104H/A168P/G203E/	t60c/c573g	309	N
		P266S	Ö	310	ľ
	254	N35G/E104H/A168P/D234N	t60c/c573g		A
	255	N35G/E104H/A168P/S286N/	t60c/c573g	311	ľ
		S329H		312	ľ
	256	N35G/E104H/A168P/S330H	t60c/c573g		F
	257	N35G/E104H/A168P/W337R	t60c/c573g	313	ľ
	258	N35G/N66D/E104H/S164E/	t60c/c573g	21.4	S
		A168P/G267T	/	314	ľ
	259	N35G/E104H/A168P/P233V	t60c/c573g	315	N
	260	R34E/N35G/E104H/R145T/	t60c/c573g	316	N
	261	A168P	+60-1-573-	317	N
	261	S24Q/N35G/E104H/A168P/ V237I	t60c/c573g	318	N
	262	Y32S/N35G/E64S/E104H/	t60c/c573g	319	Ņ
	202	A168P	100c/c3/3g	317	1
	263	N35G/E104H/A168P/V333R	t60c/c573g	320	S
	264	N35G/E104H/G144S/A168P/	t60c/c573g		7
		V333Q	g	321	N
	265	V28H/N35G/P45K/E104H/	t60c/c573g		7
		A168P	2	322	N
	266	N35G/E104H/A168P/P327K	t60c/c573g	323	ľ
	267	N35G/N66Q/E104H/A168P	t60c/c573g	324	ľ
	268	N35G/E104H/A168P/G203E	t60c/c573g	325	ľ
	269	N35G/E104H/A168P/S339W	t60c/c573g		A
	270	N35G/P45K/N46E/E104H/	t60c/c573g	326	N
		A150Y/A168P		227	A
	271	N35G/E104H/R130S/A168P	t60c/c573g	327	N
	272	N35G/E104H/R145T/A168P	t60c/c573g/g891a	328 329	l l
	273	N35G/E104H/A168P/S231K	t60c/c573g	330	ľ
	274	N35G/T40A/E104H/A168P/ D234E/P327M	t60c/c573g	331	N
		19237131 32 / IVI		551	-

TABLE 2-continued

GH61 Variants with Improved Activity Compared to Variant 1

Var. No.	Amino Acid Changes	Silent Nucleotide Changes
275	N35G/E104H/A168P/S231H	t60c/c573g
276	N35G/E104H/A168P/N317M	t60c/c573g
277	N35G/E104H/A168P/S330Y	t60c/c573g
278	N35G/E104H/A168P/S329I	t60c/c573g
279	N35G/E104H/A168P/S329R	t60c/c573g
280	N35G/N66D/E104H/A168P/	t60c/c573g
201	P322R/S329L	160 / 2004/ 572
281	N35G/E104H/A168P/P327F	t60c/c288t/c573g
282	N35G/P45D/E104H/A168P	t60c/c573g
283	N35G/E104H/A168P/S332R	t60c/c573g
284	N35G/E104H/A116S/A168P	t60c/c573g
285	N35G/T40A/E104H/A168P/ V230I/P327M	t60c/c573g
286	N35G/T49A/E104H/A168P	t60c/c573g
287	N35G/E104H/A168P/N317T	t60c/c573g
288	N35G/N46Y/E104H/A168P	t60c/c573g
289	N35G/E104H/A168P/G203V	t60c/c573g
290	N35G/E104H/A168P/S329L	t60c/c573g
291	N35G/E104H/R145N/A168P/	t60c/c573g
	S329H	_
292	N35G/A56S/E104H/A168P	t60c/c573g
293	N35G/T40S/T49R/E104H/	t60c/c573g
	A168P/D234E/P327M	
294	N35G/E104H/Q161R/A168P	t60c/c573g
295	N35G/E104H/A168P/S332F	t60c/c573g
296	N35G/P45R/T49A/E104H/	t60c/c573g
	A168P/N317R/T320A	_
297	N35G/E104H/A168P/V237I	t60c/c573g
298	N35G/Q44K/T80V/E104H/	t60c/c573g
270	A168P	1000,03735
299	N35G/E104H/A168P/E336S	+60a/a573 a
		t60c/c573g
300	N35G/E104H/A168P/P233T	t60c/c573g
301	N35G/E104H/A168P/S329Y	t60c/c573g
302	N35G/E104H/A168P/P327L	t60c/c573g
303	N35G/E104H/A168P/N317I	t60c/c573g
304	N35G/E104H/R130H/A168P	t60c/c573g
305	N35G/Q44K/E104H/A168P	t60c/c573g
306	N35G/N66D/E104H/A168P	t60c/c573g
307	N35G/E104H/A168P/S329V	t60c/c573g
308	N35G/E104H/A168P/W337F	t60c/c573g
309	N35G/E104H/A168P/N317H	t60c/c573g
310	N35G/T40L/E104H/S128K/	t60c/c573g
	A168P	
311	N35G/E104H/A168P/A326V	t60c/c573g
312	N35G/T80V/E104H/A168P/	t60c/c573g
	P303T	
313	N35G/E104H/A168P/S231A/	t60c/c573g
	S295L	
314	N35G/E104H/A116Q/A168P	t60c/c573g
315	N35G/E104H/A168P/S330C	t60c/c573g
316	N35G/T40S/E101T/E104H/	t60c/c573g
	A168P/P327M	-
317	N35G/E104H/A168P//A326Q	t60c/c573g
318	N35G/N46R/E104H/A168P	t60c/c573g
319	N35G/P45K/E104H/A168P/	t60c/c573g
517	A219R/S232E	1000,03735
320	S24Q/N35G/E104H/A168P/	t60c/c573g
320	V237I/P303T	100c/c3/3g
221		160-1-572-
321	N35G/E104H/A168P/G203E/	t60c/c573g
222	T281A	160 / 572
322	N35G/A56N/E104H/A168P	t60c/c573g
323	N35G/E104H/A168P/E336G	t60c/c573g
324	N35G/E104H/A168P/E336R	t60c/c573g
325	N35G/T40S/E104H/S128K/	t60c/c573g
	A142G/A168P	
326	N35G/Q44K/S67T/E104H/	t60c/c198t/c573g
	A168P	-
327	N35G/E104H/A168P/N317A	t60c/c573g
328	N35G/E104H/G155N/A168P	t60c/c573g
329	N35G/E104H/Q161E/A168P	t60c/c573g
330	N35G/E104H/N118S/A168P	t60c/c573g
		ē
331	N35G/P45T/V97Q/E104H/	t60c/c573g
	A168P/G267S	

TABLE 2-continued

TABLE 2-continued

TABLE 2-continued		CIVILIA STATE OF THE VICTOR OF			
GH6	51 Variants with Improved Activity Compared to Variant 1		GH61 Variants with Improved Activity Compared to Variant 1		
Var. No.	Amino Acid Changes	Silent Nucleotide Changes	Var. No.	Amino Acid Changes	Silent Nucleotide Changes
332	V28H/N35G/E104H/A168P	t60c/c573g	390	N35G/N46R/E104H/A168P/	t60c/c573g
333	N35G/E104H/A168P/Q184L	t60c/c573g	201	G203E/A263V	
334	N35G/E104H/A168P/N317V	t60c/c573g	391	N35G/P45R/E104H/A168P	t60c/c573g
335	N35G/Q44L/E104H/A168P	t60c/c573g	392	N35G/S67G/E104H/A168P	t60c/c573g
336	N35G/E104H/A168P/S330G	t60c/c573g	393	N35G/E104H/A168P/R199E	t60c/c573g
337	N35G/E104H/A168P/T320A/	t60c/c573g	394	N35G/G69T/E104H/A168P	t60c/c573g
	V333W		395	N35G/E104H/A168P/G203E/	t60c/c573g
338	N35G/E104H/A168P/E336A	t60c/c573g		G268A/G269A/G270A	
339	N35G/E104H/A168P/N335S	t60c/c573g	396	N35G/E104H/A168P/P266S	t60c/c573g
340	N35G/N66M/E104H/A168P	t60c/c573g	397	N35G/E104H/A168P/V324M	t60c/c573g
341	N35G/T54G/E104H/A168P	t60c/c573g	398	N35G/E104H/A168P/G245A	t60c/c573g
342	N35G/E104H/A168P/N317S	t60c/c573g	399	N35G/N66R/E104H/A168P	t60c/c573g
343	N35G/E64L/E104H/A168P	t60c/c573g	400	N35G/E104H/A168P/T236E	t60c/c573g
344	N35G/E104H/S164E/A168P/ A271T	t60c/c573g	401	S24Q/N35G/Q44K/T80H/ E104H/A168P	t60c/c573g
345	N35G/N66A/E104H/A168P	t60c/c573g	402	N35G/E104H/S128D/A168P	t60c/c573g
346	N35G/G83R/E104H/A168P	t60c/c573g	403	N35G/N66D/S78D/E104H/	t60c/c573g
347	N35G/E104H/A168P/N317Q/	t60c/c573g	403	A168P/S253D	1000/03/38
347	T320A	100c/c3/3g	404		t60c/c573g
240		+60-1-573-		N35G/E104H/R130Y/A168P	
348	N35G/E104H/K141A/A168P	t60c/c573g	405	N35G/E104H/A168P/K310I	t60c/c573g
349	N35G/P71T/E104H/A168P	t60c/c573g	406	N35G/E104H/R145E/A168P	t60c/c573g
350	N35G/P71S/E104H/A168P	t60c/c573g	407	N35G/N66D/E104H/S164E/	t60c/c573g
351	N35G/E104H/R130G/A168P	t60c/c573g		A168P/S282D	
352	N35G/E104H/R145Q/A168P	t60c/c573g	408	N35G/E104H/K141P/A168P	t60c/c573g
353	N35G/T70A/E104H/A168P	t60c/c573g	409	N35G/E104H/A168P/Q184R	t60c/c573g
354	N35G/E104H/A168P/K218R	t60c/c573g	410	N35G/E104H/A168P/S231T	t60c/c573g
355	N35G/E104H/A168P/Q184E	t60c/c573g	411	N35G/N66V/E104H/A168P	t60c/c573g
356	N35G/E104H/R130K/A168P	t60c/c573g	412	N35G/E104H/A142L/A168P	t60c/c573g
357	N35G/Q58H/E104H/A168P	t60c/c573g	413	N35G/E104H/R145H/A168P	t60c/c573g
358	Y32S/N35G/E104H/A168P	t60c/c573g	414	N35G/E104H/A168P/K218L	t60c/c573g
359	N35G/E104H/A168P/S329T	t60c/c573g	415	N35G/E104H/K141T/A168P	t60c/c573g
360	N35G/E104H/A168P/S330I	t60c/c573g	416	N35G/E104H/A168P/P233F	t60c/c573g
		ē			
361	Y32S/N35G/P71A/E104H/ A168P	t60c/c573g	417	N35G/T40S/E104H/A168P/ P327M	t60c/c573g
362	N35G/E104H/A168P/S330T	t60c/c573g	418	N35G/T54M/E104H/A168P	t60c/c573g
363	N35G/G82A/E104H/A168P	t60c/c573g	419	S24T/N35G/E104H/S164E/	t60c/c573g
364	N35G/T80V/E104H/A168P	t60c/c573g		A168P	
365	N35G/E104H/A168P/S295T	t60c/c573g	420	N35G/P45T/E104H/A168P	t60c/c573g
366	N35G/N66G/E104H/A168P	t60c/c573g	421	N35G/N66D/E104H/S164E/	t60c/c573g
367	N35G/E104H/R145L/A168P	t60c/c573g		A168P/S231T/S253T	
368	N35G/S67H/E104H/A168P/	t60c/c573g	422	N35G/G69H/E104H/A168P	t60c/c573g
	V230M	2	423	N35G/E104H/S128Y/A168P	t60c/c573g
369	N35G/E104H/G136E/A168P	t60c/c573g	424	N35G/T49Q/E104H/A168P	t60c/c573g
370	N35G/T54S/E104H/A168P	t60c/c573g	425	N35G/T49A/E104H/A168P/	t60c/c573g
371	N35G/P45S/E104H/A168P	t60c/c573g	123	O184H	1000,03738
372	N35G/E104H/A168P/A326M	2	426	N35G/E104H/A168P/G203Y	t60c/c573g
		t60c/c573g/c882t			
373	N35G/N66D/N95E/E104H/	t60c/c573g	427	N35G/Q44K/N66V/E104H/	t60c/c573g
274	S164E/A168P/G267D	t60a/a572 =	420	A168P	+60-/-572
374	N35G/E104H/A168P/S332C	t60c/c573g	428	N35G/E104H/A137M/A168P	t60c/c573g
375	N35G/E104H/S128L/A168P	t60c/c573g	429	N35G/E104H/A168P/P327C	t60c/c573g
376	N35G/T54W/E104H/A168P	t60c/c573g	430	N35G/E104H/A168P/T236R	t60c/c573g
377	N35G/E104H/A168P/G268A/	t60c/c573g	431	N35G/I51A/E104H/A168P	t60c/c573g
	G269A/G270A		432	N35G/S67H/E104H/A168P	t60c/c573g
378	N35G/Q44K/E104H/A168P/	t60c/c573g	433	N35G/E104H/A168P/A326C	t60c/c573g
	S231T	-	434	N35G/T49A/E104H/S128N/	t60c/c573g
379	R34E/N35G/E104H/A168P/	t60c/c573g		A168P	8
	A280D		435	N35G/T49R/E104H/A168P/	t60c/c573g
380	N35G/E104H/A168P/A297T	t60c/g399a/c573g	155	K218L/N317Q	
			126	N35G/E104H/A168P/P266S/	+60a/a572 =
381	N35G/E104H/K141P/R145Q/	t60c/c573g	436		t60c/c573g
382	A168P N35G/P45E/E104HVK141R/	t60c/c573g	437	G267V N35G/E104H/A168P/V237I/	t60c/c573g
	A168P			P303T	
383	N35G/N66T/E104H/A168P	t60c/c573g	438	N35G/T49E/E104H/A168P	t60c/c573g
384	N35G/E104H/S164E/A168P/ S295D	t60c/c573g	439	N35G/P45R/E104H/A168P/	t60c/c573g
205		160-1-572		T320A	160-7-572
385	N35G/E104H/A168P/N317F	t60c/c573g	440	N35G/N66L/E104H/A168P	t60c/c573g
386	N35G/E104H/A168P/N317Q	t60c/c573g	441	N35G/P45R/E104H/A168P/	t60c/c573g
387	N35G/T40G/T49R/S78C/	t60c/c573g		K218L/N317Q	
	E104H/A142G/A168P	-	442	N35G/E104H/R145V/A168P	t60c/c573g
					0
388	N35G/G82S/E104H/A168P	t60c/c573g	443	N35G/N66D/E104H/A168P/	t60c/c573g

TABLE 2-continued

GH61 Variants with Improved Activity Compared to Variant 1				
Var. No.	Amino Acid Changes	Silent Nucleotide Changes		
444	N35G/T80L/E104H/A168P	t60c/c573g		
445	N35G/A55G/E104H/A168P	t60c/c573g		
446	N35G/E104H/A168P/S330A	t60c/c573g		
447	N35G/E104H/K141N/A168P/ P266S	t60c/c573g		
448	N35G/E104H/A142S/A168P	t60c/c573g		
449	N35G/E104H/A168P/Q184G	t60c/c573g		
450	N35G/E104H/N118E/A168P	t60c/c573g		
451	N35G/E104H/A168P/A212M	t60c/c573g		
452	N35G/E104H/A168P/G267D	t60c/c573g		
453	N35G/K93N/E104H/R130Y/ A168P	t60c/c573g		
454	N35G/P45R/T49Y/E104H/ A168P/N317D	t60c/c573g		
455	N35G/E104H/A168P/S329Q	t60c/c573g		
456	N35G/E104H/A168P/V230Q	t60c/c573g		
457	N35G/P45K/E104H/A168P/ A219R	t60c/c573g		
458	N35G/E104H/A142G/A168P	t60c/c573g		
459	N35G/E104H/A168P/S205T	t60c/c573g		
460	N35G/S78D/E104H/S164E/ A168P	t60c/c573g		
461	N35G/E104H/R130E/A168P	t60c/c573g		
462	N35G/E104H/A168P/Q184H	t60c/c573g		
463	N35G/E104H/A116P/A168P	t60c/c573g		
464	N35G/E104H/A142D/A168P	t60c/c573g		
465	V28H/N35G/N46E/Q58H/	t60c/c573g		
102	E104H/A168P	:00 0 :0575g		
466	N35G/E104H/A168P/A280T	t60c/c573g		
467	R34E/N35G/E104H/A168P/ A280T	t60c/c573g		
468	N35G/E104H/A168P/E336L	t60c/c573g		
469	N35G/T49D/E104H/A168P	t60c/c573g		
470	N35G/E104H/A168P/A219T	t60c/c573g		
471	N35G/E104H/A142W/A168P	t60c/c573g		
472	N35G/E104H/A168P/P303T/	t60c/c573g		
	G305D N35G/Q44V/E104H/A168P			
473 474	N35G/Q44V/E104H/A168P/N187D	t60c/c573g		
474	N35G/E104H/G136H/A168P	t60c/c573g t60c/c573g		
473 476	S24Q/N35G/Q44K/E104H/	t60c/c573g		
	A168P/P303T/S332D	_		
477	N35G/E104H/A168P/Q184N	t60c/c573g		
478	N35G/E104H/A168P/S332L	t60c/c573g		
479	S24T/N35G/N66D/S78D/ E104H/A168P/S205T/S253T	t60c/c573g		
480	N35G/E104H/A168P/P327A	t60c/c573g		
481	N35G/T40A/T49Q/S78C/ E104H/A168P	t60c/c573g		
482	N35G/T40L/E104H/A142G/ A168P	t60c/c573g		
483	N35G/T49Y/E104H/A168P/ N317R	t60c/c573g		
484	R34E/N35G/K93T/E104H/ R130E/R145T/A168P/R199E/ K218T/A280D	t60c/c573g		

[0130] Positions that were changed in variants with improved GH61 activity listed in Table 2 include 24, 28, 32, 34, 35, 40, 44, 45, 46, 49, 51, 54, 55, 56, 58, 64, 66, 67, 69, 70, 71, 78, 80, 82, 83, 88, 93, 95, 101, 104, 116, 118, 128, 130, 136, 137, 141, 142, 144, 145, 150, 155, 161, 164, 168, 184, 187, 199, 203, 205, 212, 218, 219, 230, 231, 232, 233, 234, 236, 237, 245, 253, 263, 266, 267, 268, 269, 270, 271, 280, 281, 282, 290, 295, 297, 303, 305, 310, 317, 320, 324, 326, 327, 329, 330, 332, 333, 336, 337, and 339, wherein the amino acid positions are numbered with reference to SEQ ID NO:2.

[0131] Residues that were changed in variants with improved GH61 activity listed in Table 2 include S24, V28,

Y32, R34, N35, T40, Q44, P45, N46, T49, 151, T54, A55, A56, Q58, E64, N66, S67, G69, T70, P71, S78, T80, G82, G83, V88, K93, N95, E101, E104, A116, N118, S128, R130, G136, A137, K141, A142, G144, R145, A150, G155, Q161, S164, A168, Q184, N187, R199, G203, S205, A212, K218, A219, V230, S231, S232, P233, D234, T236, V237, G245, S253, A263, P266, G267, G268, G269, G270, A271, A280, T281, S282, R290, S295, A297, P303, G305, K310, N317, T320, V324, A326, P327, S329, S330, S332, V333, E336, W337, and 5339, wherein the amino acid positions are numbered with reference to SEQ ID NO:2.

[0132] Substitutions occurring in variants with improved GH61 activity listed in Table 2 include S24Q, V28H, Y32S, R34E, N35G, T40A/G/L/S, Q44K, P45D/E/K/R/S, N46E/R, T49A/Q/R/Y, I51A, T54G/M/S/W, A55G, A56S, Q58H/P, E64L/S, N66A/D/G/L/M/Q/R/V, S67G/H/T, G69T, T70A, P71A, S78C/D, T80H/L/V, G82A/S, G83R, V88I, K93N/T, N95E, E101T, E104H, A116Q/S, N118E/S, S128K/L/N, R130E/G/H/K/Y, G136H, A137M/S, K141A/N/P/R, A142D/G/L, G144S, R145H/L/N/Q/T, A150Y, G155N, Q161E/R, S164E, A168P, Q184E/H/L/N/R, N187D, R199E, G203E/V/Y, S205T, A212M, K218L/T, A219R/T, V230I/Q, S231A/H/K/I, S232E, P233F/T, D234E/M/N, T236E, V237I, G245A, S253D/T, A263V, P266S, G267D/V, G268A, G269A, G270A, A271T, A280D/T, T281A, S282D, R290K, S295D/L/T, A297T, P303T, G305D, K310I, N317D/H/I/M/Q/R, T320A, V324M, A326C/Q/V, P327F/ K/L/M, S329H/I/Q/T/Y, S330A/H/I/T/V, S332C/F/R, V333Q, E336L/R/S, W337R, and S339W.

[0133] In some embodiments, the changed residues and substitutions of the GH61 variants of this invention may be combined in a manner that produces an effect that is cumulative or synergistic. Cumulative effects occur when adding an additional mutation increases the effect beyond those of the mutations already present. Synergistic effects occur when having two more mutations in a variant produces an effect than is greater than the product of the mutations when incorporated by themselves. This invention includes without limitation any and all combinations of any two, three, four, five, six, seven, eight, nine, ten, or more than ten of the mutations listed in Table 1, Table 2, or both Tables.

[0134] Useful combinations of mutated positions include but are not limited to N35/T40/E104/A168/P327; N35/P45/ E104/A168/N317; N35/E104/A168/N317; N35/E104/ A168/N317/S329; N35/E104/A137/A168/S232; N35/E104/ A168/N317/T320; N35/E104/A168/D234; N35/T40/E104/ A142/A168; N35/E104/R145/A168; N35/T40/S78N88/ E104/S128K/A168/D234; N35/E104/A168/S330; N35/ E104/A168/G203/P266; N35/E104/A168/D234; N35/E104/ A168/S330; N35/E104/A168/W337; R34/N35/E104/R145/ Y32/N35/E64/E104/A168; V28/N35/P45/E104/ A168; N35/E104/G144/A168/V333; N35/N66/E104/A168; N35/E104/A168/P327; N35/E104/A168/G203; N35/E104/ A168/S339; N35/P45/N46/E104/A150/A168; N35/E104/ A168/S231; N35/T40/E104/A168/D234/P327; N35/E104/ A168/S231; N35/E104/A168/N317; N35/E104/A168/S330; N35/E104/A168/S329; N35/E104/A168/P327; N35/P45/ E104/A168: N35/E104/A116/A168; N35/T40/E104/ A168N230/P327; and N35/E104/A168/S332.

[0135] Useful combinations of mutated residues further include but are not limited to N35/E104/A168/G203; N35/E104/R145/A168/S329; N35/T40/T49/E104/A168/D234/P327; N35/A56/E104/A168; N35/E104/Q161/A168; N35/E104/A168/S332; N35/P45/T49/E104/A168/N317/T320;

N35/E104/A168/V237: N35/E104/A168/E336: N35/E104/ A168/P233; N35/E104/R130/A168; N35/E104/A168/P327; N35/E104/A168/N317; N35/Q44/E104/A168; N35/E104/ A168/A326; N35/E104/A168/N317; N35/T40/E104/S128/ A168; N35/T80/E104/A168/P303; N35/E104/A116/A168; N35/E104/A168/S231/S295; N35/T40/E101/E104/A168/ P327; N35/P45/E104/A168/A219/S232; N35/N46/E104/ N35/E104/A168/A326; N35/E104/A168/G203/ T281; N35/E104/A168/E336; N35/T40/E104/S128/A142/ A168; N35/E104/N118/A168; N35/E104/G155/A168; S24/ N35/E104/A168/V237/P303; N35/E104/O161/A168; N35/ Q44/S67/E104/A168; V28/N35/E104/A168; N35/E104/ A168/Q184; N35/T54/E104/A168; N35/N66/E104/A168; N35/E64/E104/A168; N35/E104/S164/A168/A271; N35/ N66/E104/A168: N35/G83/E104/A168: N35/E104/K141/ A168; and N35/E104/A168/N317/T320.

Useful combinations of mutated residues include but are not limited to N35/E104/R130/A168; N35/E104/ R145/A168; N35/T70/E104/A168; N35/E104/R130/A168; N35/E104/A168/Q184; N35/E104/A168/S329; N35/T49/ E104/A168; Y32/N35/E104/A168; N35/E104/A168/S330; N35/Q58/E104/A168; Y32/N35/P71/E104/A168; N35/ E104/A168/S330; N35/T80/E104/A168; N35/G82/E104/ A168; N35/E104/A168/S295; N35/N66/E104/A168; N35/ T54/E104/A168; N35/P45/E104/A168; N35/E104/S128/ A168; N35/N66/N95/E104/S164/A168; /G267; N35/T54/ E104/A168; N35/P45/E104/K141/A168; N35/E104/A168/ S332; N35/E104/A168/A297; N35/E104/K141/R145/A168; N35/Q44/E104/A168/S231; N35/T40/T49/S78/E104/A142; N35/E104/S164/A168/S295; N35/E104/A168/ N317; N35/P45/E104/A168; N35/G82/E104/A168; N35/ N46/E104/A168/G203/A263; N35/Q58/E104/A168; N35/ G69/E104/A168; N35/S67/E104/A168; N35/E104/A168/ N35/E104/A168/G203/G268/G269/G270; E104/A168/V324; N35/E104/A168/P266; N35/E104/A168/ G245; N35/N66/E104/A168; and S24/N35/Q44/T80/E104/

[0137] Useful combinations of mutated residues further include but are not limited to N35/E104/A168/T236; N35/ E104/A168/K310; N35/E104/R130/A168; N35/N66/S78/ E104/A168/S253; N35/N66/E104/S164/A168/S282; N35/ E104/A142/A168; N35/E104/R145/A168; N35/E104/ A168/S231; N35/E104/A168/O184; N35/E104/A168/ K218; N35/E104/A168/P233; N35/T49/E104/A168/O184; N35/T40/E104/A168/P327; N35/T54/E104/A168; N35/ N66/E104/S164/A168/S231/S253; N35/E104/A168/G203; N35/T49/E104/A168; N35/E104/A168/P266/G267; N35/ Q44/N66/E104/A168; N35/S67/E104/A168; N35/E104/ A137/A168; N35/T49/E104/S128/A168; N35/T49/E104/ A168/K218/N317; N35/I51/E104/A168; N35/E104/A168/ A326; N35/P45/E104/A168/T320; N35/N66/E104/A168; N35/E104/A168/V237/P303; N35/P45/E104/A168/K218/ N317; N35/T80/E104/A168; N35/A55/E104/A168; N35/ E104/K141/A168/P266; N35/E104/A168/S330; N35/N66/ E104/A168/R290; N35/E104/N118/A168; N35/E104/A168/ A212; N35/K93/E104/R130/A168; N35/E104/A168/G267; N35/P45/T49/E104/A168/N317; N35/E104/A168/V230; N35/E104/A168/S329; N35/P45/E104/A168/A219; N35/ S78/E104/S164/A168; N35/E104/A168/S205; N35/E104/ A168/Q184; V28/N35/N46/Q58/E104/A168; N35/E104/ A142/A168; N35/E104/A168/E336; N35/E104/A168/ N35/E104/A168/A219; N35/E104/A168/P303/ G305; R34/N35/E104/A168/A280; N35/E104/A168/N187; N35/E104/G136/A168; N35/E104/A168/Q184; N35/T49/

E104/A168/N317; N35/T40/T49/S78/E104/A168; R34/N35/K93/E104/R130/R145/A168/R199/K218/A280; N35/T40/E104/A142/A168; and N35/N66/E104/A168.

[0138] Useful combinations of mutations further include but are not limited to N35G/T40A/E104H/A168P/P327M; N35G/P45D/E104H/A168P/N317R; N35G/E104H/A168P/ N35G/E104H/A168P/N317D/S329Y; N317R; E104H/A137S/A168P/S232E; N35G/E104H/A168P/ N317R/T320A; N35G/E104H/A168P/D234E; N35G/T40S/ E104H/A142G/A168P; N35G/E104H/R145L/A168P; N35G/T40S/S78C/V88I/E104H/S128K/A168P/D234M; N35G/E104H/A168P/S330V; N35G/E104H/A168P/ N35G/E104H/A168P/D234N: N35G/ G203E/P266S: E104H/A168P/S330H; N35G/E104H/A168P/W337R; R34E/N35G/E104H/R145T/A168P; Y32S/N35G/E64S/ E104H/A168P; V28H/N35G/P45K/E104H/A168P; N35G/ E104H/G144S/A168P/V333Q; N35G/N66Q/E104H/ A168P: N35G/E104H/A168P/P327K; N35G/E104H/ A168P/G203E; N35G/E104H/A168P/S339W; N35G/P45K/ N46E/E104H/A150Y/A168P; N35G/E104H/A168P/ N35G/T40A/E104H/A168P/D234E/P327M; S231K: N35G/E104H/A168P/S231H; N35G/E104H/A168P/ N35G/E104H/A168P/S330Y; N317M: N35G/E104H/ A168P/S329I; N35G/E104H/A168P/P327F; N35G/P45D/ E104H/A168P; N35G/E104H/A116S/A168P; N35G/T40A/ E104H/A168P/V230I/P327M; and N35G/E104H/A168P/ S332R.

[0139] Useful combinations of mutations further include but are not limited to N35G/E104H/A168P/G203V; N35G/ E104H/R145N/A168P/S329H; N35G/T40S/T49R/E104H/ /P327M: N35G/A56S/E104H/A168P: A168P/D234E: N35G/E104H/Q161R/A168P; N35G/E104H/A168P/ S332F; N35G/P45R/T49A/E104H/A168P/N317R/T320A; N35G/E104H/A168P/V237I; N35G/E104H/A168P/E336S; N35G/E104H/A168P/P233T; N35G/E104H/R130H/ N35G/E104H/A168P/P327L; N35G/E104H/ A168P/N317I; N35G/Q44K/E104H/A168P; N35G/E104H/ A168P/A326V; N35G/E104H/A168P/N317H; N35G/T40L/ E104H/S128K/A168P; N35G/T80V/E104H/A168P/P303T; N35G/E104H/A116Q/A168P; N35G/E104H/A168P/ S231A/S295L; N35G/T40S/E101T/E104H/A168P/P327M; N35G/P45K/E104H/A168P/A219R/S232E; N35G/N46R/ E104H/A168P; N35G/E104H/A168P/A326Q; E104H/A168P/G203E/T281A; N35G/E104H/A168P/ E336R; N35G/T40S/E104H/S128K/A142G/A168P; N35G/ E104H/N118S/A168P; N35G/E104H/G155N/A168P; S24Q/N35G/E104H/A168P/V237I/P303T; N35G/E104H/ Q161E/A168P; N35G/Q44K/S67T/E104H/A168P; V28H/ N35G/E104H/A168P; N35G/E104H/A168P/Q184L; N35G/T54G/E104H/A168P; N35G/N66M/E104H/A168P; N35G/E64L/E104H/A168P; N35G/E104H/S164E/A168P/ A271T; N35G/N66A/E104H/A168P; N35G/G83R/E104H/ A168P; N35G/E104H/K141A/A168P; and N35G/E104H/ A168P/N317Q/T320A.

[0140] Useful combinations of mutations further include but are not limited to N35G/E104H/R130G/A168P; N35G/E104H/R145Q/A168P; N35G/T70A/E104H/A168P; N35G/E104H/R130K/A168P; N35G/E104H/A168P/Q184E; N35G/E104H/A168P/S329T; N35G/E104H/A168P/S330I; N35G/O58H/E104H/A168P; N35G/E104H/A168P; N35G/E104H/A168P; N35G/E104H/A168P; N35G/E104H/A168P; N35G/E104H/A168P/S330T; N35G/T80V/E104H/A168P; N35G/G82A/E104H/A168P; N35G/E104H/A168P/S295T; N35G/N66G/E104H/A168P; N35G/T54S/E104H/

A168P; N35G/P45S/E104H/A168P; N35G/E104H/S128L/ A168P; N35G/N66D/N95E/E104H/S164E/A168P/G267D; N35G/T54W/E104H/A168P; N35G/P45E/E104H/K141R/ A168P; N35G/E104H/A168P/S332C; N35G/E104H/ N35G/E104H/K141P/R145Q/A168P; A168P/A297T; N35G/Q44K/E104H/A168P/S231T; N35G/T40G/T49R/ S78C/E104H/A142G; /A168P; N35G/E104H/S164E/ A168P/S295D; N35G/E104H/A168P/N317Q; N35G/P45R/ E104H/A168P; N35G/G82S/E104H/A168P; N35G/N46R/ E104H/A168P/G203E/A263V; N35G/Q58P/E104H/ A168P; N35G/G69T/E104H/A168P; N35G/S67G/E104H/ N35G/E104H/A168P/R199E; N35G/E104H/ A168P/G203E/G268A/G269A/G270A; N35G/E104H/ A168P/V324M; N35G/E104H/A168P/P266S; N35G/ E104H/A168P/G245A; N35G/N66R/E104H/A168P; and S24Q/N35G/Q44K/T80H/E104H/A168P.

[0141] Useful combinations of mutations further include but are not limited to N35G/E104H/A168P/T236E; N35G/ E104H/A168P/K310I; N35G/E104H/R130Y/A168P; N35G/N66D/S78D/E104H/A168P/S253D; N35G/N66D/ E104H/S164E/A168P/S282D; N35G/E104H/A142L/ A168P: N35G/E104H/R145H/A168P; N35G/E104H/ A168P/S231T; N35G/E104H/A168P/Q184R; N35G/ E104H/A168P/K218L; N35G/E104H/A168P/P233F; N35G/T49A/E104H/A168P/Q184H; N35G/T40S/E104H/ A168P/P327M; N35G/T54M/E104H/A168P; N35G/N66D/ E104H/S164E/A168P/S231T/S253T; N35G/E104H/ A168P/G203Y; N35G/T49Q/E104H/A168P; N35G/E104H/ A168P/P266S/G267V; N35G/Q44K/N66V/E104H/A168P; N35G/S67H/E104H/A168P; N35G/E104H/A137M/A168P; N35G/T49A/E104H/S128N/A168P; N35G/T49R/E104H/ A168P/K218L/N317Q; N35G/I51A/E104H/A168P; N35G/ N35G/P45R/E104H/A168P/ E104H/A168P/A326C; T320A; N35G/N66L/E104H/A168P; N35G/E104H/A168P/ V237I/P303T; N35G/P45R/E104H/A168P/K218L/N317Q; N35G/T80L/E104H/A168P; N35G/A55G/E104H/A168P; N35G/E104H/K141N/A168P/P266S; N35G/E104H/ N35G/N66D/E104H/A168P/R290K: A168P/S330A: N35G/E104H/N118E/A168P; N35G/E104H/A168P/ A212M; N35G/K93N/E104H/R130Y/A168P; E104H/A168P/G267D; N35G/P45R/T49Y/E104H/A168P/ N317D; N35G/E104H/A168P/V230Q; N35G/E104H/ A168P/S329Q; N35G/P45K/E104H/A168P/A219R; N35G/ S78D/E104H/S164E/A168P; N35G/E104H/A168P/S205T; N35G/E104H/A168P/Q184H; V28H/N35G/N46E/Q58H/ E104H/A168P; N35G/E104H/A142D/A168P; E104H/A168P/E336L; N35G/E104H/A168P/A280T; N35G/E104H/A168P/A219T; N35G/E104H/A168P/P303T/ G305D; R34E/N35G/E104H/A168P/A280T; N35G/E104H/ A168P/N187D; N35G/E104H/G136H/A168P; N35G/ E104H/A168P/Q184N; N35G/T49Y/E104H/A168P/ N317R; N35G/T40A/T49Q/S78C/E104H/A168P; R34E/ N35G/K93T/E104H/R130E/R145T/A168P/R199E/K218T/ A280D; N35G/T40L/E104H/A142G/A168P; and N35G/ N66G/E104H/A168P.

Production of GH61 Variant Proteins

[0142] In some embodiments, the GH61 variant proteins of this invention are produced by recombinant expression in a host cell. Any suitable method for recombinant expression in any suitable host cell finds use in the present invention. In some embodiments, a nucleotide sequence encoding the protein is obtained, and introduced into a suitable host cell by way of a suitable transfer vector or expression vector. In

some embodiments, the nucleotide sequence is operably linked to a promoter that promotes expression in the host cell. The promoter sequence is often selected to optimize in a cell that is not *M. thermophila*, in which case the promoter is typically heterologous to the GH61 variant protein encoding sequence. In some embodiments, the host cell is a eukaryotic cell and the GH61 variant protein comprises a heterologous signal peptide at the N-terminus.

[0143] Optionally, in some embodiments, the encoding sequence is codon-optimized for the host cell (e.g., a particular species of yeast cell). Any suitable method for obtaining codon-optimized sequences find use in the present invention (e.g., GCG CodonPreference, Genetics Computer Group Wisconsin Package; Codon W, John Peden, University of Nottingham; and McInerney, Bioinform., 14:372-73 [1998]).

[0144] General reference texts relating to gene expression include but are not limited to the most recent editions of Protocols in Molecular Biology (Ausubel et al. eds.); Molecular Cloning: A Laboratory Manual (Sambrook et al. eds.); Advances In Fungal Biotechnology For Industry, Agriculture, And Medicine (Tkacz and Lange, 2004); and Fungi: Biology and Applications (K. Kavanagh ed., 2005). [0145] In some embodiments, culture broth from GH61 protein-producing cells is collected and combined directly with cellulase enzymes in a saccharification reaction. In some alternative embodiments, the broth is fractionated to any extent desired to provide partially or substantially purified GH61 protein, following the activity during the separation process using a GH61 activity assay, using standard protein separation techniques, and following GH61 activity during fractionation with a suitable GH61 activity assay. Such protocols may combine one or more of the following methods (but are not limited to these particular methods): salt precipitation, solid phase binding, affinity chromatography, ion exchange chromatography, molecular size separation, and/or filtration. Protein separation techniques are generally described in Protein Purification: Principles, High Resolution Methods, and Applications, (J. C. Janson, ed., 2011); High Throughput Protein Expression and Purification: Methods and Protocols (S. A. Doyle ed., 2009).

[0146] The present invention provides GH61 variant protein having an amino acid sequence that is at least about 60%, at least about 65%, at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID NO:2 or a fragment of SEQ ID NO:2 having GH61 activity. In some embodiments, the amino acid sequence of the variant proteins have one or more amino acid substitutions with respect to SEQ ID NO:2 or said fragment. In some embodiments, the substitution(s) that are present in the amino acid sequence result in the variant protein having increased GH61 activity in a saccharification reaction by certain cellulase enzymes under specified conditions, compared with a reference protein comprising SEQ ID NO:2 or said fragment, without any of the substitutions.

[0147] In some embodiments, GH61 variant proteins of this invention comprise one or more of SEQ ID NOS:5, 6, 8, 9,11, and/or 12, or biologically-active fragments of these sequences having GH61 activity. These correspond to Variants 1 (SEQ ID NOS:5 and 6), Variant 5 (SEQ ID NOS: 8 and 9), and Variant 9 (SEQ ID NOS: 11 and 12). In some

embodiments, the variants have more than about 2-fold, 3-fold, or more than 3-fold GH61 activity compared with wild-type GH61a (i.e., SEQ ID NO:2). The combined effect of multiple rounds of optimization yield GH61 variant proteins that have about 3-fold, about 5-fold, about 8-fold, or about 10-fold activity compared with the original parental sequence (SEQ ID NO:2).

[0148] Also provided are polynucleotides encoding such GH61 variant proteins, expression vectors comprising such polynucleotides, and host cells that have been transfected with such vectors so as to express the GH61 variant proteins that are encoded.

Fragments and Variants

[0149] GH61 variant proteins of this invention may comprise one or more substitutions, deletions, or additions in the sequence in addition to the substitutions highlighted above. By way of illustration, the GH61 protein may be longer or shorter by at least about 5, 10, 20, 40, 75, 100, 125, 150, or 200 amino acids; or by about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 15%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, or 80% of the total number of amino acids in the polypeptide, compared with SEQ ID NO:2. The variant or any of these fragments may also be part of a fusion protein in which a portion having GH61 activity is joined to one or more other sequences. Providing the protein retains a degree of GH61 activity or other commercial applicability, the variations may comprise any combination of amino acid substitutions at any position that is not specifically indicated otherwise. Depending on the circumstances, a conservative amino acid substitution may be preferred over other types of substitutions.

[0150] Where an amino acid substitution is a "conservative" substitution, the substituted amino acid that shares one or more chemical property with the amino acid it is replacing. Shared properties include the following: Basic amino acids: arginine (R), lysine (K), histidine (H); acidic amino acids: glutamic acid (E) and aspartic acid (D); uncharged polar amino acids: glutamine (Q) and asparagine (N); hydrophobic amino acids: leucine (L), isoleucine (I), valine (V); aromatic amino acids: phenylalanine (F), tryptophan (W), and tyrosine (Y); sulphur-containing amino acids: cysteine (C), methionine (M); small amino acids: glycine (G), alanine (A), serine (S), threonine (T), proline (P), cysteine (C), and methionine (M).

Obtaining Functional Fragments and Variants

[0151] Functional fragments of GH61 protein variants of this invention can be identified by standard methodology for mapping function within a polypeptide. In some embodiments, recombinant protein is expressed that has effectively been trimmed at the N- or C-terminus, and then tested in a GH61 activity assay. Trimming can continue until activity is lost, at which point the minimum functional unit of the protein would be identified. Fragments containing any portion of the protein down to the identified size would typically be functional, as would be fusion constructs containing at least the functional core of the protein.

[0152] To generate further variants that incorporate one or more amino acid changes in a GH61 encoding sequence, the skilled artisan can change particular nucleotides, and then retest the expressed protein for GH61 activity.

[0153] An effective way to generate a large collection of functional variants is to use a random mutation strategy. The standard texts Protocols in Molecular Biology (Ausubel et al. eds.) and Molecular Cloning: A Laboratory Manual (Sambrook et al. eds.) describe techniques employing chemical mutagenesis, cassette mutagenesis, degenerate oligonucleotides, mutually priming oligonucleotides, linkerscanning mutagenesis, alanine-scanning mutagenesis, and error-prone PCR. Other efficient methods include the E. coli mutator strains of Stratagene (See e.g., Greener et al., Methods Mol. Biol. 57:375 [1996]) and the DNA shuffling technique of Maxygen (See e.g., Patten et al., Curr. Opin. Biotechnol., 8:724 [1997]; Harayama, Tr. Biotechnol., 16:76 [1998]; U.S. Pat. Nos. 5,605,793 and 6,132,970). To increase variation, a technology can be used that generates more abrupt changes, such as DNA shuffling techniques.

[0154] Mutagenesis may be performed in accordance with any of the techniques known in the art, including random and site-specific mutagenesis. Directed evolution can be performed with any of the techniques known in the art to screen for production of variants including shuffling. Mutagenesis and directed evolution methods are well known in the art (See e.g., U.S. Pat. Nos. 5,605,793, 5,830,721, 6,132,970, 6,420,175, 6,277,638, 6,365,408, 6,602,986, 7,288,375, 6,287,861, 6,297,053, 6,576,467, 6,444,468, 5,811238, 6,117,679, 6,165,793, 6,180,406, 6,291,242, 6,995,017, 6,395,547, 6,506,602, 6,519,065, 6,506,603, 6,413,774, 6,573,098, 6,323,030, 6,344,356, 6,372,497, 7,868,138, 5,834,252, 5,928,905, 6,489,146, 6,096,548, 6,387,702, 6,391,552, 6,358,742, 6,482,647, 6,335,160, 6,653,072, 6,355,484, 6,03,344, 6,319,713, 6,613,514, 6,455,253, 6,579,678, 6,586,182, 6,406,855, 6,946,296, 7,534,564, 7,776,598, 5,837,458, 6,391,640, 6,309,883, 7,105,297, 7,795,030, 6,326,204, 6,251,674, 6,716,631, 6,528,311, 6,287,862, 6,335,198, 6,352,859, 6,379,964, 7,148,054, 7,629,170, 7,620,500, 6,365,377, 6,358,740, 6,406,910, 6,413,745, 6,436,675, 6,961,664, 7,430,477, 7,873,499, 7,702,464, 7,783,428, 7,747,391, 7,747,393, 7,751,986, 6,376,246, 6,426,224, 6,423,542, 6,479,652, 6,319,714, 6,521,453, 6,368,861, 7,421,347, 7,058,515, 7,024,312, 7,620,502, 7,853,410, 7,957,912, 7,904,249, and all related US and non-US counterparts; Ling et al., Anal. Biochem., 254(2):157-78 [1997]; Dale et al., Meth. Mol. Biol., 57:369-74 [1996]; Smith, Ann. Rev. Genet., 19:423-462 [1985]; Botstein et al., Science, 229:1193-1201 [1985]; Carter, Biochem. J., 237:1-7 [1986]; Kramer et al., Cell, 38:879-887 [1984]; Wells et al., Gene, 34:315-323 [1985]; Minshull et al., Curr. Op. Chem. Biol., 3:284-290 [1999]; Christians et al., Nat. Biotechnol., 17:259-264 [1999]; Crameri et al., Nature, 391:288-291 [1998]; Crameri, et al., Nat. Biotechnol., 15:436-438 [1997]; Zhang et al., Proc. Nat. Acad. Sci. U.S.A., 94:4504-4509 [1997]; Crameri et al., Nat. Biotechnol., 14:315-319 [1996]; Stemmer, Nature, 370:389-391 [1994]; Stemmer, Proc. Nat. Acad. Sci. USA, 91:10747-10751 [1994]; WO 95/22625; WO 97/0078; WO 97/35966; WO 98/27230; WO 00/42651; WO 01/75767; and WO 2009/152336, all of which are incorporated herein by ref-

[0155] There are commercially available services and kits available to the skilled reader to use in obtaining variants of the claimed proteins. By way of illustration, systems specifically designed for mutagenesis projects include the following: the GeneTailorTM Site-Directed Mutagenesis System sold by InVitrogenTM Life Technologies; the BD

DiversifyTM PCR Random Mutagenesis KitTM, sold by BD Biosciences/Clontech; the Template Generation SystemTM, sold by MJ Research Inc., the XL1-RedTM mutator strain of *E. coli*, sold by Stratagene; and the GeneMorph® Random Mutagenesis Kit, also sold by Stratagene. By employing any of these systems in conjunction with a suitable GH61 activity assay, variants can be generated and tested in a high throughput manner.

[0156] Alternatively or in addition, the user may conduct further evolution of the encoded protein (See e.g., U.S. Pat. No. 7,981,614; US Pat. Appln. Publ. No. 2011/0034342; U.S. Pat. No. 7,795,030; U.S. Pat. No. 7,647,184; U.S. Pat. No. 6,939,689; and U.S. Pat. No. 6,773,900).

[0157] After each iteration of mutagenesis, the user can test and select the desired clones retaining GH61 activity. Optionally, the selected clones can be subject to further rounds of mutagenesis, until the desired degree of variation from the original sequence has been achieved.

Cellulase Enzymes and Compositions

[0158] The GH61 proteins of this invention are useful for increasing the yield of fermentable sugars in a saccharification reaction with one or more cellulase enzymes. The cellulase enzymes can be produced in the same cell as the GH61 protein or in a different cell. In either case, the cellulase enzymes can be expressed from a recombinant encoding region or from a constitutive gene. The cellulase enzymes can be provided in the form of a culture broth (with or without the microorganism producing the enzyme(s)) or supernatant, or purified to any extent desired.

[0159] The terms "cellulase" and "cellulase enzyme" broadly refer to enzymes that catalyze the hydrolysis of the beta-1,4-glycosidic bonds joining individual glucose units in a cellulose containing substrate. Examples of cellulase enzymes suitable for use with the GH61 proteins of this invention are described in more detail later in this section. [0160] Endoglucanases (EGs), comprise a group of cellulase enzymes classified as E.C. 3.2.1.4. These enzymes catalyze the hydrolysis of internal beta-1,4 glycosidic bonds of cellulose. In some embodiments, the present invention comprises an endogenous M. thermophila endoglucanase such as M. thermophila EG2 (See, WO 2007/109441) or a variant thereof. In some additional embodiments, the EG is from S. avermitilis, having a sequence set forth in GenBank accession NP_821730, or a variant thereof (See e.g., US Pat. Appln. Publ. No. 2010/0267089 A1). In some additional embodiments, the EG is a Thermoascus aurantiacus EG or variant thereof. In some further embodiments, the EG is an endogenous EG from a bacteria, a yeast, or a filamentous fungus other than M. thermophila. Indeed, it is contemplated that any suitable EG will find use in combination with the GH61 proteins provided herein. It is not intended that the present invention be limited to any specific EG.

[0161] Beta-glucosidases (BGL), comprise a group of cellulase enzymes classified as E.C. 3.2.1.21. These enzymes hydrolyze cellobiose to glucose. In some embodiments, the BGL is an endogenous *M. thermophila* enzyme, or a variant thereof (See e.g., US Pat. Appln. Publ. No. 2011/0129881 A1; and US Pat. Appln. Publ. No. 2011/0124058 A1). In some alternative embodiments, the BGL is from *Azospirillum irakense* (CelA), or a variant thereof (See e.g., US Pat. Appln. Publ. No. 2011/0114744 A1; and PCT/US2010/038902). Indeed, it is contemplated that any suitable BGL will find use in combination with the GH61

proteins provided herein. It is not intended that the present invention be limited to any specific BGL.

[0162] Cellobiohydrolases comprise a group of cellulase enzymes classified as E.C. 3.2.1.91. Type 1 cellobiohydrolase (CBH1) hydrolyzes cellobiose processively from the reducing end of cellulose chains. Type 2 cellobiohydrolase (CBH2) hydrolyzes cellobiose processively from the nonreducing end of cellulose chains. In some embodiments, the CBH1 and/or CBH2 enzymes used in the present invention are endogenous to M. thermophila, while in some other embodiments, the CBH1 and/or CBH2 enzymes used in the present invention are obtained from bacteria, yeast, and/or a filamentous fungus other than M. thermophila. Indeed, it is contemplated that any suitable CBHs will find use in combination with the GH61 proteins provided herein. It is not intended that the present invention be limited to any specific CBHs. The invention provides compositions comprising a GH61 variant protein in combination with at least one, at least two, at least three, or more than three cellulases selected from EG, BGL, CBH1, CBH2, xylosidase, and/or xylanase. In some embodiments, enzymes are purified or partly purified before combining them, so that the combined mass of the GH61, EG, BGL, CBH1 and CBH2 is at least about 50% or at least about 70% of the total cell-free protein in compositions.

[0163] In addition to one or more cellulase enzymes such as those listed above, in some embodiments, GH61 variant enzymes are combined with other enzymes to produce mixtures with industrial applicability. Such combinations are useful, for example, in rendering a cellulose-containing source into an intermediate that is more amenable to hydrolysis by the cellulase enzymes in the mixture. For example, in some embodiments, enzymes are selected to digest or hydrolyze other components of a particular cellulosic biomass, such as hemicellulose, arabinogalactan, pectin, rhamnogalacturonan and/or lignin.

[0164] In some embodiments, the compositions comprise enzymes selected from endoxylanases (EC 3.2.1.8); β-xylosidases (EC 3.2.1.37); alpha-L-arabinofuranosidases (EC 3.2.1.55); alpha-glucuronidases (EC 3.2.1.139); acetylxylanesterases (EC 3.1.1.72); feruloyl esterases (EC 3.1.1.73); coumaroyl esterases (EC 3.1.1.73); alpha-galactosidases (EC 3.2.1.22); beta-galactosidases (EC 3.2.1.23); beta-mannanases (EC 3.2.1.78); beta-mannosidases (EC 3.2.1.25); endo-polygalacturonases (EC 3.2.1.15); pectin methyl esterases (EC 3.1.1.11); endo-galactanases (EC 3.2.1.89); pectin acetyl esterases (EC 3.1.1.6); endo-pectin lyases (EC 4.2.2.10); pectate lyases (EC 4.2.2.2); alpha rhamnosidases (EC 3.2.1.40); exo-poly-alpha-galacturonosidase (EC 3.2.1. 82); 1,4-alpha-galacturonidase (EC 3.2.1.67); exopolygalacturonate lyases (EC 4.2.2.9); rhamnogalacturonan endolyases EC (4.2.2.B3); rhamnogalacturonan acetylesterases (EC 3.2.1.B11); rhamnogalacturonan galacturonohydrolases (EC 3.2.1.B11); endo-arabinanases (EC 3.2.1.99); laccases (EC 1.10.3.2); manganese-dependent peroxidases (EC 1.10. 3.2); amylases (EC 3.2.1.1), glucoamylases (EC 3.2.1.3), proteases, lipases, and lignin peroxidases (EC 1.11.1.14). Any combination of one, two, three, four, five, or more than five enzymes find use in the compositions of the present invention.

[0165] Cellulase mixtures for efficient enzymatic hydrolysis of cellulose are known (See e.g., Viikari et al., Adv. Biochem. Eng. Biotechnol., 108:121-45 [2007]; and US Pat. Publns. 2009/0061484; US 2008/0057541; and US 2009/

0209009, each of which is incorporated herein by reference). In some embodiments, mixtures of purified naturally occurring or recombinant enzymes are combined with cellulosic feedstock or a product of cellulose hydrolysis. In some embodiments, one or more cell populations, each producing one or more naturally occurring or recombinant cellulases, are combined with cellulosic feedstock or a product of cellulose hydrolysis.

[0166] In some embodiments, the GH61 variant polypeptides of the present invention are present in mixtures comprising enzymes other than cellulases that degrade cellulose, hemicellulose, pectin, and/or lignocellulose.

[0167] In some embodiments, the present invention provides at least one GH61 variant and at least one endoxylanase. Endoxylanases (EC 3.2.1.8) catalyze the endo hydrolysis of 1,4-beta-D-xylosidic linkages in xylans. This enzyme may also be referred to as endo-1,4-beta-xylanase or 1,4-beta-D-xylan xylanohydrolase. In some embodiments, an alternative is EC 3.2.1.136, a glucuronoarabinoxylan endoxylanase, an enzyme that is able to hydrolyze 1,4 xylosidic linkages in glucuronoarabinoxylans.

[0168] In some embodiments, the present invention provides at least one GH61 variant and at least one beta-xylosidase. Beta-xylosidases (EC 3.2.1.37) catalyze the hydrolysis of 1,4-beta-D-xylans, to remove successive D-xylose residues from the non-reducing termini. This enzyme may also be referred to as xylan 1,4-beta-xylosidase, 1,4-beta-D-xylan xylohydrolase, exo-1,4-beta-xylosidase or xylobiase.

[0169] In some embodiments, the present invention provides at least one GH61 variant and at least one α -L-arabinofuranosidase. Alpha-L-arabinofuranosidases (EC 3.2.1.55) catalyze 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-arabinofuranosidase, alpha-L-arabinofuranosidase, alpha-L-arabinofuranosidase, alpha-L-arabinofuranosidase, alpha-L-arabinofuranosidase and alpha-L-arabinanase.

[0170] In some embodiments, the present invention provides at least one GH61 variant and at least one alphaglucuronidase. Alpha-glucuronidases (EC 3.2.1.139) catalyze the hydrolysis of an alpha-D-glucuronoside to D-glucuronate and an alcohol.

[0171] In some embodiments, the present invention provides at least one GH61 variant and at least one acetylxylanesterase. Acetylxylanesterases (EC 3.1.1.72) catalyze the hydrolysis of acetyl groups from polymeric xylan, acetylated xylose, acetylated glucose, alpha-napthyl acetate, and p-nitrophenyl acetate.

[0172] In some embodiments, the present invention provides at least one GH61 variant and at least one feruloyl esterase. Feruloyl esterases (EC 3.1.1.73) have 4-hydroxy-3-methoxycinnamoyl-sugar hydrolase activity (EC 3.1.1.73) that catalyzes the hydrolysis of the 4-hydroxy-3-methoxycinnamoyl (feruloyl) group from an 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.

[0173] In some embodiments, the present invention provides at least one GH61 variant and at least one coumaroyl esterase. Coumaroyl esterases (EC 3.1.1.73) catalyze a reaction of the form: coumaroyl-saccharide+H₂O=coumarate+saccharide. In some embodiments, the saccharide is an oligosaccharide or a polysaccharide. This enzyme may also be referred to as trans-4-coumaroyl esterase, trans-p-coumaroyl esterase, p-coumaroyl esterase or p-coumaric acid esterase. The enzyme also falls within EC 3.1.1.73; it may also be referred to as a "feruloyl esterase."

[0174] In some embodiments, the present invention provides at least one GH61 variant and at least one alphagalactosidase. Alpha-galactosidases (EC 3.2.1.22) catalyze the hydrolysis of terminal, non-reducing alpha-D-galactose residues in alpha-D-galactosides, including galactose oligosaccharides, galactomannans, galactans and arabinogalactans. This enzyme may also be referred to as "melibiase." [0175] In some embodiments, the present invention provides at least one GH61 variant and at least one betagalactosidase. Beta-galactosidases (EC 3.2.1.23) catalyze the hydrolysis of terminal non-reducing beta-D-galactose residues in beta-D-galactosides. In some embodiments, the polypeptide is also capable of hydrolyzing alpha-L-arabino-

[0176] In some embodiments, the present invention provides at least one GH61 variant and at least one betamannanase. Beta-mannanases (EC 3.2.1.78) catalyze the random hydrolysis of 1,4-beta-D-mannosidic linkages in mannans, galactomannans and glucomannans. This enzyme may also be referred to as "mannan endo-1,4-beta-mannosidase" or "endo-1,4-mannanase."

sides. This enzyme may also be referred to as exo-(1->4)-

beta-D-galactanase or lactase.

[0177] In some embodiments, the present invention provides at least one GH61 variant and at least one betamannosidase. Beta-mannosidases (EC 3.2.1.25) catalyze the hydrolysis of terminal, non-reducing beta-D-mannose residues in beta-D-mannosides. This enzyme may also be referred to as mannanase or mannase.

[0178] In some embodiments, the present invention provides at least one GH61 variant and at least one glucoamylase. Glucoamylases (EC 3.2.1.3) catalyzes the release of D-glucose from non-reducing ends of oligo- and polysaccharide molecules. Glucoamylase is also generally considered a type of amylase known as amylo-glucosidase.

[0179] In some embodiments, the present invention provides at least one GH61 variant and at least one amylase. Amylases (EC 3.2.1.1) are starch cleaving enzymes that degrade starch and related compounds by hydrolyzing the alpha-1,4 and/or alpha-1,6 glucosidic linkages in an endo- or an exo-acting fashion. Amylases include alpha-amylases (EC 3.2.1.1); beta-amylases (3.2.1.2), amylo-amylases (EC 3.2.1.3), alpha-glucosidases (EC 3.2.1.20), pullulanases (EC 3.2.1.41), and isoamylases (EC 3.2.1.68). In some embodiments, the amylase is an alpha-amylase.

[0180] In some embodiments one or more enzymes that degrade pectin are included in enzyme mixtures that comprise at least one GH61 variant of the present invention. Pectinases catalyze the hydrolysis of pectin into smaller units such as oligosaccharide or monomeric saccharides. In some embodiments, the enzyme mixtures comprise any pectinase, for example an endo-polygalacturonase, a pectin methyl esterase, an endo-galactanase, a pectin acetyl esterase, an endo-pectin lyase, pectate lyase, alpha rhamnosidase, an exo-galacturonase, an exo-polygalacturonate

lyase, a rhamnogalacturonan hydrolase, a rhamnogalacturonan lyase, a rhamnogalacturonan acetyl esterase, a rhamnogalacturonan galacturonohydrolase and/or a xylogalacturonase.

[0181] In some embodiments, the present invention provides at least one GH61 variant and at least one endopolygalacturonase. Endo-polygalacturonases (EC 3.2.1.15) catalyze the random hydrolysis of 1,4-alpha-D-galactosiduronic linkages in pectate and other galacturonass. This enzyme may also be referred to as "polygalacturonase pectin depolymerase," "pectinase," "endopolygalacturonase," "pectolase," "pectin hydrolase," "pectin polygalacturonase," "poly-alpha-1,4-galacturonide glycanohydrolase," "endogalacturonase," "endo-D-galacturonase" or "poly(1,4-alpha-D-galacturonide) glycanohydrolase."

[0182] In some embodiments, the present invention provides at least one GH61 variant and at least one pectin methyl esterase. Pectin methyl esterases (EC 3.1.1.11) catalyze the reaction: pectin+n $\rm H_2O=n$ methanol+pectate. The enzyme may also been known as "pectin esterase," "pectin demethoxylase," "pectin methylesterase," "pectase," "pectinoesterase," or "pectin pectylhydrolase."

[0183] In some embodiments, the present invention provides at least one GH61 variant and at least one endogalactanase. Endo-galactanases (EC 3.2.1.89) catalyze the endohydrolysis of 1,4-beta-D-galactosidic linkages in arabinogalactans. The enzyme may also be known as "arabinogalactan endo-1,4-beta-galactosidase," "endo-1,4-beta-galactanase," "galactanase," "arabinogalactanase," or "arabinogalactan 4-beta-D-galactanohydrolase."

[0184] In some embodiments, the present invention provides at least one GH61 variant and at least one pectin acetyl esterase. Pectin acetyl esterases catalyze the deacetylation of the acetyl groups at the hydroxyl groups of GaIUA residues of pectin.

[0185] In some embodiments, the present invention provides at least one GH61 variant and at least one endo-pectin lyase. Endo-pectin lyases (EC 4.2.2.10) catalyze the eliminative cleavage of (1 \rightarrow 4)-alpha-D-galacturonan methyl ester to give oligosaccharides with 4-deoxy-6-O-methyl- α -D-galact-4-enuronosyl groups at their non-reducing ends. The enzyme may also be known as "pectin lyase," "pectin trans-eliminase," "endo-pectin lyase," "polymethylgalacturonic transeliminase," "endo-pectin methyltranseliminase," "pectolyase," "PL," "PNL," "PMGL," or "(1 \rightarrow 4)-6-O-methylalpha-D-galacturonan lyase."

[0186] In some embodiments, the present invention provides at least one GH61 variant and at least one pectate lyase. Pectate lyases (EC 4.2.2.2) catalyze the eliminative cleavage of (1→4)-alpha-D-galacturonan to give oligosaccharides with 4-deoxy-alpha-D-galact-4-enuronosyl groups at their non-reducing ends. The enzyme may also be known "polygalacturonic transeliminase," "pectic acid transeliminase," "polygalacturonate lyase," "endopectin methyltranseliminase," "pectate transeliminase," "endogalacturonate transeliminase," "pectic acid lyase," "PGA lyase," "PPase-N," "endo-alpha-1,4-polygalacturonic acid lyase," "pectin trans-eliminase," "polygalacturonic acid lyase," "pectin trans-eliminase," "polygalacturonic acid trans-eliminase," "polygalacturonic acid trans-eliminase," or "(1→4)-alpha-D-galacturonan lyase."

[0187] In some embodiments, the present invention provides at least one GH61 variant and at least one alpha-

rhamnosidase. Alpha-rhamnosidases (EC 3.2.1.40) catalyze the hydrolysis of terminal non-reducing alpha-L-rhamnose residues in alpha-L-rhamnosides or alternatively in rhamnogalacturonan. This enzyme may also be known as "alpha-L-rhamnosidase T," "alpha-L-rhamnosidase N," or "alpha-L-rhamnoside rhamnohydrolase."

[0188] In some embodiments, the present invention provides at least one GH61 variant and at least one exogalacturonase. Exo-galacturonases (EC 3.2.1.82) hydrolyze pectic acid from the non-reducing end, releasing digalacturonate. The enzyme may also be known as "exo-polyalpha-galacturonosidase," "exopolygalacturonosidase," or "exopolygalacturanosidase."

[0189] In some embodiments, the present invention provides at least one GH61 variant and at least one β -galacturan 1,4-alpha galacturonidase. Exo-galacturonases (EC 3.2.1. 67) catalyze a reaction of the following type: (1,4- α -D-galacturonide)n+H $_2$ O=(1,4- α -D-galacturonide)n-i+D-galacturonate. The enzyme may also be known as "poly [1->4) alpha-D-galacturonide] galacturonohydrolase," "exo-polygalacturonase," "poly(galacturonate) hydrolase," "exo-D-galacturonase," "exo-D-galacturonase," "exo-D-galacturonase," "poly(1,4-alpha-D-galacturonide) galacturonhydrolase."

[0190] In some embodiments, the present invention provides at least one GH61 variant and at least one exopolygalacturonate lyase. Exopolygalacturonate lyases (EC 4.2. 2.9) catalyze eliminative cleavage of 4-(4-deoxy-alpha-D-galact-4-enuronosyl)-D-galacturonate from the reducing end of pectate (i.e., de-esterified pectin). This enzyme may be known as "pectate disaccharide-lyase," "exopectate exolyase," "exopectic acid transeliminase," "exopectate lyase," "exopolygalacturonic acid-trans-eliminase," "PATE," "exo-PATE," "exo-PGL," or "(1→4)-alpha-D-galacturonan reducing-end-disaccharide-lyase."

[0191] In some embodiments, the present invention provides at least one GH61 variant and at least one rhamnogalacturonanase. Rhamnogalacturonanases hydrolyze the linkage between galactosyluronic acid and rhamnopyranosyl in an endo-fashion in strictly alternating rhamnogalacturonan structures, consisting of the disaccharide [(1,2-alpha-L-rhamnoyl-(1,4)-alpha-galactosyluronic acid].

[0192] In some embodiments, the present invention provides at least one GH61 variant and at least one rhamnogalacturonan lyase. Rhamnogalacturonan lyases cleave alpha-L-Rhap-(1→4)-alpha-D-GalpA linkages in an endo-fashion in rhamnogalacturonan by beta-elimination.

[0193] In some embodiments, the present invention provides at least one GH61 variant and at least one rhamnogalacturonan acetyl esterase. Rhamnogalacturonan acetyl esterases catalyze the deacetylation of the backbone of alternating rhamnose and galacturonic acid residues in rhamnogalacturonan.

[0194] In some embodiments, the present invention provides at least one GH61 variant and at least one rhamnogalacturonan galacturonohydrolase. Rhamnogalacturonan galacturonohydrolases hydrolyze galacturonic acid from the non-reducing end of strictly alternating rhamnogalacturonan structures in an exo-fashion. This enzyme may also be known as "xylogalacturonan hydrolase."

[0195] In some embodiments, the present invention provides at least one GH61 variant and at least one endoarabinanase. Endo-arabinanases (EC 3.2.1.99) catalyze endohydrolysis of 1,5-alpha-arabinofuranosidic linkages in

1,5-arabinans. The enzyme may also be known as "endo-arabinase," "arabinan endo-1,5-alpha-L-arabinosidase," "endo-1,5-alpha-L-arabinanase," "endo-alpha-1,5-arabanase," "endo-arabanase," or "1,5-alpha-L-arabinan 1,5-alpha-L-arabinanohydrolase."

[0196] In some embodiments, the present invention provides at least one GH61 variant and at least one enzyme that participates in lignin degradation in an enzyme mixture. Enzymatic lignin depolymerization can be accomplished by lignin peroxidases, manganese peroxidases, laccases, and/or cellobiose dehydrogenases (CDH), often working in synergy. These extracellular enzymes are often referred to as "lignin-modifying enzymes" or "LMEs." Three of these enzymes comprise two glycosylated heme-containing peroxidases, namely lignin peroxidase (LIP), Mn-dependent peroxidase (MNP), and copper-containing phenoloxidase laccase (LCC).

[0197] In some embodiments, the present invention provides at least one GH61 variant and at least one laccase. Laccases are copper containing oxidase enzymes that are found in many plants, fungi and microorganisms. Laccases are enzymatically active on phenols and similar molecules and perform a one electron oxidation. Laccases can be polymeric and the enzymatically active form can be a dimer or trimer.

[0198] In some embodiments, the present invention provides at least one GH61 variant and at least one Mndependent peroxidase. The enzymatic activity of Mn-dependent peroxidase (MnP) in is dependent on Mn2+. Without being bound by theory, it has been suggested that the main role of this enzyme is to oxidize Mn2+ to Mn3+(See e.g, Glenn et al., Arch. Biochem. Biophys., 251:688-696 [1986]). Subsequently, phenolic substrates are oxidized by the Mn3+ generated.

[0199] In some embodiments, the present invention provides at least one GH61 variant and at least one lignin peroxidase. Lignin peroxidase is an extracellular heme peroxidase that catalyses the oxidative depolymerization of dilute solutions of polymeric lignin in vitro. Some of the substrates of LiP, most notably 3,4-dimethoxybenzyl alcohol (veratryl alcohol, VA), are active redox compounds that have been shown to act as redox mediators. VA is a secondary metabolite produced at the same time as LiP by ligninolytic cultures of *P. chrysosporium* and without being bound by theory, has been proposed to function as a physiological redox mediator in the LiP-catalyzed oxidation of lignin in vivo (See e.g., Harvey, et al., FEBS Lett., 195:242-246 [1986]).

[0200] In some embodiments, the present invention provides at least one GH61 variant and at least one protease, amylase, glucoamylase, and/or a lipase that participates in cellulose degradation.

[0201] As used herein, the term "protease" includes enzymes that hydrolyze peptide bonds (peptidases), as well as enzymes that hydrolyze bonds between peptides and other moieties, such as sugars (glycopeptidases). Many proteases are characterized under EC 3.4, and are suitable for use in the invention. Some specific types of proteases include, cysteine proteases including pepsin, papain and serine proteases including chymotrypsins, carboxypeptidases and metalloendopeptidases.

[0202] As used herein, the term "lipase" includes enzymes that hydrolyze lipids, fatty acids, and acylglycerides, including phospoglycerides, lipoproteins, diacylglycerols, and the

like. In plants, lipids are used as structural components to limit water loss and pathogen infection. These lipids include waxes derived from fatty acids, as well as cutin and suberin. [0203] In some additional embodiments, the present invention provides at least one GH61 variant and at least one expansin or expansin-like protein, such as a swollenin (See e.g., Salheimo et al., Eur. J. Biochem., 269:4202-4211 [2002]) or a swollenin-like protein. Expansins are implicated in loosening of the cell wall structure during plant cell growth. Expansins have been proposed to disrupt hydrogen bonding between cellulose and other cell wall polysaccharides without having hydrolytic activity. In this way, they are thought to allow the sliding of cellulose fibers and enlargement of the cell wall. Swollenin, an expansin-like protein contains an N-terminal Carbohydrate Binding Module Family 1 domain (CBD) and a C-terminal expansin-like domain. In some embodiments, an expansin-like protein or swollenin-like protein comprises one or both of such domains and/or disrupts the structure of cell walls (such as disrupting cellulose structure), optionally without producing detectable amounts of reducing sugars.

[0204] In some embodiments, the present invention provides at least one GH61 variant and at least one polypeptide product of a cellulose integrating protein, scaffoldin or a scaffoldin-like protein, for example CipA or CipC from Clostridium thermocellum or Clostridium cellulolyticum, respectively. Scaffoldins and cellulose integrating proteins are multi-functional integrating subunits which may organize cellulolytic subunits into a multi-enzyme complex. This is accomplished by the interaction of two complementary classes of domains (i.e. a cohesion domain on scaffoldin and a dockerin domain on each enzymatic unit). The scaffoldin subunit also bears a cellulose-binding module that mediates attachment of the cellulosome to its substrate. A scaffoldin or cellulose integrating protein for the purposes of this invention may comprise one or both such domains.

[0205] In some embodiments, the present invention provides at least one GH61 variant and at least one cellulose induced protein or modulating protein, for example as encoded by a cip1 or cip2 gene or similar genes from *Trichoderma reesei* (See e.g., Foreman et al., J. Biol. Chem., 278:31988-31997 [2003]).

[0206] In some embodiments, the present invention provides at least one GH61 variant and at least one member of each of the classes of the polypeptides described above, several members of one polypeptide class, or any combination of these polypeptide classes to provide enzyme mixtures suitable for various uses.

[0207] In some embodiments, the enzyme mixture comprises other types of cellulases, selected from but not limited to cellobiohydrolase, endoglucanase, beta-glucosidase, and glycoside hydrolase 61 protein (GH61) cellulases. These enzymes may be wild-type or recombinant enzymes. In some embodiments, the cellobiohydrolase is a type 1 cellobiohydrolase (e.g., a *T. reesei* cellobiohydrolase I). In some embodiments, the endoglucanase comprises a catalytic domain derived from the catalytic domain of a Streptomyces avermitilis endoglucanase (See e.g., US Pat. Appln. Pub. No. 2010/0267089; U.S. Pat. No. 8,206,960; and U.S. Pat. No. 8,088,608, each of which is incorporated herein by reference). In some embodiments, at least one cellulase in the mixtures of the present invention is derived from Acidothermus cellulolyticus, Thermobifida fusca, Humicola grisea, Myceliophthora thermophila, Chaetomium thermophilum, Acremonium sp., Thielavia sp, Trichoderma reesei, Aspergillus sp., or a Chrysosporium sp. In some embodiments, cellulase enzymes of the cellulase mixture work together resulting in decrystallization and hydrolysis of the cellulose from a biomass substrate to yield fermentable sugars, such as but not limited to glucose.

[0208] Some cellulase mixtures for efficient enzymatic hydrolysis of cellulose are known (See e.g., Viikari et al., Adv. Biochem. Eng. Biotechnol., 108:121-45 [2007]; and US Pat. Appln. Publn. Nos. US 2009/0061484, US 2008/0057541, and US 2009/0209009, each of which is incorporated herein by reference in their entireties). In some embodiments, mixtures of purified naturally occurring or recombinant enzymes are combined with cellulosic feedstock or a product of cellulose hydrolysis. Alternatively or in addition, one or more cell populations, each producing one or more naturally occurring or recombinant cellulase, are combined with cellulosic feedstock or a product of cellulose hydrolysis.

[0209] In some embodiments, the enzyme mixture comprises commercially available purified cellulases. Commercial cellulases are known and available (e.g., C2730 cellulase from *Trichoderma reesei* ATCC No. 25921 available from Sigma-Aldrich, Inc.) Any suitable commercially available enzyme finds use in the present invention.

[0210] In some embodiments, the enzyme mixture comprises at least one isolated GH61 variant as provided herein and at least one or more isolated enzymes, including but not limited to at least one isolated CBH1a, isolated CBH2b, isolated endoglucanase (EG) (e.g., EG2 and/or EG1), and/or isolated beta-glucosidase (BGL). In some embodiments, at least 5%, at least 10%, at last 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% of the enzyme mixture is GH61. In some embodiments, the enzyme mixture further comprises a cellobiohydrolase type 1a (e.g., CBH1a), and GH61, wherein the enzymes together comprise at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or at least 80% of the enzyme mixture. In some embodiments, the enzyme mixture further comprises a beta-glucosidase (BGL), GH61, and CBH, wherein the three enzymes together comprise at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% of the enzyme mixture. In some embodiments, the enzyme mixture further comprises an endoglucanase (EG), GH61, CBH2b, CBH1a, BGL, wherein the five enzymes together comprise at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% of the enzyme mixture. In some embodiments, the enzyme mixture comprises GH61, CBH2b, CBH1, BGL, and at least one EG, in any suitable proportion for the desired reaction.

[0211] In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight (wherein the total weight of the cellulases is 100%): about 20%-10% of GH61, about 20%-10% of GH61, about 30%-25% of CBH1a, about 10%-30% of GH61, about 20%-10% of EG, and about 20%-25% of CBH2b. In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight: about 20%-10% of GH61, about 25%-

15% of BGL, about 20%-30% of CBH1a, about 10%-15% of EG, and about 25%-30% of CBH2b. In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight: about 30%-20% of GH61, about 15%-10% of BGL, about 25%-10% of CBH1a, about 25%-10% of CBH2b, about 15%-10% of EG. In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight: about 40-30% of GH61, about 15%-10% of BGL, about 20%-10% of CBH1a, about 20%-10% of CBH2b, and about 15%-10% of EG.

[0212] In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight: about 50-40% of GH61, about 15%-10% of BGL, about 20%-5% of CBH1a, about 15%-10% of CBH2b, and about 10%-5% of EG. However, in some embodiments, the enzyme mixture composition comprises no EG (e.g., EG2). In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight: about 10%-15% of GH61, about 20%-25% of BGL, about 30%-20% of CBH1a, about 15%-5% of EG, and about 25%-35% of CBH2b. In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight: about 15%-5% of GH61, about 15%-10% of BGL, about 45%-30% of CBH1a, about 25%-5% of EG, and about 40%-10% of CBH2b. In some embodiments, the enzyme mixture composition comprises isolated cellulases in the following proportions by weight: about 10% of GH61, about 15% of BGL, about 40% of CBH1a, about 25% of EG, and about 10% of CBH2b.

[0213] In some embodiments, the enzyme mixtures provided herein further comprise at least one xylan-active enzyme and/or at least one ester-active enzyme. In some embodiments, the enzyme mixture compositions comprise about 0-25% xylanase (e.g., about 2%-5%, about 1%-10%, about 10%-15%, about 15%-25%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% xylanase) by weight. In some embodiments, the enzyme mixture compositions comprise about 0-15% xylosidase (e.g., about 2%-5%, about 1%-10%, about 10%-15%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% xylosidase) by weight. In some embodiments, the enzyme mixture compositions comprise about 0-15% esterase (e.g., about 2%-5%, about 1%-10%, about 10%-15%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% esterase) by weight. It is contemplated that any suitable combination of enzymes and suitable enzyme concentrations will find use in the present invention, as applied using various saccharification reactions and conditions.

[0214] In some embodiments, the enzyme component comprises more than one CBH1a, CBH2b, EG, BGL, and/or GH61 variant enzyme (e.g., 2, 3 or 4 different enzymes), in any suitable combination. In some embodiments, an enzyme mixture composition of the invention further comprises at least one additional protein and/or enzyme. In some embodiments, enzyme mixture compositions of the present invention further comprise at least one additional enzyme other than at least one GH61 variant, BGL, CBH1a, wild-type

GH61, and/or CBH2b. In some embodiments, the enzyme mixture compositions of the invention further comprise at least one additional cellulase, other than at least one GH61 variant, BGL, CBH1a, GH61, and/or CBH2b as described herein. In some embodiments, the GH61 polypeptide variant of the invention is also present in mixtures with noncellulase enzymes that degrade cellulose, hemicellulose, pectin, and/or lignocellulose.

[0215] In some embodiments, GH61 polypeptide variant of the present invention is used in combination with other optional ingredients such as at least one buffer, surfactant, and/or scouring agent. In some embodiments, at least one buffer is used with the GH61 polypeptide variant of the present invention (optionally combined with other enzymes) to maintain a desired pH within the solution in which the GH61 variant is employed. The exact concentration of buffer employed depends on several factors which the skilled artisan can determine. Suitable buffers are well known in the art. In some embodiments, at least one surfactant is used in with the GH61 variant of the present invention. Suitable surfactants include any surfactant compatible with the GH61 variant and, optionally, with any other enzymes being used in the mixture. Exemplary surfactants include, but are not limited to anionic, non-ionic, and ampholytic surfactants. Suitable anionic surfactants include, but are not limited to, linear or branched alkylbenzenesulfonates; alkyl or alkenyl ether sulfates having linear or branched alkyl groups or alkenyl groups; alkyl or alkenyl sulfates; olefinsulfonates; alkanesulfonates, and the like. Suitable counter ions for anionic surfactants include, for example, alkali metal ions, such as sodium and potassium; alkaline earth metal ions, such as calcium and magnesium; ammonium ion; and alkanolamines having from 1 to 3 alkanol groups of carbon number 2 or 3. Ampholytic surfactants suitable for use in the practice of the present invention include, for example, quaternary ammonium salt sulfonates, betaine-type ampholytic surfactants, and the like. Suitable nonionic surfactants generally include polyoxalkylene ethers, as well as higher fatty acid alkanolamides or alkylene oxide adduct thereof, fatty acid glycerine monoesters, and the like. Mixtures of surfactants also find use in the present invention, as is known in the art.

Exemplary Mixtures of Cellulolytic Enzymes and Cofactors

[0216] As a further guide to the reader, yet without implying any limitation in the practice of the present invention, exemplary mixtures of components that may be used as catalysts in a saccharification reaction to generate fermentable sugars from a cellulosic substrate are provided herein. Concentrations are given in wt/vol of each component in the final reaction volume with the cellulose substrate. Also provided are percentages of each component (wt/wt) in relation to the total mass of the components that are listed for addition into each mixture (the "total protein"). This may be a mixture of purified enzymes and/or enzymes in a culture supernatant.

[0217] By way of example, the invention embodies mixtures that comprise at least four, at least five, or all six of the following components. In some embodiments, cellobiohydrolase 1 (CBH1) finds use; in some embodiments CBH1 is present at a concentration of about 0.14 to about 0.23 g/L (about 15% to about 25% of total protein). Exemplary CBH1 enzymes include, but are not limited to *T. emersonii* CBH1 (wild-type) (e.g., SEQ ID NO:125), *M. thermophila* CBH1a

(wild-type) (e.g., SEQ ID NO:128), and the variants CBH1a-983 (SEQ ID NO:134) and CBH1a-145 (SEQ ID NO:131). In some embodiments, cellobiohydrolase 2 (CBH2) finds use; in some embodiments, CBH2 is present at a concentration of about 0.14 to about 0.23 g/L (about 15% to about 25% of total protein). Exemplary CBH2 enzymes include but are not limited to CBH2b from M. thermophila (wild-type) (e.g., SEQ ID NO:137). In some embodiments, endoglucanase 2 (EG2) finds use; in some embodiments, EG2 is present at a concentration of 0 to about 0.05 g/L (0 to about 5% of total protein). Exemplary EGs include, but are not limited to M. thermophila EG2 (wildtype) (e.g., SEQ ID NO:113). In some further embodiments, endoglucanase 1 (EG1) finds use; in some embodiments, EG1 is present at a concentration of about 0.05 to about 0.14 g/L (about 5% to about 15% of total protein). Exemplary EG1s include, but are not limited to M. thermophila EG1b (wild-type) (e.g., SEQ ID NO:110). In some embodiments, beta-glucosidase (BGL) finds use in the present invention; in some embodiments, BGL is present at a concentration of about 0.05 to about 0.09 g/L (about 5% to about 10% of total protein). Exemplary beta-glucosidases include, but are not limited to M. thermophila BGL1 (wild-type) (e.g., SEQ ID NO:116), variant BGL-900 (SEQ ID NO:122), and variant BGL-883 (SEQ ID NO:119). In some further embodiments, GH61 protein and/or protein variants find use; in some embodiments, GH61 enzymes are present at a concentration of about 0.23 to about 0.33 g/L (about 25% to about 35% of total protein). Exemplary GH61s include, but are not limited to M. thermophila GH61a wild-type (SEQ ID NO:2), Variant 1 (SEQ ID NO:5), Variant 5 (SEQ ID NO:8) and/or Variant 9 (SEQ ID NO:11), and/or any other GH61a variant proteins, as well as any of the other GH61 enzymes (e.g., GH61b, GH61c, GH61d, GH61e, GH61f, GH61g, GH61h, GH161i, GH61j, GH61k, GH61l, GH61m, GH61n, GH61o, GH61p, GH61q, GH61r, GH61s, GH61t, GH61u, GH61v, GH61w, GH61x, and/or GH61y) as provided herein.

[0218] In some embodiments, one, two or more than two enzymes are present in the mixtures of the present invention. In some embodiments, GH61p is present at a concentration of about 0.05 to about 0.14 g/L (e.g, about 1% to about 15% of total protein). Exemplary M. thermophila GH61p enzymes include those set forth in SEQ ID NOS:70 and 73. In some embodiments, GH61f is present at a concentration of about 0.05 to about 0.14 g/L (about 1% to about 15% of total protein). An exemplary M. thermophila GH61f is set forth in SEQ ID NO:29. In some additional embodiments, at least one additional GH61 enzyme provided herein (e.g., GH61b, GH61c, GH61d, GH61e, GH61g, GH61h, GH61i, GH61j, GH61k, GH61l, GH61m, GH61n, GH61n, GH61o, GH61q, GH61r, GH61s, GH61t, GH61u, GH61v, GH61w, GH61x, and/or GH61y, finds use at an appropriate concentration (e.g., about 0.05 to about 0.14 g/L [about 1% to about 15% of total protein]).

[0219] In some embodiments, at least one xylanase at a concentration of about 0.05 to about 0.14 g/L (about 1% to about 15% of total protein) finds use in the present invention. Exemplary xylanases include but are not limited to the *M. thermophila* xylanase-3 (SEQ ID NO:149), xylanase-2 (SEQ ID NO:152), xylanase-1 (SEQ ID NO:155), xylanase-6 (SEQ ID NO:158), and xylanase-5 (SEQ ID NO:161).

[0220] In some additional embodiments, at least one beta-xylosidase at a concentration of about 0.05 to about $0.14~\rm g/L$

(e.g., about 1% to about 15% of total protein) finds use in the present invention. Exemplary beta-xylosidases include but are not limited to the *M. thermophila* beta-xylosidase (SEQ ID NO:164).

[0221] In still some additional embodiments, at least one acetyl xylan esterase at a concentration of about 0.05 to about 0.14 g/L (e.g., about 1% to about 15% of total protein) finds use in the present invention. Exemplary acetylxylan esterases include but are not limited to the *M. thermophila* acetylxylan esterase (SEQ ID NO:167).

[0222] In some further additional embodiments, at least one ferulic acid esterase at a concentration of about 0.05 to about 0.14 g/L (e.g., about 1% to about 15% of total protein) finds use in the present invention. Exemplary ferulic esterases include but are not limited to the *M. thermophila* ferulic acid esterase (SEQ ID NO:170).

[0223] In some embodiments, the enzyme mixtures comprise at least one GH61 variant protein as provided herein and at least one cellulase, including but not limited to any of the enzymes described herein. In some embodiments, the enzyme mixtures comprise at least one GH61 variant protein and at least one wild-type GH61 protein. In some embodiments, the enzyme mixtures comprise at least one GH61 variant protein and at least one non-cellulase enzyme. Indeed, it is intended that any combination of enzymes will find use in the enzyme compositions comprising at least one GH61 variant of the present invention.

[0224] The concentrations listed above are appropriate for a final reaction volume with the biomass substrate in which all of the components listed (the "total protein") is about 0.75 g/L, and the amount of glucan is about 93 g/L, subject to routine optimization. The user may empirically adjust the amount of each component and total protein for cellulosic substrates that have different characteristics and/or are processed at a different concentration. Any one or more of the components may be supplemented or substituted with variants with common structural and functional characteristics, as described below.

[0225] Without implying any limitation, the following mixtures further describe some embodiments of the present invention.

[0226] Some mixtures comprise CBH1a within a range of about 15% to about 30% total protein, typically about 20% to about 25%; CBH2 within a range of about 15% to about 30%, typically about 17% to about 22%; EG2 within a range of about 1% to about 10%, typically about 2% to about 5%; BGL1 within a range of about 5% to about 15%, typically about 8% to about 12%; GH61a within a range of about 10% to about 40%, typically about 20% to about 30%; EG1b within a range of about 5% to about 25%, typically about 10% to about 18%; and GH61f within a range of 0% to about 30%; typically about 5% to about 20%.

[0227] In some mixtures, exemplary BGL1s include the BGL1 variant 900 (SEQ ID NO:122) and/or variant 883 (SEQ ID NO:119). In some embodiments, other enzymes are *M. thermophila* wild-type: CBH1a (SEQ ID NO:128), CBH2b (SEQ ID NO:137), EG2 (SEQ ID NO:113), GH61a (SEQ ID NO:2), EG1b (SEQ ID NO:110) and GH61f (SEQ ID NO:29). Any one or more of the components may be supplemented or substituted with variants having common structural and functional characteristics with the component being substituted or supplemented, as described below. In a saccharification reaction, the amount of glucan is generally about 50 to about 300 g/L, typically about 75 to about 150

g/L. The total protein is about 0.1 to about 10 g/L, typically about 0.5 to about 2 g/L, or about 0.75 g/L.

[0228] Some mixtures comprise CBH1 within a range of about 10% to about 30%, typically about 15% to about 25%; CBH2b within a range of about 10% to about 25%, typically about 15% to about 20%; EG2 within a range of about 1% to about 10%, typically about 2% to about 5%; EG1b within a range of about 2% to about 25%, typically about 6% to about 14%; GH61a within a range of about 5% to about 50%, typically about 10% to about 35%; and BGL1 within a range of about 2% to about 15%, typically about 5% to about 12%. Also included is copper sulfate to generate a final concentration of Cu⁺⁺ of about 4 μM to about 200 μM, typically about 25 μM to about 60 μM. However, it is not intended that the added copper be limited to any particular concentration, as any suitable concentration finds use in the present invention and will be determined based on the reaction conditions.

[0229] In an additional mixture, an exemplary CBH1 is wild-type CBH1 from T. emersonii (SEQ ID NO:125), as well as wild-type M. thermophila CBH1a (SEQ ID NO:128), Variant 983 (SEQ ID NO:134), and Variant 145 (SEQ ID NO:131); exemplary CBH2 enzymes include the wild-type (SEQ ID NO:137), Variant 962 (SEQ ID NO:146), Variant 196 (SEQ ID NO:140), and Variant 287 (SEQ ID NO:143); an exemplary EG2 is the wild-type M. thermophila (SEQ ID NO:113); an exemplary EG1b is the wildtype (SEQ ID NO: 110); exemplary GH61a enzymes include wild-type M. thermophila (SEQ ID NO:2), Variant 1 (SEQ ID NO:5), Variant 5 (SEQ ID NO:11), and Variant 9 (SEQ ID NO:11); and exemplary BGLs include wild-type M. thermophila BGL (SEQ ID NO:116), Variant 883 (SEQ ID NO:119), and Variant 900 (SEQ ID NO:122). Any one or more of the components may be supplemented or substituted with other variants having common structural and functional characteristics with the component being substituted or supplemented, as described below. In a saccharification reaction, the amount of glucan is generally about 50 to about 300 g/L, typically about 75 to about 150 g/L. The total protein is about 0.1 to about 10 g/L, typically about 0.5 to about 2 g/L, or about 0.75 g/L.

[0230] Any or all of the components listed in the mixtures referred to above may be supplemented or substituted with variant proteins that are structurally and functionally related, as described herein.

[0231] In some embodiments, the CBH1 cellobiohydrolase used in mixtures of the present invention comprises at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to either SEQ ID NO:128 (M. thermophila), SEQ ID NO:125 (T. emersonii), or a fragment of either SEQ ID NO:128 or SEQ ID NO:125 having cellobiohydrolase activity, as well as variants of M. thermophila CBH1a (e.g., SEQ ID NO:131 and/or SEQ ID NO:133), and variant fragment(s) having cellobiohydrolase activity. Exemplary CBH1 enzymes include, but are not limited to those described in US Pat. Appln. Publn. No. 2012/0003703 A1, which is hereby incorporated herein by reference in its entirety for all purposes.

[0232] In some embodiments, the CBH2b cellobiohydrolase used in the mixtures of the present invention comprises at least about 80%, at least about 85%, at least about 90%,

at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO:127 or a fragment of SEQ ID NO:127, as well as at least one variant *M. thermophila* CBH2b enzyme (e.g., SEQ ID NO:140, 143, and/or 146) and/or variant fragment(s) having cellobiohydrolase activity. Exemplary CBH2b enzymes are described in U.S. Patent Appln. Ser. Nos. 61/479,800, 13/459,038, both of which are hereby incorporated herein by reference in their entirety for all purposes.

[0233] In some embodiments, the EG2 endoglucanase used in the mixtures of the present invention comprises at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO:113 or a fragment of SEQ ID NO:113 having endoglucanase activity. Exemplary EG2 enzymes are described in U.S. patent application Ser. No. 13/332,114, and WO 2012/088159, both of which are hereby incorporated herein by reference in their entirety for all purposes.

[0234] In some embodiments, the EG1b endoglucanase used in the mixtures of the present invention comprises at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO:110 or a fragment of SEQ ID NO:110 having endoglucanase activity.

[0235] In some embodiments, the BGL1 beta-glucosidase used the mixtures of the present invention comprises at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 99%, or 100% identical to SEQ ID NOS:116, 119, and/or 122, or a fragment of SEQ ID NOS:116, 119, and/or 122 having beta-glucosidase activity. Exemplary BGL1 enzymes include, but are not limited to those described in US Pat. Appln. Publ. No. 2011/0129881, WO 2011/041594, and US Pat. Appln. Publ. No. 2011/0124058 A1, all of which are hereby incorporated herein by reference in their entireties for all purposes.

[0236] In some embodiments, the GH61f protein used in the mixtures of the present invention comprises at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 99%, or 100% identical to SEQ ID NO:29, or a fragment of SEQ ID NO:29 having GH61 activity, assayed as described elsewhere in this disclosure.

[0237] In some embodiments, the GH61p protein used in the mixtures of the present invention comprises at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 99%, or 100% identical to SEQ ID NO:70, SEQ ID NO:73, or a fragment of such sequence having GH61p activity.

[0238] In some embodiments, the xylanase used in the mixtures of the present invention comprises at least about

80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO:149, SEQ ID NO:151, or a fragment of such sequence having xylanase activity.

GH61 Activity Assays

[0239] The cellulase enhancing activity of GH61 proteins of the invention can be determined using any suitable GH61 activity assay. For example, in some embodiments, a purified and/or recombinant GH61 protein of this invention is obtained, and then assayed for GH61 activity by combining it with cellulase enzymes in a saccharification reaction, and determining if there is an increase in glucose yield, as compared to the same saccharification reaction conducted without the GH61.

[0240] In one approach, GH61 activity can be assayed by combining a cellulosic substrate with cellulase enzymes (e.g., 5-10 mg total weight of cellulase enzymes per gram of substrate) in the presence and absence of GH61 protein. In some embodiments, the cellulase enzymes comprise a defined set of recombinant cellulase enzymes from *M. thermophila*.

[0241] In another approach, broth from a culture of wild-type *M. thermophila* is used (with and without supplementation with GH61 protein and/or GH61 variants). GH61 activity is evidenced by enhanced glucose yield in the presence of exogenous GH61 (i.e., beyond any enhancement resulting from endogenous GH61 in the broth). It is also possible to use a broth supplemented with one or more purified enzymes.

[0242] Suitable enzymes include isolated recombinant enzymes cloned from *M. thermophila*, including but not limited to EG, BGL, CBH1, and/or CBH2, in any combination suitable for the chosen substrate to yield a measurable product.

[0243] In one exemplary assay for measuring GH61 activity from *M. thermophila* derived GH61 proteins and variant proteins, the cellulase enzymes used are *M. thermophila* BGL1 (e.g., SEQ ID NOS:116, 119, and/or 122); See e.g., Badhan et al., Biores. Technol., 98:504-10 [2007]); *M. thermophila* CBH1 (SEQ ID NOS:128, 131, and/or 134); and *M. thermophila* CBH2 (SEQ ID NOS:137, 140, 143 and/or 146). In some embodiments, endoglucanase is also used, such as *M. thermophila* EG2 (SEQ ID NO:113; See e.g., Rosgaard et al., Prog., 22:493-8 [2006]; and Badhan et al., supra).

[0244] Alternatively, commercially available preparations comprising a mixture of cellulase enzymes may be used, such as LaminexTM and SpezymeTM (Genencor), RohamentTM (Rohm GmbH), and CelluzymeTM, CerefloTM and UltrafloTM (Novozymes).

[0245] Assays with cellulose enzymes are typically done at 50° C., but in some embodiments, other temperatures find use (e.g., 35, 45, 55, 60, or 65° C.). In some embodiments, the GH61 enzymes and any other desired enzymes are combined with the substrate and incubated so as to produce fermentable sugars. The sugars are then recovered and quantitated for yield of glucose. One suitable substrate is wheat straw (e.g., pre-treated wheat straw). Other cellulosic substrates listed in this disclosure may be used as an alternative, including corn stover pretreated with sulfuric acid (See e.g., U.S. Pat. No. 7,868,227). Assay methods are

known in the art. For example, the method of Harris et al., (Harris et al., Biochem., 49:3305-3316 [2010], incorporated herein by reference) finds use. In this method, corn stover is pretreated with sulfuric acid, washed, incubated with cellulase enzymes and GH61 for several days, and then the yield of sugars quantitated by refraction. Another method is described in U.S. Pat. No. 7,868,227 (incorporated herein by reference). In this method, the cellulosic substrate is PCS (corn stover pretreated with heat and dilute sulfuric acid, as described in WO 2005/074647; and a cellulose enzyme mixture is Cellucast®, a blend of cellulase enzymes from the fungus Trichoderma reesei (Sigma-Aldrich). Hydrolysis of PCS is conducted in a total reaction volume of 1.0 mL and a PCS concentration of 50 mg/mL in 1 mM manganese sulfate, 50 mM sodium acetate buffer pH 5.0. The test protein is combined with the base cellulase mixture at relative concentrations between 0 and 100% total protein. The protein composition is incubated with the PCS at 65° C. for 7 days. The combined yield of glucose and cellobiose is measured by refractive index detection.

[0246] GH61 activity is calculated as an increase in glucose production from the substrate by the cellulase(s) in the presence of GH61 protein, in comparison with the same reaction mixture in the absence of GH61 protein. Typically, the increase is dose-dependent within at least a 3-fold range of concentrations. GH61 activity can be expressed as a degree of "synergy".

Use of GH61 Variant Protein to Promote Saccharification

[0247] The GH61 variant proteins of the present invention can be used industrially to promote or otherwise modulate the activity of cellulase enzymes.

[0248] In some embodiments, suitably prepared lignocellulose is subjected to enzymatic hydrolysis using one or more cellulase enzymes in the presence of one or more GH61 variant proteins or preparations according to this invention. Thus, in some embodiments, saccharification reactions are carried out by exposing biomass to GH61 variant protein and cellulases, which work in concert to break down the biomass. Typically, the cellulases include at least one endoglucanase (EG), at least one beta-glucosidase (BGL), at least one Type 1 cellobiohydrolase (CBH1), and/or at least one Type 2 cellobiohydrolase (CBH2). In some alternative embodiments, a minimum enzyme mixture is used, for example, comprising GH61 protein in combination with BGL and either CBH1 or CBH2, or both, but with substantially no EG.

[0249] Hydrolysis of the hemicellulose and cellulose components of a lignocellulosic feedstock yields a lignocellulosic hydrolysate comprising xylose and glucose. Other sugars typically present include galactose, mannose, arabinose, fucose, rhamnose, or a combination thereof. Regardless of the means of hydrolyzing the lignocellulosic feedstock (e.g., full acid hydrolysis or chemical pretreatment with or without subsequent enzymatic hydrolysis), the xylose and glucose generally make up a large proportion of the sugars present. In some embodiments, if the lignocellulosic hydrolysate is a hemicellulose hydrolysate resulting from acid pretreatment, xylose will likely be the predominant sugar and lesser amounts of glucose will be present. The relative amount of xylose present in the lignocellulosic hydrolysate will depend on the feedstock and the pretreatment that is employed.

[0250] The cells and compositions of the present invention (including culture broth and/or cell lysates) find use in the production of fermentable sugars from cellulosic biomass. The biomass substrate may be converted to a fermentable sugar by (a) optionally pretreating a cellulosic substrate to increase its susceptibility to hydrolysis; (b) contacting the optionally pretreated cellulosic substrate of step (a) with a composition, culture medium or cell lysate containing at least one GH61 variant and any additional cellulases under conditions suitable for the production of cellobiose and fermentable sugars such as glucose.

[0251] In some embodiments, each of the at least one GH61 variant and additional cellulase enzymes described herein are partially or substantially purified, and the purified proteins are added to the biomass. Alternatively or in addition, the various individual enzymes are recombinantly expressed in different cells, and the media containing the secreted proteins are added to the biomass. The GH61 variant protein(s) and cellulase enzymes are then reacted with the biomass at a suitable temperature for a suitable period.

[0252] In some embodiments, sugars produced by methods of this invention are used to produce an end product such as an alcohol, such as ethanol. Other end-products may be produced, such as acetone, amino acid(s) (e.g., glycine, or lysine), organic acids (e.g., lactic acid, acetic acid, formic acid, citric acid, oxalic acid, or uric acid), glycerol, diols (e.g., 1,3 propanediol or butanediol), or at least one hydrocarbon with 1 to 20 carbon atoms. In some embodiments, cellulosic biomass is treated with at least one composition of the present invention to prepare an animal feed.

[0253] In some embodiments, when GH61 protein (e.g., at least one GH61 variant) is used to increase the yield of fermentable sugars in a saccharification reaction, at least one divalent metal cation or additional cofactor or adjunct compound is added to the reaction at a concentration of about 1 to 100 uM. In some embodiments, the divalent metal cation (e.g., copper) is included at a concentration of about 1 to 90 uM, about 10 to 80 uM, about 15 to 75 uM, about 20 to 70 uM, about 30 to 60 uM, about 40 to 50 uM, about 5 to 10 uM, about 10 to 20 μ M, about 15 to 25 uM, about 20 to 30 uM, about 25 to 35 uM, about 30 to 40 uM, about 35 to 45 uM, about 40 to 50 uM, about 45 to 55 uM, about 50 to 60 uM, about 55 to 65 uM, about 60 to 70 uM, about 65 to 75 uM, about 70 to 80 uM, about 75 to 85 uM, about 80 to 90 uM, about 85 to 95 uM, about 90 to 100 uM, about 95 to 100 uM. or about 1 uM. about 2 uM. about 3 uM. about 4 uM. about 5 uM, about 6 uM, about 7 uM, about 8 uM, about 9 uM, about 10 uM, about 11 uM, about 12 uM, about 13 uM, about 14 uM, about 15 uM, about 16 uM, about 17 uM, about 18 uM, about 19 uM, about 20 uM, about 25 uM, about 30 uM, about 35 uM, about 40 uM, about 45 uM, about 50 uM, about 55 uM, about 60 uM, about 65 uM, about 70 uM, about 75 uM, about 80 uM, about 85 uM, about 90 uM, about 95 uM, or about 100 uM. Divalent cations present in the reaction include, but are not limited to Cu++, Mn++, Co++, Mg++, Ni++, Zn++, and Ca++ at concentrations of 0.001 to 50 mM, 1 μ M to 1 mM, or 10-50 μ M. Indeed, it is not intended that the concentration of divalent metal cation(s) be limited to any particular value, as any suitable concentration finds use in the present invention and will depend upon the reaction conditions, as known in the

Fermentation of Sugars

[0254] In some embodiments, once a suitable cellulosic biomass substrate has been treated with cellulase(s) and at least one GH61 variant protein(s) according to this invention, sugars and other components in the product are fermented to produce various fermentation end products, including but not limited to biofuels, such as ethanol or alcohol mixtures. Depending on the substrate used, other components (e.g., long-chain esters) may also be present.

[0255] Fermentation is the process of extracting energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor. Alcoholic fermentation is a process in which sugars such as xylulose, glucose, fructose, and sucrose are converted into a fermentation end product, including but not limited to biofuel. For example, the fermentation product may comprise alcohol (such as ethanol or butanol) and/or a sugar alcohol, such as xylitol.

[0256] In some embodiments, enzyme compositions comprising at least one GH61 variant of the present invention is reacted with a biomass substrate in the range of about 25° C. to 100° C., about 30° C. to 90° C., about 30° C. to 80° C., and about 30° C. to 70° C. In some embodiments, the biomass is reacted with the enzyme compositions at about 25° C., at about 30° C., at about 35° C., at about 40° C., at about 45° C., at about 50° C., at about 55° C., at about 60° C., at about 65° C., at about 70° C., at about 75° C., at about 80° C., at about 85° C., at about 90° C., at about 95° C. and at about 100° C. In general, the pH range is from about pH 3.0 to 8.5, pH 3.5 to 8.5, pH 4.0 to 7.5, pH 4.0 to 7.0 and pH 4.0 to 6.5. The incubation time may vary for example from 1.0 to 240 hours, from 5.0 to 180 hrs and from 10.0 to 150 hrs. For example, the incubation time is generally at least 1 h, at least 5 hrs, at least 10 hrs, at least 15 hrs, at least 25 hrs, at least 50 h, at least 100 hrs, at least 180, or longer. Incubation of the cellulase under these conditions and subsequent contact with the substrate may result in the release of substantial amounts of fermentable sugars from the substrate (e.g., glucose when the cellulase is combined with beta-glucosidase). For example at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more fermentable sugar may be available as compared to the release of sugar by a wild-type polypeptide.

[0257] Any suitable micro-organism finds use in converting sugar in the sugar hydrolysate to ethanol or other fermentation products. These include yeast from the genera Saccharomyces, Hansenula, Pichia, Kluyveromyces, and Candida. Commercially available yeasts also find use, including but not limited to ETHANOLRED® SAFDISTIL®, THERMOSACC®, FERMIOL®, FERMIVIN®, or SuperstartTM.

[0258] In some embodiments, the yeast is genetically engineered to ferment both hexose and pentose sugars to at least one end-product, including but not limited to ethanol. Alternatively, in some embodiments, the yeast is a strain that has been made capable of xylose and glucose fermentation by one or more non-recombinant methods, such as adaptive evolution or random mutagenesis and selection. For example, in some embodiments, the fermentation is performed with recombinant *Saccharomyces*. In some embodiments, the recombinant yeast is a strain that has been made capable of xylose fermentation by recombinant incorporation of genes encoding xylose reductase (XR) and xylitol

dehydrogenase (XDH) (See e.g., U.S. Pat. Nos. 5,789,210, 5,866,382, 6,582,944 and 7,527,927; and EP 450 530) and/or gene(s) encoding one or more xylose isomerase (XI) (See e.g., U.S. Pat. Nos. 6,475,768 and 7,622,284). In some additional embodiments, the modified yeast strain overexpresses an endogenous and/or heterologous gene encoding xylulokinase (XK). Other yeast can ferment hexose and pentose sugars to at least one end-product, including but not limited to ethanol, such as yeast of the genera *Hansenula*, *Pichia*, *Kluyveromyces* and *Candida* (See e.g., WO 2008/130603).

[0259] A typical temperature range for the fermentation of xylose to ethanol using Saccharomyces spp. is between about 25° C. to about 37° C., although the temperature may be higher (up to 55° C.) if the yeast is naturally or genetically modified to be thermostable. The pH of a typical fermentation employing Saccharomyces spp. is between about 3 and about 6, depending on the pH optimum of the fermentation microorganism. The sugar hydrolysate may also be supplemented with additional nutrients required for growth and fermentation performance of the fermentation microorganism. For example, yeast extract, specific amino acids, phosphate, nitrogen sources, salts, trace elements and vitamins (See e.g., Verduyn et al., Yeast 8:501-170 [1992]; Jorgensen, Appl. Biochem. Biotechnol., 153:44-57 [2009]; and Zhao et al., J. Biotechnol., 139:55-60 [2009]). In some embodiments, the fermentation is conducted under anaerobic conditions, although aerobic or microaerobic conditions also find use.

Use of Copper, Gallic Acid, and Biomass Pretreatment Filtrate to Enhance GH61 Activity

[0260] In some embodiments, GH61 proteins and variants exhibit increased activity in a saccharification reaction when Cu⁺⁺, gallic acid, and/or pretreatment filtrate are added. In some embodiments, wild-type GH61a (SEQ ID NO:2) and/or Variant 1 (SEQ ID NO:5) are used. Similarly, in some embodiments, the present invention encompasses the supplemental addition of Cu⁺⁺, gallic acid, and/or pretreatment filtrate as an enhancing agent in saccharification reactions conducted using any of the GH61a variants shown in Tables 1 and 2, any of the other GH61 proteins described herein, and any active variant or fragment thereof such as may be obtained using any suitable method, including but not limited to the methods provided herein. In some embodiments, enhancing GH61 activity allows saccharification reactions to proceed more quickly and/or with less GH61 or cellulase enzyme.

[0261] In some embodiments, Cu⁺⁺, gallic acid, and other potential cofactors are tested by titrating into a saccharification reaction comprising a GH61 protein, one or more cellulase enzymes (e.g., CBH1, CBH2, and/or BGL), and a cellulosic substrate, and measuring the relative rate of glucose production. Controls may include the combination of GH61 protein, cellulase enzymes, and substrate in the absence of the putative cofactor (to test the relative enhancement), and combinations of cellulase enzymes and substrate with or without cofactor in the absence of GH61 protein (to determine the effect of the putative cofactor on other enzymes in the reaction).

[0262] As shown herein, in some embodiments, Cu⁺⁺ can enhance the activity of GH61a Variant 1 (SEQ ID NO:5). The source of Cu⁺⁺ used in the example was CuSO₄, although any effective copper source can be used as an

alternative. Effective supplemental copper sources include copper salts and metallic copper, or mixtures thereof. Copper salts include copper(II) (Cu⁺) salts and copper(I) (Cu⁺) salts. Copper in metallic copper(0) and copper(I) salts can be oxidized to Cu⁺⁺ in water by oxygen (e.g., by oxygen present in air). Suitable copper(II) and copper(I) salts include sulfates, chlorides, oxides, hydroxides, nitrates, carbonates, hydroxycarbonates (basic carbonates), oxychlorides, and acetates. Suitable sources of metallic copper include metallic copper refined from copper ores, including copper vessels and piping in contact with water and oxygen (e.g., in air).

[0263] In some embodiments, as shown herein, gallic acid and/or pretreated biomass filtrate can also be used to enhance the activity of GH61 protein. In some embodiments, the gallic acid and/or pretreated biomass filtrate are titrated to the optimal dose for the reaction conditions used. Thus, an effective concentration of gallic acid can be determined empirically by titrating it into the reaction mixture, depending on the enzymes being used and the total biomass. In some embodiments, in which gallic acid is utilized, an effective concentration of gallic acid is within the range of about 0.1 to 20 mM, about 0.5 to 5 mM, or about 1 to 2 mM. However, it is not intended that the present invention be limited to any particular concentration of gallic acid, as any suitable concentration finds use in the present invention, depending upon the reaction conditions.

[0264] A cofactor of GH61 in a reaction volume such as Cu⁺⁺ is said to be "supplemented" if it has been added into the reaction volume as a separate reagent, which is in addition to any metal ions that may be bound to GH61 or other reactants beforehand. Depending on the amount or molar ratio of cofactors such as Cu⁺⁺ already present in a GH61 preparation, addition of such cofactors into the reaction may increase the amount of glucose produced per weight of GH61 by 25%, 50%, 2-fold, or more.

[0265] Effective concentrations of supplemented Cu⁺⁺ in the reaction volume may be readily determined empirically as described herein. Depending on reaction conditions, effective supplemented concentrations include but are not limited to 1 μ M to 200 μ M, 4 μ M to 100 μ M, 10 μ M to 100 μ M, or at least 1 μ M, 4 μ M, 10 μ M, 20 μ M, 30 μ M, 40 μ M, or 50 µM in the reaction volume (i.e., the concentration of supplemented copper in the reaction volume). However, it is not intended that the present invention be limited to any particular copper concentration or range of concentrations, as any suitable concentration finds use and will depend upon the reaction conditions used. In some embodiments, prior to or without copper supplementation, copper is present in the GH61 protein preparation, the other enzymes, the cellulase fermentation production media, the pretreated biomass, and/ or any other component of the reaction volume (i.e., in some embodiments, there are other sources of copper present in the reaction than any copper added to the reaction as a supplement). Thus, in some embodiments, the reaction is conducted without the supplemental addition of copper as described herein.

[0266] In some embodiments, inclusion of copper and/or gallic acid in the reaction mixture at an effective concentration or ratio, less GH61 protein is needed to produce the same amount of fermentable sugars from the same cellulase enzymes. In some embodiments, this provides a cost reduction associated with saccharification reactions.

Vectors, Promoters, Other Expression Elements, Host Cells, and Signal Peptides.

[0267] There are numerous general texts that describe molecular biological techniques including the use of vectors, promoters, in vitro amplification methods including the polymerase chain reaction (PCR) and the ligase chain reaction (LCR) (See e.g., Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Volume 152 Academic Press, Inc., San Diego, Calif. (Berger); Sambrook et al., Molecular Cloning-A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989; and *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., Current Protocols [as supplemented through 2009]). Introduction of a vector or a DNA construct into a host cell can be effected by any suitable method, including but not limited to calcium phosphate transfection, DEAE-Dextran mediated transfection, electroporation, or other common techniques (See Davis et al., 1986, Basic Methods in Molecular Biology). General references on cell culture techniques and nutrient media for fungal host cells include Gene Manipulations in Fungi, Bennett, J. W. et al., Ed., Academic Press, 1985; More Gene Manipulations in Fungi, Bennett, J. W. et al., Ed., Academic Press, 1991; and The Handbook of Microbiological Media, CRC Press, Boca Raton, Fla., 1993.

Vectors

[0268] The present invention makes use of recombinant constructs comprising at least one sequence encoding at least one GH61 variant as described above. In some embodiments, the present invention provides expression vectors comprising at least one GH61 variant polynucleotide operably linked to a heterologous promoter. Expression vectors of the present invention may be used to transform an appropriate host cell to permit the host to express the GH61 variant protein. Methods for recombinant expression of proteins in fungi and other organisms are well known in the art, and a number expression vectors are available or can be constructed using routine methods (See, e.g., Tkacz and Lange, 2004, Advances in fungal biotechnology for industry, agriculture, and medicine, Kluwer Academic/Plenum Publishers, New York; Zhu et al., Plasmid 6:128-33 [2009]; and Kavanagh, K. 2005, Fungi: biology and applications, Wiley, all of which are incorporated herein by reference).

[0269] Nucleic acid constructs of the present invention comprise a vector, such as, a plasmid, a cosmid, a phage, a virus, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), and the like, into which a nucleic acid sequence of the invention has been inserted. Polynucleotides of the present invention can be incorporated into any one of a variety of expression vectors suitable for expressing a polypeptide. Suitable vectors include, but are not limited to chromosomal, nonchromosomal and synthetic DNA sequences (e.g., derivatives of SV40); bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adenovirus, adeno-associated virus, retroviruses and many others. Any vector that transduces genetic material into a cell, and, if replication is desired, which is replicable and viable in the relevant host can be used.

[0270] In some embodiments, the construct further comprises regulatory sequences, including, for example, a pro-

moter, operably linked to the protein encoding sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art.

Promoters

[0271] In order to obtain high levels of expression in a particular host it is often useful to express the GH61 variant of the present invention under the control of a heterologous promoter. A promoter sequence may be operably linked to the 5' region of the GH61 variant coding sequence using routine methods.

[0272] Examples of useful promoters for expression of GH61 enzymes include promoters from fungi. In some embodiments, a promoter sequence that drives expression of a gene other than a GH61 gene in a fungal strain may be used. As a non-limiting example, a fungal promoter from a gene encoding an endoglucanase may be used. In some embodiments, a promoter sequence that drives the expression of a GH61 gene in a fungal strain other than the fungal strain from which the GH61 variant was derived may be used. As a non-limiting example, if the GH61 variant is derived from C1, a promoter from a *T. reesei* GH61 gene may be used or a promoter as described in WO 2010/107303, such as but not limited to the sequences identified as SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or SEQ ID NO:29 in WO 2010/107303.

[0273] Examples of other suitable promoters useful for directing the transcription of the nucleotide constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes for Aspergillus oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, Aspergillus niger neutral alpha-amylase, Aspergillus niger acid stable alpha-amylase, Aspergillus niger or Aspergillus awamori glucoamylase (glaA), Rhizomucor miehei lipase, Aspergillus oryzae alkaline protease, Aspergillus oryzae triose phosphate isomerase, Aspergillus nidulans acetamidase, and Fusarium oxysporum trypsin-like protease (WO 96/00787, which is incorporated herein by reference), as well as the NA2-tpi promoter (a hybrid of the promoters from the genes for Aspergillus niger neutral alpha-amylase and Aspergillus oryzae triose phosphate isomerase), promoters such as cbh1, cbh2, egl1, egl2, pepA, hfb1, hfb2, xyn1, amy, and glaA (Nunberg et al., Mol. Cell Biol., 4:2306-2315 [1984]; Boel et al., EMBO J., 3:1581-85 [1984]; and European Pat. Publ. 137280, all of which are incorporated herein by reference), and mutant, truncated, and hybrid promoters thereof. In a yeast host, useful promoters can be from the genes for Saccharomyces cerevisiae enolase (eno-1), Saccharomyces cerevisiae galactokinase (gall), Saccharomyces cerevisiae alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP), and S. cerevisiae 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are known (See e.g., Romanos et al., Yeast 8:423-488 [1992], incorporated herein by reference. Promoters associated with chitinase production in fungi may be used (See, e.g., Blaiseau and Lafay, Gene 120243-248 [1992] (filamentous fungus Aphanocladium album); Limon et al., Curr. Genet, 28:478-83 (Trichoderma harzianum), both of which are incorporated herein by reference).

[0274] Promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses and which can be used in some embodiments of the invention include SV40 promoter, $E.\ coli$ lac or trp promoter, phage lambda P_L promoter, tac promoter, T7 promoter, and the like. In bac-

terial host cells, suitable promoters include the promoters obtained from the *E. coli* lac operon, *Streptomyces coeli-color* agarase gene (dagA), *Bacillus subtilis* levansucranse gene (sacB), *Bacillus licheniformis* α-amylase gene (amyl), *Bacillus stearothermophilus* maltogenic amylase gene (amyM), *Bacillus amyloliquefaciens* α-amylase gene (amyQ), *Bacillus subtilis* xylA and xylB genes and prokaryotic beta-lactamase gene.

[0275] Any other promoter sequence that drives expression in a suitable host cell may be used. Suitable promoter sequences can be identified using well known methods. In one approach, a putative promoter sequence is linked 5' to a sequence encoding a reporter protein, the construct is transfected into the host cell (e.g., M. thermophila) and the level of expression of the reporter is measured. Expression of the reporter can be determined by measuring, for example, mRNA levels of the reporter sequence, an enzymatic activity of the reporter protein, or the amount of reporter protein produced. For example, promoter activity may be determined by using the green fluorescent protein as coding sequence (See e.g., Henriksen et al, Microbiol., 145:729-34 [1999], incorporated herein by reference) or a lacZ reporter gene (Punt et al., Gene, 197:189-93 [1997], incorporated herein by reference). Functional promoters may be derived from naturally occurring promoter sequences by directed evolution methods (See, e.g. Wright et al., Human Gene Therapy, 16:881-892 [2005], incorporated herein by reference.

[0276] Additional promoters include those from *M. thermophila*, provided in U.S. Prov. Patent Appln. Ser. Nos. 61/375,702, 61/375,745, 61/375,753, 61/375,755, and 61/375,760, all of which were filed on Aug. 20, 2010, and are hereby incorporated by reference in their entireties, as well as WO 2010/107303.

Other Expression Elements

[0277] Cloned GH61 variants may also have a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleic acid sequence encoding the polypeptide. Any terminator that is functional in the host cell of choice may be used in the present invention.

[0278] For example, exemplary transcription terminators for filamentous fungal host cells can be obtained from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* alpha-glucosidase, and *Fusarium oxysporum* trypsin-like protease. Suitable transcription terminators are known in the art (See e.g., U.S. Pat. No. 7,399,627, incorporated herein by reference).

[0279] Exemplary terminators for yeast host cells include those 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 known in the art (See e.g., Romanos et al., Yeast 8:423-88 [1992]).

[0280] A suitable leader sequence may be part of a cloned GH61 variant sequence, which 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 nucleic acid sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice

may be used. Exemplary 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).

[0281] In some embodiments, sequences also contain a polyadenylation sequence, which is a sequence operably linked to the 3' terminus of the nucleic acid sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention. Exemplary polyadenylation sequences for filamentous fungal host cells can be from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Fusarium oxysporum* trypsin-like protease, and *Aspergillus niger* alpha-glucosidase. Useful polyadenylation sequences for yeast host cells are known in the art (See e.g., Guo and Sherman, Mol. Cell. Biol., 15:5983-5990 [1995]).

[0282] The expression vector of the present invention optionally contains one or more selectable markers, which facilitate easy selection of transformed cells. A selectable marker is a typically gene, the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, amdS (acetamidase), argB (ornithine carbamoyltransferase), bar (phosphinothricin acetyltransferase), hph (hygromycin phosphotransferase), niaD (nitrate reductase), pyrG (orotidine-5'-phosphate decarboxylase), sC (sulfate adenyltransferase), and trpC (anthranilate synthase), as well as equivalents thereof. Embodiments for use in an Aspergillus cell include the amdS and pyrG genes of Aspergillus nidulans or Aspergillus orvzae and the bar gene of Streptomyces hygroscopicus. Suitable markers for yeast host cells include but are not limited to ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3.

Host Cells

[0283] In some embodiments, at least one GH61 variant protein of the present invention is expressed from a nucleic acid that has been recombinantly introduced into a suitable host cell line. In some embodiments, the host cell also expresses other proteins of interest, particularly one or more cellulase enzymes that work in concert with at least one GH61 variant protein in the process of saccharification. The cellulase enzymes may be constitutively expressed by the parent strain of the host cell, or they may be expressed from other recombinant nucleic acids that were introduced serially or simultaneously with the GH61 variant encoding sequence.

[0284] Rather than expressing at least one GH61 variant protein and at least one additional cellulase enzyme in the same cell, in some embodiments, the invention is practiced by producing at least one GH61 variant protein in one host cell, and producing one or more cellulases together in another host cell, or in a plurality of host cells. Once such cells have been engineered, cells expressing GH61 protein and cells expressing cellulase enzymes can be combined and

cultured together to produce compositions of this invention containing both GH61 variant proteins and other cellulase enzymes. Alternatively, the culture supernatant or broth from each cell line can be collected separately, optionally fractionated to enrich for the respective activities, and then mixed together to produce the desired combination.

[0285] Suitable fungal host cells include, but are not limited to Ascomycota, Basidiomycota, Deuteromycota, Zygomycota, and Fungi imperfecti. In some embodiments, preferred fungal host cells are yeast cells, and filamentous fungal cells, including all filamentous forms of the subdivision Eumycotina and Oomycota. Filamentous fungi are characterized by a vegetative mycelium with a cell wall composed of chitin, cellulose and other complex polysaccharides, and are morphologically distinct from yeast. In some embodiments, *Trichoderma* is a source of one or more cellulases for use in combination with GH61 variant proteins.

[0286] Any suitable host cell finds use in the present invention, including but not limited to host cells that are species of Achlya, Acremonium, Aspergillus, Aureobasidium, Azospirillum, Bjerkandera, Cellulomonas, Cephalosporium, Ceriporiopsis, Chrysosporium, Clostridium, Coccidioides, Cochliobolus, Coprinus, Coriolus, Corynascus, Cryphonectria, Cryptococcus, Dictyostelium, Diplodia, Elizabethkingia, Endothia, Erwinia, Escherichia, Fusarium, Gibberella, Gliocladium, Gluconacetobacter, Humicola, Hypocrea, Kuraishia, Mucor, Myceliophthora, Neurospora, Nicotiana, Paenibacillus, Penicillium, Periconia, Phaeosphaeria, Phlebia, Piromyces, Podospora, Prevotella, Pyricularia, Rhizobium, Rhizomucor, Rhizopus, Ruminococcus, Saccharomycopsis, Salmonella, Schizophyllum, Scytalidium, Septoria, Sporotrichum, Streptomyces, Talaromyces, Thermoanaerobacter, Thermoascus, Thermotoga, Thielavia, Tolypocladium, Trametes, Trichoderma, Tropaeolum, Uromyces, Verticillium, Volvariella, Wickerhamomyces, or corresponding teleomorphs, or anamorphs, and synonyms or taxonomic equivalents thereof.

[0287] An exemplary host cell is yeast, including but not limited to Candida, Hansenula, Saccharomyces, Schizosaccharomyces, Pichia, Kluyveromyces, or Yarrowia. In some embodiments, the yeast cell is Hansenula polymorpha, Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Saccharomyces diastaticus, Saccharomyces norbensis, Saccharomyces kluyveri, Schizosaccharomyces pombe, Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia kodamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia quercuum, Pichia pijperi, Pichia stipitis, Pichia methanolica, Pichia angusta, Kluyveromyces lactis, Candida albicans, or Yarrowia lipolytica.

[0288] Another exemplary host cell is a *Myceliophthora* species, such as *M. thermophila*. As used herein, the term "C1" refers to *Myceliophthora thermophila*, including a fungal strain described by Garg (See, Garg, Mycopathol., 30: 3-4 [1966]). As used herein, "*Chrysosporium lucknowense*" includes the strains described in U.S. Pat. Nos. 6,015, 707, 5,811,381 and 6,573,086; US Pat. Pub. Nos. 2007/0238155, US 2008/0194005, US 2009/0099079; International Pat. Pub. Nos., WO 2008/073914 and WO 98/15633, all of which are incorporated herein by reference, and include, without limitation, *Chrysosporium lucknowense* Garg 27K, VKM-F 3500 D (Accession No. VKM F-3500-D), C1 strain UV13-6 (Accession No. VKM F-3632

D), C1 strain NG7C-19 (Accession No. VKM F-3633 D), and C1 strain UV18-25 (VKM F-3631 D), all of which have been deposited at the All-Russian Collection of Microorganisms of Russian Academy of Sciences (VKM), Bakhurhina St. 8, Moscow, Russia, 113184, and any derivatives thereof. Although initially described as Chrysosporium lucknowense, C1 may currently be considered a strain of Myceliophthora thermophila. Other C1 strains include cells deposited under accession numbers ATCC 44006, CBS (Centraalbureau voor Schimmelcultures) 122188, CBS 251. 72, CBS 143.77, CBS 272.77, CBS122190, CBS122189, and VKM F-3500D. Exemplary C1 derivatives include modified organisms in which one or more endogenous genes or sequences have been deleted or modified and/or one or more heterologous genes or sequences have been introduced. Derivatives include, but are not limited to UV18#100f Δalp1, UV18#100f Δpyr5 Δalp1, UV18#100.f Δalp1 Δpep4 Δalp2, UV18#100.f Δpyr5 Δalp1 Δpep4 Δalp2 and UV18#100.f Δpyr4 Δpyr5 Δalp1 Δpep4 Δalp2, as described in WO2008073914 and WO2010107303, each of which is incorporated herein by reference.

[0289] In some embodiments, the host cell is a *Trichoderma* species, such as *T. longibrachiatum*, *T. viride*, *Hypocrea jecorina* or *T. reesei*, *T. koningii*, and *T. harzianum*

[0290] In some embodiments, the host cell is a Aspergillus species, such as A. awamori, A. funigatus, A. japonicus, A. nidulans, A. niger, A. aculeatus, A. foetidus, A. oryzae, A. sojae, and A. kawachi.

[0291] In some additional embodiments, the host cell is a Fusarium species, such as F. bactridioides, F. cerealis, F. crookwellense, F. culmorum, F. graminearum, F. graminum. F. oxysporum, F. roseum, and F. venenatum.

[0292] The host cell may also be a *Neurospora* species, such as *N. crassa*. Alternatively, the host cell is a *Humicola* species, such as *H. insolens*, *H. grisea*, and *H. lanuginosa*. Alternatively, the host cell is a *Mucor* species, such as *M. miehei* and *M. circinelloides*. Alternatively, the host cell is a *Rhizopus* species, such as *R. oryzae* and *R. niveus*. Alternatively, the host cell is a *Penicillum* species, such as *P. purpurogenum*, *P. chrysogenum*, and *P. verruculosum*.

[0293] In some embodiments, the host cell is a *Thielavia* species, such as *T. terrestris*. Alternatively, the host cell is a *Tolypocladium* species, such as *T. inflatum* and *T. geodes*. Alternatively, the host cell is a the *Trametes* species, such as *T. villosa* and *T. versicolor*.

[0294] In some embodiments, the host cell is of a *Chrysosporium* species, such as *C. lucknowense*, *C. keratinophilum*, *C. tropicum*, *C. merdarium*, *C. inops*, *C. pannicola*, and *C. zonatum*. In a particular embodiment the host is *C. lucknowense*. Alternatively, the host cell is an algae such as *Chlamydomonas* (e.g., *C. reinhardtii*) or *Phormidium* (P. sp. ATCC29409).

[0295] In some alternative embodiments, the host cell is a prokaryotic cell. Suitable prokaryotic cells include Grampositive, Gram-negative and Gram-variable bacterial cells. Examples of bacterial host cells include, but are not limited to Bacillus (e.g., B. subtilis, B. licheniformis, B. megaterium, B. stearothermophilus and B. amyloliquefaciens), Streptomyces (e.g., S. ambofaciens, S. achromogenes, S. avermitilis, S. coelicolor, S. aureofaciens, S. aureus, S. fungicidicus, S. griseus, and S. lividans), and Streptococcus (e.g., S. equisimiles, S. pyogenes, and S. uberis) species.

[0296] Any suitable eukaryotic or prokaryotic species finds use as host cells, including but not limited to Aspergillus aculeatus, Azospirillum irakense KBC1, Bacillus sp. GL1, Cellulomonas biazotea, Clostridium thermocellum, Thermoanaerobacter brockii, Coccidioides posadasii, Dictyostelium discoideum, Elizabethkingia meningoseptica, Erwinia chrysanthemi, Escherichia coli, Gluconacetobacter xylinus, Hypocrea jecorina, Kuraishia capsulata, Nicotiana tabacum, Paenibacillus sp. C7, Penicillium brasilianum, Periconia sp. BCC 2871, Phaeosphaeria avenaria, Prevotella albensis, Rhizobium leguminosarum, Rhizomucor miehei, Ruminococcus albus, Saccharomycopsis fibuligera, Salmonella typhimurium, Septoria lycopersici, Streptomyces coelicolor, Talaromyces emersonii, Thermotoga maritima, Tropaeolum majus, Uromyces viciae-fabae, and Wickerhamomyces anomalus.

[0297] Strains that may be used in the practice of the invention (both prokaryotic and eukaryotic strains) may be obtained from any suitable source, including but not limited to the American Type Culture Collection (ATCC), or other biological depositories such as Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM), Centraalbureau Voor Schimmelcultures (CBS), and the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL).

[0298] In some embodiments, host cells are genetically modified to have characteristics that improve genetic manipulation, protein secretion, protein stability or other properties desirable for expression or secretion of a protein. For example, knock-out of Alp1 function results in a cell that is protease deficient. Knock-out of pyr5 function results in a cell with a pyrimidine deficient phenotype. Host cells may be modified to delete endogenous cellulase protein-encoding sequences or otherwise eliminate expression of one or more endogenous cellulases. Expression of one or more unwanted endogenous cellulases may be inhibited to increase the proportion of cellulases of interest, for example, by chemical or UV mutagenesis and subsequent selection. Homologous recombination can be used to induce targeted gene modifications by specifically targeting a gene in vivo to suppress expression of the encoded protein.

Signal Peptides

[0299] In general, polypeptides are secreted from the host cell after being expressed as a pre-protein including a signal peptide (i.e., an amino acid sequence linked to the amino terminus of a polypeptide which directs the encoded polypeptide into the cell's secretory pathway).

[0300] In some embodiments, the secreted part of a GH61 variant is linked at the N-terminal to a heterologous signal peptide, depending on the host cell and other factors. Effective signal peptide coding regions for filamentous fungal host cells include but are not limited to signal peptide coding regions obtained from *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Rhizomucor miehei* aspartic proteinase, *Humicola insolens* cellulase, *Humicola lanuginosa* lipase, and *T. reesei* cellobiohydrolase II (TrCBH2).

[0301] Effective signal peptide coding regions for bacterial host cells include but are not limited to signal peptide coding regions obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* beta-lactamase, *Bacillus stearothermophilus*

neutral proteases (nprT, nprS, nprM), and *Bacillus subtilis* prsA. Further signal peptides are known in the art (See e.g., described by Simonen and Palva, Microbiol. Rev., 57:109-137 [1993]).

[0302] Useful signal peptides for yeast host cells also include those from the genes for *Saccharomyces cerevisiae* alpha-factor, *Saccharomyces cerevisiae* SUC2 invertase (see Taussig and Carlson, Nucl. Acids Res., 11:1943-54 [1983]; SwissProt Accession No. P00724; and Romanos et al., Yeast 8:423-488 [1992]). Variants of these signal peptides and other signal peptides are suitable. In addition, the signal peptides provided herein find use in the present invention.

EXPERIMENTAL

[0303] The present invention is described in further detail in the following Examples, which are not in any way intended to limit the scope of the invention as claimed.

[0304] In the experimental disclosure below, the following abbreviations apply: ppm (parts per million); M (molar); mM (millimolar), uM and μM (micromolar); nM (nanomolar); mol (moles); gm and g (gram); mg (milligrams); ug and μg (micrograms); L and 1 (liter); ml and mL (milliliter); cm (centimeters); mm (millimeters); um and µm (micrometers); sec. (seconds); min(s) (minute(s)); h(s) and hr(s) (hour(s)); U (units); MW (molecular weight); rpm (rotations per minute); ° C. (degrees Centigrade); DNA (deoxyribonucleic acid); RNA (ribonucleic acid); HPLC (high pressure liquid chromatography); MES (2-N-morpholino ethanesulfonic acid); FIOPC (fold improvements over positive control); YPD (10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose); SOE-PCR (splicing by overlapping extension PCR); PEG (polyethylene glycol); TWEEN®-20 (TWEEN® non-ionic surfactant; Sigma-Aldrich); ARS (ARS Culture Collection or NRRL Culture Collection, Peoria, Ill.); Axygen (Axygen, Inc., Union City, Calif.); Lallemand (Lallemand Ethanol Technology, Milwaukee, Wis.); Dual Biosystems (Dual Biosystems AG, Schlieven, Switzerland); US Biological (United States Biological, Swampscott, Mass.); Megazyme (Megazyme International Ireland, Ltd., Wicklow, Ireland); Genetix (Genetix USA, Inc., Beaverton, Oreg.); Sigma-Aldrich (Sigma-Aldrich, St. Louis, Mo.); Dasgip (Dasgip Biotools, LLC, Shrewsbury, Mass.); Difco (Difco Laboratories, BD Diagnostic Systems, Detroit, Mich.); PCRdiagnostics (PCRdiagnostics, by E. coli SRO, Slovak Republic); Agilent (Agilent Technologies, Inc., Santa Clara, Calif.); Molecular Devices (Molecular Devices, Sunnyvale, Calif.); Symbio (Symbio, Inc., Menlo Park, Calif.); Newport (Newport Scientific, Australia); and Bio-Rad (Bio-Rad Laboratories, Hercules, Calif.).

[0305] The *M. thermophila* strains included in the development of the present invention included a "Strain CF-400" (Δcdh1), which is a derivative of C1 strain ("UV18#100fΔalp1Δpyr5"), modified by deletion of cdh1, wherein cdh1 comprises the polynucleotide sequence of SEQ ID NO:5 of U.S. Pat. No. 8,236,551. "Strain CF-401" (Δcdh1 Δcdh2) (ATCC No. PTA-12255), is a derivative of the C1 strain modified by deletion of both a cdh1 and a cdh2, wherein cdh2 comprises the polynucleotide sequence of SEQ ID NO:7 of U.S. Pat. No. 8,236,551. "Strain CF-402" (+Bgl1) is a derivative of the C1 strain further modified for overexpression of an endogenous beta-glucosidase 1 enzyme (Bgl1). "Strain CF-403" is a derivative of the C1 strain modified with a deletion of cdh1 and further modified to overexpress bgl1. "Strain CF-404" is a derivative of the

C1 strain further modified to overexpress bgl1 with a deletion of both cdh1 and cdh2. "Strain CF-416" is a derivative of the CF-404 strain, further modified to overexpress wild-type GH61a enzyme.

[0306] The following sequences are referred to herein and find use in the present invention

Wild-type M. thermophila C1 GH61a cDNA sequence: (SEQ ID NO: 1) ATGTCCAAGGCCTCTGCTCTCCTCGCTGGCCTGACGGGCGCGCCCTCGT CGCTGCACATGGCCACGTCAGCCACATCGTCGTCAACGGCGTCTACTACA $\tt GGAACTACGACCCCACGACAGACTGGTACCAGCCCAACCCGCCAACAGTC$ $\tt ATCGGCTGGACGGCAGCCGATCAGGATAATGGCTTCGTTGAACCCAACAG$ CTTTGGCACGCCAGATATCATCTGCCACAAGAGCGCCACCCCCGGCGGCG GCCACGCTACCGTTGCTGCCGGAGACAAGATCAACATCGTCTGGACCCCC GAGTGGCCCGAATCCCACATCGGCCCCGTCATTGACTACCTAGCCGCCTG ${\tt CAACGGTGACTGCGAGACCGTCGACAAGTCGTCGCTGCGCTGGTTCAAGA}$ CTGCGCGCCAACGGCAACAGCTGGCTCGTCCAGATCCCGTCGGATCTCAA GGCCGGCAACTACGTCCTCCGCCACGAGATCATCGCCCTCCACGGTGCTC $\tt AGAGCCCCAACGGCGCCCAGGCCTACCCGCAGTGCATCAACCTCCGCGTC$ ACCGGCGGCGCAGCAACCTGCCCAGCGGCGTCGCCGGCACCTCGCTGTA CAAGGCGACCGGGCATCCTCTTCAACCCCTACGTCTCCTCCCCGG $\tt ATTACACCGTCCCCGGCCCGGCCCTCATTGCCGGCGCCCAGCTCGATC$ GCCCAGAGCACGTCGGTCGCCACTGCCACCGGCACGGCCACCGTTCCCGG CGGCGGCGCCAACCCTACCGCCACCACCACCGCCGCCACCTCCGCCG CCCCGAGCACCACCTGAGGACGACCACTACCTCGGCCGCGCAGACTACC GCCCGCCCTCCGGCGATGTGCAGACCAAGTACGGCCAGTGTGGTGGCAA CGGATGGACGGCCCGACGGTGTGCGCCCCCGGCTCGAGCTGCTCCGTCC TCAACGAGTGGTACTCCCAGTGTTTGTAA

Wild-type M. thermophila C1 GH61a polypeptide sequence:

(SEQ ID NO: 2)
MSKASALLAGLTGAALVAAHGHVSHIVVNGVYYRNYDPTTDWYQPNPPTV
IGWTAADQDNGFVEPNSFGTPDIICHKSATPGGGHATVAAGDKINIVWTP
EWPESHIGPVIDYLAACNGDCETVDKSSLRWFKIDGAGYDKAAGRWAADA
LRANGNSWLVQIPSDLKAGNYVLRHEIIALHGAQSPNGAQAYPQCINLRV
TGGGSNLPSGVAGTSLYKATDPGILFNPYVSSPDYTVPGPALIAGAASSI
AQSTSVATATGTATVPGGGGANPTATTTAATSAAPSTTLRTTTTSAAQTT
APPSGDVQTKYGQCGGNGWTGPTVCAPGSSCSVLNEWYSQCL

Wild-type M. thermophila C1 GH61a polypeptide sequence without the signal sequence:

(SEQ ID NO: 3)
HGHVSHIVVNGVYYRNYDPTTDWYQPNPPTVIGWTAADQDNGFVEPNSFG
TPDIICHKSATPGGGHATVAAGDKINIVWTPEWPESHIGPVIDYLAACNG
DCETVDKSSLRWFKIDGAGYDKAAGRWAADALRANGNSWLVQIPSDLKAG

NYVLRHEIIALHGAQSPNGAQAYPQCINLRVTGGGSNLPSGVAGTSLYKA
TDPGILFNPYVSSPDYTVPGPALIAGAASSIAQSTSVATATGTATVPGGG
GANPTATTTAATSAAPSTTLRTTTTSAAQTTAPPSGDVQTKYGQCGGNGW
TGPTVCAPGSSCSVLNEWYSOCL

GH61a Variant 1 cDNA sequence: (SEQ ID NO: 4) ATGTCCAAGGCCTCTGCTCTCCTCGCTGGCCTGACGGGCCCGGCCCTCGT CGCTGCACACGGCCACGTCAGCCACATCGTCGTCAACGGCGTCTACTACA GGGGCTACGACCCCACGACAGACTGGTACCAGCCCAACCCGCCAACAGTC ATCGGCTGGACGGCAGCCGATCAGGATAATGGCTTCGTTGAACCCAACAG CTTTGGCACGCCAGATATCATCTGCCACAAGAGCGCCACCCCGGCGGCG GCCACGCTACCGTTGCTGCCGGAGACAAGATCAACATCGTCTGGACCCCC GAGTGGCCCCACTCCCACATCGGCCCCGTCATTGACTACCTAGCCGCCTG CAACGGTGACTGCGAGACCGTCGACAAGTCGTCGCTGCGCTGGTTCAAGA CTGCGCGCCAACGGCAACAGCTGGCTCGTCCAGATCCCGTCGGATCTCAA GCCCGGCAACTACGTCCTCCGCCACGAGATCATCGCCCTCCACGGTGCTC AGAGCCCCAACGGCGCCCAGGCGTACCCGCAGTGCATCAACCTCCGCGTC ACCGGCGGCGGCAGCAACCTGCCCAGCGGCGTCGCCGGCACCTCGCTGTA CAAGGCGACCGGGCATCCTCTTCAACCCCTACGTCTCCTCCCCGG ATTACACCGTCCCCGGCCCGGCCCTCATTGCCGGCGCCGCCAGCTCGATC GCCCAGAGCACGTCGGTCGCCACTGCCACCGGCACGGCCACCGTTCCCGG CGGCGGCGCCAACCCTACCGCCACCACCACCGCCGCCACCTCCGCCG CCCCGAGCACCACCTGAGGACGACCACTACCTCGGCCGCGCAGACTACC GCCCGCCCTCCGGCGATGTGCAGACCAAGTACGGCCAGTGTGGTGGCAA CGGATGGACGGGCCCGACGGTGTGCGCCCCCGGCTCGAGCTGCTCCGTCC

GH61a Variant 1 polypeptide sequence:

(SEQ ID NO: 5)

MSKASALLAGLTGAALVAAHGHVSHIVVNGVYYRGYDPTTDWYQPNPPTV

IGWTAADQDNGFVEPNSFGTPDIICHKSATPGGGHATVAAGDKINIVWTP

EWPHSHIGPVIDYLAACNGDCETVDKSSLRWFKIDGAGYDKAAGRWAADA

LRANGNSWLVQIPSDLKPGNYVLRHEIIALHGAQSPNGAQAYPQCINLRV

TGGGSNLPSGVAGTSLYKATDPGILFNPYVSSPDYTVPGPALIAGAASSI

AQSTSVATATGTATVPGGGGANPTATTTAATSAAPSTTLRTTTTSAAQTT

APPSGDVOTKYGOCGGNGWTGPTVCAPGSSCSVLNEWYSOCL

TCAACGAGTGGTACTCCCAGTGTTTGTAA

GH61a Variant 1 polypeptide sequence without the signal sequence:

(SEQ ID NO: 6)
HGHVSHIVVNGVYYRGYDPTTDWYQPNPPTVIGWTAADQDNGFVEPNSFG

TPDIICHKSATPGGGHATVAAGDKINIVWTPEWPHSHIGPVIDYLAACNG
DCETVDKSSLRWFKIDGAGYDKAAGRWAADALRANGNSWLVQIPSDLKPG

-continued
NYVLRHEIIALHGAQSPNGAQAYPQCINLRVTGGGSNLPSGVAGTSLYKA
TDPGILFNPYVSSPDYTVPGPALIAGAASSIAQSTSVATATGTATVPGGG
GANPTATTTAATSAAPSTTLRTTTTSAAQTTAPPSGDVQTKYGQCGGNGW
TGPTVCAPGSSCSVLNEWYSQCL

GH61a Variant 5 cDNA sequence (SEQ ID NO: 7) ${\tt ACACAAATGTCCAAGGCCTCTGCTCTCCTCGCTGGCCTGACGGGCGCGGC}$ CCTCGTCGCTGCACACGGCCACGTCAGCCACATCGTCGTCAACGGCGTCT ACTACAGGAACTACGACCCCACGACAGACTGGTACCAGCCCAACCCGCCA ACAGTCATCGGCTGGACGGCAGCCGATCAGGATAATGGCTTCGTTGAACC CAACAGCTTTGGCACGCCAGATATCATCTGCCACAAGAGCGCCACCCCCG GCGGCGGCCACGCTACCGTTGCTGCCGGAGACAAGATCAACATCGTATGG ACCCCGAGTGGCCCCACTCCCACATCGGCCCCGTCATTGACTACCTAGC CGCCTGCAACGGTGACTGCGAGACCGTCGACAAGTCGTCGCTGCGCTGGT GACGCTCTGCGCGCCAACGGCAACAGCTGGCTCGTCCAGATCCCGTCGGA TCTCGCGGCCGGCAACTACGTCCTCCGCCACGAGATCATCGCCCTCCACG GTGCTCAGAGCCCCAACGGCGCCCAGGCGTACCCGCAGTGCATCAACCTC $\tt CGCGTCACCGGCGGCGGCAGCAACCTGCCCAGCGGCGTCGCCGGCACCTC$ $\tt GCTGTACAAGGCGACCGGGCATCCTCTTCAACCCCTACGTCTCCT$ $\tt CCCCGGATTACACCGTCCCCGGCCCGGCCCTCATTGCCGGCGCCGCCAGC$ ${\tt TCGATCGCCCAGAGCACGTCGGTCGCCACTGCCACCGGCACGGCCACCGT}$ TCCCGGCGGCGCCGCCAACCCTACCGCCACCACCACCGCCGCCACCT CCGCCGCCCGAGCACCACCCTGAGGACGACCACTACCTCGGCCGCGCAG ACTACCGCCCCGCCCTCCGGCGATGTGCAGACCAAGTACGGCCAGTGTGG TGGCAACGGATGGACGGCCCGACGGTGTGCGCCCCCGGCTCGAGCTGCT CCGTCCTCAACGAGTGGTACTCCCAGTGTTTGTAA

GH61a Variant 5 polypeptide sequence:

(SEQ ID NO: 8)

MSKASALLAGLTGAALVAAHGHVSHIVVNGVYYRNYDPTTDWYQPNPPTV

IGWTAADQDNGFVEPNSFGTPDIICHKSATPGGGHATVAAGDKINIVWTP

EWPHSHIGPVIDYLAACNGDCETVDKSSLRWFKIDGAGYDKAAGRWAADA

LRANGNSWLVQIPSDLAAGNYVLRHEIIALHGAQSPNGAQAYPQCINLRV

TGGGSNLPSGVAGTSLYKATDPGILFNPYVSSPDYTVPGPALIAGAASSI

AQSTSVATATGTATVPGGGGANPTATTTAATSAAPSTTLRTTTTSAAQTT

APPSGDVQTKYGQCGGNGWTGPTVCAPGSSCSVLNEWYSQCL

GH61a Variant 5 polypeptide sequence without the signal sequence:

(SEQ ID NO: 9)
HGHVSHIVVNGVYYRNYDPTTDWYQPNPPTVIGWTAADQDNGFVEPNSFG
TPDIICHKSATPGGGHATVAAGDKINIVWTPEWPHSHIGPVIDYLAACNG
DCETVDKSSLRWFKIDGAGYDKAAGRWAADALRANGNSWLVQIPSDLAAG
NYVLRHEIIALHGAQSPNGAQAYPQCINLRVTGGGSNLPSGVAGTSLYKA

TDPGILFNPYVSSPDYTVPGPALIAGAASSIAQSTSVATATGTATVPGGG
GANPTATTTAATSAAPSTTLRTTTTSAAQTTAPPSGDVQTKYGQCGGNGW
TGPTVCAPGSSCSVLNEWYSOCL

GH61a Variant 9 cDNA sequence: (SEQ ID NO: 10) CCTCGTCGCTGCACATGGCCACGTCAGCCACATCGTCGTCAACGGCGTCT ACTACAGGAACTACGACCCCACGACAGACTGGTACCAGCCCAACCCGCCA ACAGTCATCGGCTGGACGGCAGCCGATCAGGATAATGGCTTCGTTGAACC CAACAGCTTTGGCACGCCAGATATCATCTGCCACAAGAGCGCCACCCCCG GCGGCGGCCACGCTACCGTTGCTGCCGGAGACAAGATCAACATCCAGTGG ACCCCGAGTGGCCCGAATCCCACATCGGCCCCGTCATTGACTACCTAGC CGCCTGCAACGGTGACTGCGAGACCGTCGACAAGTCGTCGCTGCGCTGGT GACGCTCTGCGCGCCAACGGCAACAGCTGGCTCGTCCAGATCCCGTCGGA TCTCAAGGCCGGCAACTACGTCCTCCGCCACGAGATCATCGCCCTCCACG $\tt GTGCTCAGAGCCCCAACGGCGCCCAGAACTACCCGCAGTGCATCAACCTC$ $\tt CGCGTCACCGGCGGCGGCAGCAACCTGCCCAGCGGCGTCGCCGGCACCTC$ $\tt GCTGTACAAGGCGACCGACCCGGGCATCCTCTTCAACCCCTACGTCTCCT$ CCCCGGATTACACCGTCCCCGGCCCGGCCCTCATTGCCGGCGCCGCCAGC ${\tt TCGATCGCCCAGAGCACGTCGGTCGCCACTGCCACCGGCACGGCCACCGT}$ TCCCGGCGGCGCGCCAACCCTACCGCCACCACCACCGCCGCCACCT

GH61a Variant 9 polypeptide sequence:
(SEQ ID NO: 11)

MSKASALLAGLTGAALVAAHGHVSHIVVNGVYYRNYDPTTDWYQPNPPTV

IGWTAADQDNGFVEPNSFGTPDIICHKSATPGGGHATVAAGDKINIQWTP

EWPESHIGPVIDYLAACNGDCETVDKSSLRWFKIDGAGYDKAAGRWAADA

LRANGNSWLVQIPSDLKAGNYVLRHEIIALHGAQSPNGAQNYPQCINLRV

CCGCCGCCCCGAGCACCACCCTGAGGACGACCACTACCTCGGCCGCGCAG

ACTACCGCCCCGCCCTCCGGCGATGTGCAGACCAAGTACGGCCAGTGTGG
TGGCAACGGATGGACGGGCCCGACGGTTGCGCCCCCGGCTCGAGCTGCT

TGGGSNLPSGVAGTSLYKATDPGILFNPYVSSPDYTVPGPALIAGAASSI AOSTSVATATGTATVPGGGGANPTATTTAATSAAPSTTLRTTTTSAAOTT

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APPSGDVQTKYGQCGGNGWTGPTVCAPGSSCSVLNEWYSQCL

CCGTCCTCAACGAGTGGTACTCCCAGTGTTTGTAA

GH61a Variant 9 polypeptide sequence without the signal sequence:

(SEQ ID NO: 12) <u>MSKASALLAGLTGAALVAA</u>HGHVSHIVVNGVYYRNYDPTTDWYQPNPPTV

 ${\tt IGWTAADQDNGFVEPNSFGTPDIICHKSATPGGGHATVAAGDKINIQWTP}$ ${\tt EWPESHIGPVIDYLAACNGDCETVDKSSLRWFKIDGAGYDKAAGRWAADA}$

LRANGNSWLVQIPSDLKAGNYVLRHEIIALHGAQSPNGAQNYPQCINLRV

-continued

TGGGSNLPSGVAGTSLYKATDPGILFNPYVSSPDYTVPGPALIAGAASSI

 ${\tt AQSTSVATATGTATVPGGGGANPTATTTAATSAAPSTTLRTTTTSAAQTT}$

APPSGDVQTKYGQCGGNGWTGPTVCAPGSSCSVLNEWYSQCL

[0307] The polynucleotide (SEQ ID NO:13) and amino acid (SEQ ID NO:14) sequences of an *M. thermophila* GH61b are provided below. The signal sequence is shown underlined in SEQ ID NO:14. SEQ ID NO:15 provides the sequence of this GH61b without the signal sequence.

(SEQ ID NO: 13) ATGAAGCTCTCCTCTTTTCCGTCCTGGCCACTGCCCTCACCGTCGAGGG GCATGCCATCTTCCAGAAGGTCTCCGTCAACGGAGCGGACCAGGGCTCCC TCACCGGCCTCCGCGCTCCCAACAACAACAACCCCGTGCAGAATGTCAAC AGCCAGGACATGATCTGCGGCCAGTCGGGATCGACGTCGAACACTATCAT CGAGGTCAAGGCCGGCGATAGGATCGGTGCCTGGTATCAGCATGTCATCG GCGGTGCCCAGTTCCCCAACGACCCAGACAACCCGATTGCCAAGTCGCAC AAGGGCCCCGTCATGGCCTACCTCGCCAAGGTTGACAATGCCGCAACCGC CAGCAAGACGGGCCTGAAGTGGTTCAAGATTTGGGAGGATACCTTTAATC CCAGCACCAAGACCTGGGGTGTCGACAACCTCATCAACAACAACGGCTGG GTGTACTTCAACCTCCCGCAGTGCATCGCCGACGGCAACTACCTCCTCCG CGTCGAGGTCCTCGCTCTGCACTCGGCCTACTCCCAGGGCCAGGCTCAGT ${\tt TCTACCAGTCCTGCGCCCAGATCAACGTATCCGGCGGCGGCTCCTTCACG}$ $\tt CCGGCGTCGACTGTCAGCTTCCCGGGTGCCTACAGCGCCAGCGACCCCGG$ ${\tt TATCCTGATCAACATCTACGGCGCCACCGGCCAGCCCGACAACAACGGCC}$ AGCCGTACACTGCCCCTGGGCCCGCGCCCATCTCCTGC

(SEQ ID NO: 14)

MKLSLFSVLATALTVEGHAIFQKVSVNGADQGSLTGLRAPNNNNPVQNVN

SQDMICGQSGSTSNTIIEVKAGDRIGAWYQHVIGGAQFPNDPDNPIAKSH

KGPVMAYLAKVDNAATASKTGLKWFKIWEDTFNPSTKTWGVDNLINNNGW

VYFNLPQCIADGNYLLRVEVLALHSAYSQGQAQFYQSCAQINVSGGGSFT

PASTVSFPGAYSASDPGILINIYGATGQPDNNGQPYTAPGPAPISC

(SEQ ID NO: 15)
IFQKVSVNGADQGSLTGLRAPNNNNPVQNVNSQDMICGQSGSTSNTIIEV
KAGDRIGAWYQHVIGGAQFPNDPDNPIAKSHKGPVMAYLAKVDNAATASK
TGLKWFKIWEDTFNPSTKTWGVDNLINNNGWVYFNLPQCIADGNYLLRVE
VLALHSAYSQGQAQFYQSCAQINVSGGGSFTPASTVSFPGAYSASDPGIL
INIYGATGQPDNNGQPYTAPGPAPISC

[0308] The polynucleotide (SEQ ID NO:16) and amino acid (SEQ ID NO:17) sequences of an *M. thermophila* GH61c are provided below. The signal sequence is shown underlined in SEQ ID NO:17. SEQ ID NO:18 provides the sequence of this GH61c without the signal sequence.

(SEQ ID NO: 16)

ATGGCCCTCCAGCTCTTGGCGAGCTTGGCCCTCCTCTCAGTGCCGGCCCT TGCCCACGGTGGCTTGGCCAACTACACCGTCGGTGATACTTGGTACAGAG GCTACGACCCAAACCTGCCGCCGGAGACGCAGCTCAACCAGACCTGGATG ATCCAGCGGCAATGGGCCACCATCGACCCCGTCTTCACCGTGTCGGAGCC GTACCTGGCCTGCAACAACCCGGGCGCGCCGCCGCCCTCGTACATCCCCA ${\tt TCCGCGCCGGTGACAAGATCACGGCCGTGTACTGGTACTGGCTGCACGCC}$ ATCGGGCCCATGAGCGTCTGGCTCGCGCGGTGCGGCGACACGCCCGCGGC CGACTGCCGCGACGTCGACGTCAACCGGGTCGGCTGGTTCAAGATCTGGG AGGGCGGCCTGCTGGAGGGTCCCAACCTGGCCGAGGGGCTCTGGTACCAA AAGGACTTCCAGCGCTGGGACGGCTCCCCGTCCCTCTGGCCCGTCACGAT CCCCAAGGGGCTCAAGAGCGGGACCTACATCATCCGGCACGAGATCCTGT CGCTTCACGTCGCCCTCAAGCCCCAGTTTTACCCGGAGTGTGCGCATCTG AATATTACTGGGGGGGGAGACTTGCTGCCACCCGAAGAGACTCTGGTGCG GTTTCCGGGGGTTTACAAAGAGGACGATCCCTCTATCTTCATCGATGTCT ACTCGGAGGAGAACGCGAACCGGACAGATTATACGGTTCCGGGAGGGCCA ATCTGGGAAGGG

(SEQ ID NO: 17)

MALQLLASLALLSVPALAHGGLANYTVGDTWYRGYDPNLPPETQLNQTWM

IQRQWATIDPVFTVSEPYLACNNPGAPPPSYIPIRAGDKITAVYWYWLHA

IGPMSVWLARCGDTPAADCRDVDVNRVGWFKIWEGGLLEGPNLAEGLWYQ

KDFQRWDGSPSLWPVTIPKGLKSGTYIIRHEILSLHVALKPQFYPECAHL

NITGGGDLLPPEETLVRFPGVYKEDDPSIFIDVYSEENANRTDYTVPGGP

IWEG

(SEQ ID NO: 18)
NYTVGDTWYRGYDPNLPPETQLNQTWMIQRQWATIDPVFTVSEPYLACNN
PGAPPPSYIPIRAGDKITAVYWYWLHAIGPMSVWLARCGDTPAADCRDVD
VNRVGWFKIWEGGLLEGPNLAEGLWYQKDFQRWDGSPSLWPVTIPKGLKS
GTYIIRHEILSLHVALKPQFYPECAHLNITGGGDLLPPEETLVRFPGVYK
EDDPSIFIDVYSEENANRTDYTVPGGPIWEG

[0309] The polynucleotide (SEQ ID NO:19) and amino acid (SEQ ID NO:20) sequences of an *M. thermophila* GH61d are provided below. The signal sequence is shown underlined in SEQ ID NO:20. SEQ ID NO:21 provides the sequence of this GH61d without the signal sequence.

(SEQ ID NO: 19)
ATGAAGGCCCTCTCTCTCTCTGCGGCTGCCGGGGCAGTCTCTGCGCATAC
CATCTTCGTCCAGCTCGAAGCAGACGGCACGAGGTACCCGGTTTCGTACG
GGATCCGGGACCCAACCTACGACGGCCCCATCACCGACGTCACATCCAAC
GACGTTGCTTGCAACGGCGGTCCGAACCCGACGACCCCTCCAGCGACGT
CATCACCGTCACCGCGGGCACCACCGTCAAGGCCATCTGGAGGCACACCC
TCCAATCCGGCCCGGACGATCTCATGGACGCCAGCACAAGGGCCCGACC

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CTGGCCTACATCAAGAAGGTCGGCGATGCCACCAAGGACTCGGGCGTCGG
CGGTGGCTGGTTCAAGATCCAGGAGGACGGTTACAACAACGGCCAGTGGG
GCACCAGCACCGTTATCTCCAACGGCGGCGAGACACTACATTGACATCCCG
GCCTGCATCCCCGAGGGTCAGTACCTCCTCCGCGCCGAGATGATCGCCCT
CCACGCGGCCGGGTCCCCCGGCGGCGCTCAGCTCTACATGGAATGTGCCC
AGATCAACATCGTCGGCGGCTCCGGCTCGGTCCCAGCTCGACGGTCAGC
TTCCCCGGCGCGTATAGCCCCAACGACCCGGGTCTCCTCATCAACATCTA
TTCCATGTCGCCCTCGAGCTCGTACACCATCCCGGGCCCGCTTTTCA
AGTGC

(SEQ ID NO: 20)

MKALSLLAAAGAVSA
HTIFVQLEADGTRYPVSYGIRDPTYDGPITDVTSN

DVACNGGPNPTTPSSDVITVTAGTTVKAIWRHTLQSGPDDVMDASHKGPT

LAYIKKVGDATKDSGVGGGWFKIQEDGYNNGQWGTSTVISNGGEHYIDIP

ACIPEGQYLLRAEMIALHAAGSPGGAQLYMECAQINIVGGSGSVPSSTVS

FPGAYSPNDPGLLINIYSMSPSSSYTIPGPPVFKC

(SEQ ID NO: 21)
HTIFVQLEADGTRYPVSYGIRDPTYDGPITDVTSNDVACNGGPNPTTPSS

DVITVTAGTTVKAIWRHTLQSGPDDVMDASHKGPTLAYIKKVGDATKDSG
VGGGWFKIQEDGYNNGQWGTSTVISNGGEHYIDIPACIPEGQYLLRAEMI
ALHAAGSPGGAQLYMECAQINIVGGSGSVPSSTVSFPGAYSPNDPGLLIN
IYSMSPSSSYTIPGPPVFKC

[0310] The polynucleotide (SEQ ID NO:22) and amino acid (SEQ ID NO:23) sequences of an *M. thermophila* GH61e are provided below. The signal sequence is shown underlined in SEQ ID NO:23. SEQ ID NO:24 provides the sequence of this GH61d without the signal sequence.

(SEQ ID NO: 28)

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CCCGTCACGCCATCATCATCATCAGCAGCAGCAGCAACAACAGCGGCGCCC
AAGATGACCAAGAAGATCCAGGAGCCCACCATCACATCGGTCACGGACCT
CCCCACCGACGAGGGCCAAGTGGATCGCGCTCCAAAAGATCTCGTACGTGG
ACCAGACGGGCACGGCGCGGACATACGAGCCGGCGTCGCGCAAGACGCGG
TCGCCAAGAGTCTAG

(SEQ ID NO: 23)

MKSSTPALFAAGLLAQHAAAHSIFQQASSGSTDFDTLCTRMPPNNSPVTS

VTSGDMTCKVGGTKGVSGFCEVNAGDEFTVEMHAQPGDRSCANEAIGGNH

FGPVLIYMSKVDDASTADGSGDWFKVDEFGYDASTKTWGTDKLNENCGKR

TFNIPSHIPAGDYLVRAEAIALHTANQPGGAQFYMSCYQVRISGGEGGQL

PAGVKIPGAYSANDPGILVDIWGNDFNDPPGHSARHAIIIISSSSNNSGA

KMTKKIQEPTITSVTDLPTDEAKWIALQKISYVDQTGTARTYEPASRKTR

SPRV

(SEQ ID NO: 24)
HSIFQQASSGSTDFDTLCTRMPPNNSPVTSVTSGDMTCKVGGTKGVSGFC
EVNAGDEFTVEMHAQPGDRSCANEAIGGNHFGPVLIYMSKVDDASTADGS
GDWFKVDEFGYDASTKTWGTDKLNENCGKRTFNIPSHIPAGDYLVRAEAI
ALHTANQPGGAQFYMSCYQVRISGGEGGQLPAGVKIPGAYSANDPGILVD
IWGNDFNDPPGHSARHAIIIISSSSNNSGAKMTKKIQEPTITSVTDLPTD
EAKWIALQKISYVDQTGTARTYEPASRKTRSPRV

[0311] The polynucleotide (SEQ ID NO:25) and amino acid (SEQ ID NO:26) sequences of an alternative *M. thermophila* GH61e are provided below. The signal sequence is shown underlined in SEQ ID NO:26. SEQ ID NO:27 provides the sequence of this GH61e without the signal sequence.

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CCTGCCGGAGTCAAGATCCCGGGCGCGTACAGTGCCAACGACCCCGGCAT
CCTTGTCGACATCTGGGGTAACGATTTCAACGAGTACGTTATTCCGGGCC
CCCCGGTCATCGACAGCAGCTACTTC

(SEQ ID NO: 26)

MKSSTPALFAAGLLAQHAAAHSIFQQASSGSTDFDTLCTRMPPNNSPVTS

VTSGDMTCNVGGTKGVSGFCEVNAGDEFTVEMHAQPGDRSCANEAIGGNH

FGPVLIYMSKVDDASTADGSGDWFKVDEFGYDASTKTWGTDKLNENCGKR

TFNIPSHIPAGDYLVRAEAIALHTANQPGGAQFYMSCYQVRISGGEGGQL

PAGVKIPGAYSANDPGILVDIWGNDFNEYVIPGPPVIDSSYF

(SEQ ID NO: 27)
HSIFQQASSGSTDFDTLCTRMPPNNSPVTSVTSGDMTCNVGGTKGVSGFC
EVNAGDEFTVEMHAQPGDRSCANEAIGGNHFGPVLIYMSKVDDASTADGS
GDWFKVDEFGYDASTKTWGTDKLNENCGKRTFNIPSHIPAGDYLVRAEAI
ALHTANQPGGAQFYMSCYQVRISGGEGGQLPAGVKIPGAYSANDPGILVD
IWGNDFNEYVIPGPPVIDSSYF

[0312] The polynucleotide (SEQ ID NO:28) and amino acid (SEQ ID NO:29) sequences of a *M. thermophila* GH61f are provided below. The signal sequence is shown underlined in SEQ ID NO:29. SEQ ID NO:30 provides the sequence of this GH61f without the signal sequence.

CGCTCACGCGACCTTCCAGGCCCTCTGGGTCGACGGCGTCGACTACGGCG CGCAGTGTGCCCGTCTGCCCGCGTCCAACTCGCCGGTCACCGACGTGACC TCCAACGCGATCCGCTGCAACGCCAACCCCTCGCCCGCTCGGGGCAAGTG CCCGGTCAAGGCCGGCTCGACCGTTACGGTCGAGATGCATCAGCAACCCG GTGACCGCTCGTGCAGCAGCGAGGCGATCGGCGGGGGGCGCACTACGGCCCC GTGATGGTGTACATGTCCAAGGTGTCGGACGCGGCGTCGGCGGACGGGTC GTCGGGCTGGTTCAAGGTGTTCGAGGACGGCTGGGCCAAGAACCCGTCCG GCGGGTCGGGCGACGACGACTACTGGGGCACCAAGGACCTGAACTCGTGC $\tt TGCGGGAAGATGAACGTCAAGATCCCCGCCGACCTGCCCTCGGGCGACTA$ GCGCCCAGTTCTACATGACCTGCTACCAGCTCACCGTGACCGGCTCCGGC AGCGCCAGCCGCCCACCGTCTCCTTCCCGGGCGCCTACAAGGCCACCGA $\tt CCCGGGCATCCTCGTCAACATCCACGCCCCGCTGTCCGGCTACACCGTGC$ $\tt CCGGCCCGGCCGTCTACTCGGGCGGCTCCACCAAGAAGGCCGGCAGCGCC$ $\tt TGCACCGGCTGCGAGTCCACTTGCGCCGTCGGCTCCGGCCCCACCGCCAC$ $\tt GCGGCTGCACCGTCCAGAAGTACCAGCAGTGCGGCGGCCAGGGCTACACC$ GGCTGCACCAACTGCGCGTCCGGCTCCACCTGCAGCGCGGTCTCGCCGCC CTACTACTCGCAGTGCGTC

(SEQ ID NO: 29)

MKSFTLTTLAALAGNAAAHATFQALWVDGVDYGAQCARLPASNSPVTDVT

SNAIRCNANPSPARGKCPVKAGSTVTVEMHQQPGDRSCSSEAIGGAHYGP

VMVYMSKVSDAASADGSSGWFKVFEDGWAKNPSGGSGDDDYWGTKDLNSC

CGKMNVKIPADLPSGDYLLRAEALALHTAGSAGGAQFYMTCYQLTVTGSG

SASPPTVSFPGAYKATDPGILVNIHAPLSGYTVPGPAVYSGGSTKKAGSA

CTGCESTCAVGSGPTATVSQSPGSTATSAPGGGGGCTVQKYQQCGGQGYT

GCTNCASGSTCSAVSPPYYSOCV

(SEQ ID NO: 30)
HATFQALWVDGVDYGAQCARLPASNSPVTDVTSNAIRCNANPSPARGKCP
VKAGSTVTVEMHQQPGDRSCSSEAIGGAHYGPVMVYMSKVSDAASADGSS
GWFKVFEDGWAKNPSGGSGDDDYWGTKDLNSCCGKMNVKIPADLPSGDYL
LRAEALALHTAGSAGGAQFYMTCYQLTVTGSGSASPPTVSFPGAYKATDP
GILVNIHAPLSGYTVPGPAVYSGGSTKKAGSACTGCESTCAVGSGPTATV
SQSPGSTATSAPGGGGGCTVQKYQQCGGQGYTGCTNCASGSTCSAVSPPY
YSQCV

[0313] The polynucleotide (SEQ ID NO:31) and amino acid (SEQ ID NO:32) sequences of an *M. thermophila* GH61g are provided below. The signal sequence is shown underlined in SEQ ID NO:32. SEQ ID NO:33 provides the sequence of this GH61g without the signal sequence.

(SEO ID NO: 31) ATGAAGGGACTCCTCGGCGCCCCCCCCTCTCGCTGGCCGTCAGCGATGT CTCGGCCCACTACATCTTTCAGCAGCTGACGACGGCGGCGCGTCAAGCACG CTGTGTACCAGTACATCCGCAAGAACACCAACTATAACTCGCCCGTGACC GATCTGACGTCCAACGACCTCCGCTGCAATGTGGGTGCTACCGGTGCGGG CACCGATACCGTCACGGTGCGCCGGCGGATTCGTTCACCTTCACGACCG ATACGCCCGTTTACCACCAGGGCCCGACCTCGATCTACATGTCCAAGGCC CCCGGCAGCGCGTCCGACTACGACGGCAGCGGCGGCTGGTTCAAGATCAA GGACTGGGCTGACTACACCGCCACGATTCCGGAATGTATTCCCCCCGGCG ACTACCTGCTTCGCATCCAGCAACTCGGCATCCACAACCCTTGGCCCGCG GGCATCCCCCAGTTCTACATCTCTTGTGCCCAGATCACCGTGACTGGTGG CGGCAGTGCCAACCCCGGCCCGACCGTCTCCATCCCAGGCGCCTTCAAGG AGACCGACCCGGGCTACACTGTCAACATCTACAACAACTTCCACAACTAC ACCGTCCCTGGCCCAGCCGTCTTCACCTGCAACGGTAGCGGCGGCAACAA CGGCGGCGCTCCAACCCAGTCACCACCACCACCACCACCACCACCAGGC TCCAGCTGCACCGTCGCGAAGTGGGGCCAGTGCGGAGGACAGGGTTACAG CGGCTGCACCGTGTGCGCGGCCGGGTCGACCTGCCAGAAGACCAACGACT ACTACAGCCAGTGCTTGTAG

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(SEQ ID NO: 32)

MKGLLGAAALSLAVSDVSAHYIFQQLTTGGVKHAVYQYIRKNTNYNSPVT

DLTSNDLRCNVGATGAGTDTVTVRAGDSFTFTTDTPVYHQGPTSIYMSKA

PGSASDYDGSGGWFKIKDWADYTATIPECIPPGDYLLRIQQLGIHNPWPA

GIPQFYISCAQITVTGGGSANPGPTVSIPGAFKETDPGYTVNIYNNFHNY

TVPGPAVFTCNGSGGNNGGGSNPVTTTTTTTTRPSTSTAQSQPSSSPTSP

SSCTVAKWGQCGGQGYSGCTVCAAGSTCQKTNDYYSQCL

(SEQ ID NO: 33)
HYIFQQLTTGGVKHAVYQYIRKNTNYNSPVTDLTSNDLRCNVGATGAGTD

TVTVRAGDSFTFTTDTPVYHQGPTSIYMSKAPGSASDYDGSGGWFKIKDW
ADYTATIPECIPPGDYLLRIQQLGIHNPWPAGIPQFYISCAQITVTGGGS
ANPGPTVSIPGAFKETDPGYTVNIYNNFHNYTVPGPAVFTCNGSGGNNGG
GSNPVTTTTTTTTRPSTSTAQSQPSSSPTSPSSCTVAKWGQCGGQGYSGC
TVCAAGSTCQKTNDYYSQCL

[0314] The polynucleotide (SEQ ID NO:34) and amino acid (SEQ ID NO:35) sequences of an alternative *M. thermophila* GH61g are provided below. The signal sequence is shown underlined in SEQ ID NO:35. SEQ ID NO:36 provides the sequence of this GH61g without the signal sequence.

(SEQ ID NO: 34) $\tt CTGACGACGGCGCGTCAAGCACGCTGTGTACCAGTACATCCGCAAGAA$ CACCAACTATAACTCGCCCGTGACCGATCTGACGTCCAACGACCTCCGCT GCAATGTGGGTGCTACCGGTGCGGGCACCGATACCGTCACGGTGCGCGCC GGCGATTCGTTCACCTTCACGACCGATACGCCCGTTTACCACCAGGGCCC GACCTCGATCTACATGTCCAAGGCCCCCGGCAGCGCGTCCGACTACGACG GCAGCGGCGGCTGGTTCAAGATCAAGGACTGGGGTGCCGACTTTAGCAGC GGCCAGGCCACCTGGACCTTGGCGTCTGACTACACCGCCACGATTCCGGA ATGTATTCCCCCCGGCGACTACCTGCTTCGCATCCAGCAACTCGGCATCC ACAACCCTTGGCCCGCGGGCATCCCCCAGTTCTACATCTCTTGTGCCCAG ATCACCGTGACTGGTGGCGGCAGTGCCAACCCCGGCCCGACCGTCTCCAT CCCAGGCGCCTTCAAGGAGACCGACCCGGGCTACACTGTCAACATCTACA ACAACTTCCACAACTACACCGTCCCTGGCCCAGCCGTCTTCACCTGCAAC $\tt GGTAGCGGCGGCAACAACGGCGGCGCTCCAACCCAGTCACCACCACCAC$ $\tt CACCACCACCAGGCCGTCCACCAGCACCGCCCAGTCCCAGCCGTCGT$ $\tt CGAGCCCGACCAGCCCCTCCAGCTGCACCGTCGCGAAGTGGGGCCAGTGC$ GGAGGACAGGGTTACAGCGGCTGCACCGTGTGCGCGGCCGGGTCGACCTG CCAGAAGACCAACGACTACTACAGCCAGTGCTTG

(SEQ ID NO: 35)

MKGLLGAAALSLAVSDVSAHYIFQQLTTGGVKHAVYQYIRKNTNYNSPVT

DLTSNDLRCNVGATGAGTDTVTVRAGDSFTFTTDTPVYHQGPTSIYMSKA

PGSASDYDGSGGWFKIKDWGADFSSGQATWTLASDYTATIPECIPPGDYL

(SEQ ID NO: 40)

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LRIQQLGIHNPWPAGIPQFYISCAQITVTGGGSANPGPTVSIPGAFKETD
PGYTVNIYNNFHNYTVPGPAVFTCNGSGGNNGGGSNPVTTTTTTTTRPST
STAQSQPSSSPTSPSSCTVAKWGQCGGQGYSGCTVCAAGSTCQKTNDYYS
OCL

(SEQ ID NO: 36)
HYIFQQLTTGGVKHAVYQYIRKNTNYNSPVTDLTSNDLRCNVGATGAGTD
TVTVRAGDSFTFTTDTPVYHQGPTSIYMSKAPGSASDYDGSGGWFKIKDW
GADFSSGQATWTLASDYTATIPECIPPGDYLLRIQQLGIHNPWPAGIPQF
YISCAQITVTGGGSANPGPTVSIPGAFKETDPGYTVNIYNNFHNYTVPGP
AVFTCNGSGGNNGGGSNPVTTTTTTTTRPSTSTAQSQPSSSPTSPSSCTV
AKWGQCGGQGYSGCTVCAAGSTCQKTNDYYSQCL

[0315] The polynucleotide (SEQ ID NO:37) and amino acid (SEQ ID NO:38) sequences of an *M. thermophila* GH61h are provided below. The signal sequence is shown underlined in SEQ ID NO:38. SEQ ID NO:39 provides the sequence of this GH61h without the signal sequence.

(SEO ID NO: 37) ATGTCTTCCTTCACCTCCAAGGGTCTCCTTTCCGCCCTCATGGGCGCGCC AACGGTTGCCGCCCACGGTCACGTCACCAACATCGTCATCAACGGCGTCT CATACCAGAACTTCGACCCATTCACGCACCCTTATATGCAGAACCCTCCG ${\tt ACGGTTGTCGGCTGGACCGCGAGCAACACGGACAACGGCTTCGTCGGCCC}$ CGAGTCCTTCTCTAGCCCGGACATCATCTGCCACAAGTCCGCCACCAACG CTGGCGGCCATGCCGTCGCGGCCGGCCGATAAGGTCTTCATCCAGTGG GACACCTGGCCCGAGTCGCACCACGGTCCGGTCATCGACTATCTCGCCGA CTGCGGCGACGCGGGCTGCGAGAAGGTCGACAAGACCACGCTCAAGTTCT TCAAGATCAGCGAGTCCGGCCTGCTCGACGGCACTAACGCCCCCGGCAAG TGGGCGTCCGACACGCTGATCGCCAACAACAACTCGTGGCTGGTCCAGAT CCCGCCCAACATCGCCCCGGGCAACTACGTCCTGCGCCACGAGATCATCG CCCTGCACAGCGCCGGCCAGCAGAACGGCGCCCAGAACTACCCTCAGTGC TTCAACCTGCAGGTCACCGGCTCCGGCACTCAGAAGCCCTCCGGCGTCCT CGGCACCGAGCTCTACAAGGCCACCGACGCCGGCATCCTGGCCAACATCT $\tt GCCTCCGCCGTCCAGCAGACCACCTCGGCCATCACCGCCTCTGCTAGCGC$ ${\tt CATCACCGGCTCCGCTACCGCCGCCGCCCACCGCCTGCCACCACCACCGCCG}$ $\tt CCGCCGCCGCCACCACTACCACCGCTGGCTCCGGTGCTACCGCCACG$ CCCTCGACCGGCGGCTCTCCTTCTTCCGCCCAGCCTGCTCCTACCACCGC TGCCGCTACCTCCAGCCCTGCTCGCCCGACCCGCTGCGCTGGTCTGAAGA AGCGCCGTCGCCACGCCCGTGACGTCAAGGTTGCCCTC

(SEQ ID NO: 38) MSSFTSKGLLSALMGAATVAAHGHVTNIVINGVSYQNFDPFTHPYMQNPP
TVVGWTASNTDNGFVGPESFSSPDIICHKSATNAGGHAVVAAGDKVFIQW

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DTWPESHHGPVIDYLADCGDAGCEKVDKTTLKFFKISESGLLDGTNAPGK
WASDTLIANNNSWLVQIPPNIAPGNYVLRHEIIALHSAGQQNGAQNYPQC
FNLQVTGSGTQKPSGVLGTELYKATDAGILANIYTSPVTYQIPGPAIISG
ASAVQQTTSAITASASAITGSATAAPTAATTTAAAAATTTTTAGSGATAT
PSTGGSPSSAQPAPTTAAATSSPARPTRCAGLKKRRHARDVKVAL

(SEQ ID NO: 39)
AHGHVTNIVINGVSYQNFDPFTHPYMQNPPTVVGWTASNTDNGFVGPESF
SSPDIICHKSATNAGGHAVVAAGDKVFIQWDTWPESHHGPVIDYLADCGD
AGCEKVDKTTLKFFKISESGLLDGTNAPGKWASDTLIANNNSWLVQIPPN
IAPGNYVLRHEIIALHSAGQQNGAQNYPQCFNLQVTGSGTQKPSGVLGTE
LYKATDAGILANIYTSPVTYQIPGPAIISGASAVQQTTSAITASASAITG
SATAAPTAATTTAAAAATTTTTAGSGATATPSTGGSPSSAQPAPTTAAAT
SSPARPTRCAGLKKRRRHARDVKVAL

[0316] The polynucleotide (SEQ ID NO:40) and amino acid (SEQ ID NO:41) sequences of an *M. thermophila* GH61i are provided below. The signal sequence is shown underlined in SEQ ID NO:41. SEQ ID NO:42 provides the sequence of this GH61i without the signal sequence.

(SEQ ID NO: 41)

MKTLAALVVSAALVAAHGYVDHATIGGKDYQFYQPYQDPYMGDNKPDRVS

RSIPGNGPVEDVNSIDLQCHAGAEPAKLHAPAAAGSTVTLYWTLWPDSHV

GPVITYMARCPDTGCQDWSPGTKPVWFKIKEGGREGTSNTPLMTAPSAYT

YTIPSCLKSGYYLVRHEIIALHSAWQYPGAQFYPGCHQLQVTGGGSTVPS

TNLVSFPGAYKGSDPGITYDAYKAQPYTIPGPAVFTC

(SEQ ID NO: 42)
YVDHATIGGKDYQFYQPYQDPYMGDNKPDRVSRSIPGMGPVEDVNSIDLQ
CHAGAEPAKLHAPAAAGSTVTLYWTLWPDSHVGPVITYMARCPDTGCQDW
SPGTKPVWFKIKEGGREGTSNTPLMTAPSAYTYTIPSCLKSGYYLVRHEI
IALHSAWQYPGAQFYPGCHQLQVTGGGSTVPSTNLVSFPGAYKGSDPGIT
YDAYKAQPYTIPGPAVFTC

[0317] The polynucleotide (SEQ ID NO:43) and amino acid (SEQ ID NO:44) sequences of an alternative *M. thermophila* GH61i are provided below. The signal sequence is shown underlined in SEQ ID NO:44. SEQ ID NO:45 provides the sequence of this GH61i without the signal sequence.

(SEO ID NO: 43) CGGCTATGTTGACCACGCCACGATCGGTGGCAAGGATTATCAGTTCTACC AGCCGTACCAGGACCCTTACATGGGCGACACAACAAGCCCGATAGGGTTTTCC CGCTCCATCCCGGGCAACGGCCCCGTGGAGGACGTCAACTCCATCGACCT CCGGCTCGACCGTGACGCTCTACTGGACCCTCTGGCCCGACTCCCACGTC GGCCCCGTCATCACCTACATGGCTCGCTGCCCGACACCGGCTGCCAGGA GTGAGGGCACCTCCAATGTCTGGGCTGCTACCCCGCTCATGACGGCCCCC ${\tt TCCGCCTACACCTACACGATCCCGTCCTGCCTCAAGAGCGGCTACTACCT}$ CGTCCGCCACGAGATCATCGCCCTGCACTCGGCCTGGCAGTACCCCGGCG CCCAGTTCTACCCGGGCTGCCACCAGCTCCAGGTCACCGGCGGCGGCTCC ACCGTGCCCTCTACCAACCTGGTCTCCTTCCCCGGCGCCTACAAGGGGAG CGACCCCGGCATCACCTACGACGCTTACAAGGCGCAACCTTACACCATCC CTGGCCCGGCCGTGTTTACCTGC

(SEQ ID NO: 44)

MKTLAALVVSAALVAAHGYVDHATIGGKDYQFYQPYQDPYMGDNKPDRVS

RSIPGNGPVEDVNSIDLQCHAGAEPAKLHAPAAAGSTVTLYWTLWPDSHV

GPVITYMARCPDTGCQDWSPGTKPVWFKIKEGGREGTSNVWAATPLMTAP

SAYTYTIPSCLKSGYYLVRHEIIALHSAWQYPGAQFYPGCHQLQVTGGGS

TVPSTNLVSFPGAYKGSDPGITYDAYKAQPYTIPGPAVFTC

(SEQ ID NO: 45)
YVDHATIGGKDYQFYQPYQDPYMGDNKPDRVSRSIPGMGPVEDVNSIDLQ
CHAGAEPAKLHAPAAAGSTVTLYWTLWPDSHVGPVITYMARCPDTGCQDW
SPGTKPVWFKIKEGGREGTSNVWAATPLMTAPSAYTYTIPSCLKSGYYLV
RHEIIALHSAWQYPGAQFYPGCHQLQVTGGGSTVPSTNLVSFPGAYKGSD
PGITYDAYKAQPYTIPGPAVFTC

[0318] The polynucleotide (SEQ ID NO:46) and amino acid (SEQ ID NO:47) sequences of an *M. thermophila* GH61j are provided below. The signal sequence is shown

underlined in SEQ ID NO:47. SEQ ID NO:48 provides the sequence of this GH61j without the signal sequence.

(SEQ ID NO: 47)

MRYFLQLAAAAAFAVNSAAGHYIFQQFATGGSKYPPWKYIRRNTNPDWLQ

NGPVTDLSSTDLRCNVGGQVSNGTETITLNAGDEFSFILDTPVYHAGPTS

LYMSKAPGAVADYDGGGAWFKIYDWGPSGTSWTLSGTYTQRIPKCIPDGE

YLLRIQQIGLHNPGAAPQFYISCAQVKVVDGGSTNPTPTAQIPGAFHSND

PGLTVNIYNDPLTNYVVPGPRVSHW

(SEQ ID NO: 48)
HYIFQQFATGGSKYPPWKYIRRNTNPDWLQNGPVTDLSSTDLRCNVGGQV
SNGTETITLNAGDEFSFILDTPVYHAGPTSLYMSKAPGAVADYDGGGAWF
KIYDWGPSGTSWTLSGTYTQRIPKCIPDGEYLLRIQQIGLHNPGAAPQFY
ISCAQVKVVDGGSTNPTPTAQIPGAFHSNDPGLTVNIYNDPLTNYVVPGP

[0319] The polynucleotide (SEQ ID NO:49) and amino acid (SEQ ID NO:50) sequences of an *M. thermophila* GH61k are provided below. The signal sequence is shown underlined in SEQ ID NO:50. SEQ ID NO:51 provides the sequence of this GH61k without the signal sequence.

(SEQ ID NO: 49)
ATGCACCCTTCCTTCTTTTCACGCTTGGGCTGGCGAGCGTGCTTGTCCC
CCTCTCGTCTGCACACACTACCTTCACGACCCTCTTCGTCAACGATGTCA
ACCAAGGTGATGGTACCTGCATTCGCATGGCGAAGAAGAGGGCAATGTCGCC
ACCCATCCTCTCGCAGGCGGTCTCGACTCCGAAGACATGGCCTGTGGTCG
GGATGGTCAAGAACCCGTGGCATTTACGTGTCCGGCCCCAGCTGGTGCCA
AGTTGACTCTCGAGTTTCGCATGTGGCCGATGCTTCGCAGTCCGGATCG
ATCGATCCATCCCACCTTGGCGTCATGGCCATCTACCTCAAGAAGGTTTC

CGACATGAAATCTGACGCGGCCGCTGGCCCGGGCTGGTTCAAGATTTGGG ACCAAGGCTACGACTTGGCGGCCAAGAAGTGGGCCACCGAGAAGCTCATC GACAACAACGGCCTCCTGAGCGTCAACCTTCCAACCGGCTTACCAACCGG $\tt CTACTACCTCGCCCGCCAGGAGATCATCACGCTCCAAAACGTTACCAATG$ ${\tt ACAGGCCAGAGCCCCAGTTCTACGTCGGCTGCGCACAGCTCTACGTCGAG}$ GGCACCTCGGACTCACCCATCCCCTCGGACAAGACGGTCTCCATTCCCGG CCACATCAGCGACCCGGCCGACCCGGGCCTGACCTTCAACGTCTACACGG GCGACGCATCCACCTACAAGCCGCCCGGCCCCGAGGTTTACTTCCCCACC ACCACCACCACCTCCTCCTCCTCCTCCGGAAGCAGCGACAACAAGGG AGCCAGGCGCCAGCAAACCCCCGACGACAAGCAGGCCGACGGCCTCGTTC CAGCCGACTGCCTCAAGAACGCGAACTGGTGCGCCGCTGCCCTGCCG CCGTACACCGACGAGGCCGGCTGCTGGGCCGCCGCCGAGGACTGCAACAA GCAGCTGGACGCGTGCTACACCAGCGCACCCCCCTCGGGCAGCAAGGGGT GCAAGGTCTGGGAGGAGCAGGTGTGCACCGTCGTCTCGCAGAAGTGCGAG GCCGGGGATTTCAAGGGGCCCCCGCAGCTCGGGAAGGAGCTCGGCGAGGG GATCGATGAGCCTATTCCGGGGGGAAAGCTGCCCCCGGCGGTCAACGCGG GAGAGAACGGGAATCATGGCGGAGGTGGTGATGATGATGATGATGAT AATGATGAGGCCGGGGCTGGGGCAGCGTCGACTCTGGCTCCC ${\tt TGGTGCGGCCAAGACTCCCCAACCAAACTCCGAGAGGGCCCGGCGCCGTG}$ AGGCGCATTGGCGGCGACTGGAATCTGCTGAG

(SEQ ID NO: 50)

MHPSLLFTLGLASVLVPLSSAHTTFTTLFVNDVNQGDGTCIRMAKKGNVA

THPLAGGLDSEDMACGRDGQEPVAFTCPAPAGAKLTLEFRMWADASQSGS

IDPSHLGVMAIYLKKVSDMKSDAAAGPGWFKIWDQGYDLAAKKWATEKLI

DNNGLLSVNLPTGLPTGYYLARQEIITLQNVTNDRPEPQFYVGCAQLYVE

GTSDSPIPSDKTVSIPGHISDPADPGLTFNVYTGDASTYKPPGPEVYFPT

TTTTTSSSSSGSSDNKGARRQQTPDDKQADGLVPADCLVKNANWCAAALP

PYTDEAGCWAAAEDCNKQLDACYTSAPPSGSKGCKVWEEQVCTVVSQKCE

AGDFKGPPQLGKELGEGIDEPIPGGKLPPAVNAGENGNHGGGGGDDGDDD

NDEAGAGAAASTPTFAAPGAAKTPQPNSERARRREAHWRRLESAE

(SEQ ID NO: 51)
HTTFTTLFVNDVNQGDGTCIRMAKKGNVATHPLAGGLDSEDMACGRDGQE
PVAFTCPAPAGAKLTLEFRMWADASQSGSIDPSHLGVMAIYLKKVSDMKS
DAAAGPGWFKIWDQGYDLAAKKWATEKLIDNNGLLSVNLPTGLPTGYYLA
RQEIITLQNVTNDRPEPQFYVGCAQLYVEGTSDSPIPSDKTVSIPGHISD
PADPGLTFNVYTGDASTYKPPGPEVYFPTTTTTTSSSSSGSSDNKGARRQ
QTPDDKQADGLVPADCLVKNANWCAAALPPYTDEAGCWAAAEDCNKQLDA
CYTSAPPSGSKGCKVWEEQVCTVVSQKCEAGDFKGPPQLGKELGEGIDEP
IPGGKLPPAVNAGENGNHGGGGGDDGDDDNDEAGAGAASTPTFAAPGAAK
TPQPNSERARRREAHWRRLESAE

[0320] The polynucleotide (SEQ ID NO:52) and amino acid (SEQ ID NO:53) sequences of a *M. thermophila* GH611 are provided below. The signal sequence is shown underlined in SEQ ID NO:53. SEQ ID NO:54 provides the sequence of this GH611 without the signal sequence.

(SEQ ID NO: 52) ATGTTTTCTCTCAAGTTCTTTATCTTGGCCGGTGGGCTTGCTGTCCTCAC CGAGGCTCACATAAGACTAGTGTCGCCCGCCCCTTTTACCAACCCTGACC AGGGCCCCAGCCCACTCCTAGAGGCTGGCAGCGACTATCCCTGCCACAAC $\tt GGCAATGGGGGGGTTATCAGGGAACGCCAACCCAGATGGCAAAGGGTTC$ ${\tt TAAGCAGCAGCTAGCCTTCCAGGGGTCTGCCGTTCATGGGGGTGGCTCCT}$ GCCAAGTGTCCATCACCTACGACGAAAACCCGACCGCTCAGAGCTCCTTC AAGGTCATTCACTCGATTCAAGGTGGCTGCCCCGCCAGGGCCGAGACGAT CCCGGATTGCAGCGCACAAAATATCAACGCCTGCAATATAAAGCCCGATA ATGCCCAGATGGACACCCCGGATAAGTATGAGTTCACGATCCCGGAGGAT CTCCCCAGTGGCAAGGCCACCCTCGCCTGGACATGGATCAACACTATCGG CAACCGCGAGTTTTATATGGCATGCGCCCCGGTTGAGATCACCGGCGACG GCGGTAGCGAGTCGGCTCTGGCTGCCCGACATGGTCATTGCCAAC ATCCCGTCCATCGGAGGAACCTGCGCGACCGAGGAGGGGGAAGTACTACGA ATATCCCAACCCCGGTAAGTCGGTCGAAACCATCCCGGGCTGGACCGATT TGGTTCCCCTGCAAGGCGAATGCGGTGCTGCCTCCGGTGTCTCGGGCTCC GGCGGAAACGCCAGCAGTGCTACCCCTGCCGCAGGGGCCGCCCCGACTCC TGCTGTCCGCGGCCGCCGTCCCACCTGGAACGCC

(SEQ ID NO: 53)

MFSLKFFILAGGLAVLTEAHIRLVSPAPFTNPDQGPSPLLEAGSDYPCHN

GNGGGYQGTPTQMAKGSKQQLAFQGSAVHGGGSCQVSITYDENPTAQSSF

KVIHSIQGGCPARAETIPDCSAQNINACNIKPDNAQMDTPDKYEFTIPED

LPSGKATLAWTWINTIGNREFYMACAPVEITGDGGSESALAALPDMVIAN

IPSIGGTCATEEGKYYEYPNPGKSVETIPGWTDLVPLQGECGAASGVSGS

GGNASSATPAAGAAPTPAVRGRRPTWNA

(SEQ ID NO: 54)
HIRLVSPAPFTNPDQGPSPLLEAGSDYPCHNGNGGGYQGTPTQMAKGSKQ
QLAFQGSAVHGGGSCQVSITYDENPTAQSSFKVIHSIQGGCPARAETIPD
CSAQNINACNIKPDNAQMDTPDKYEFTIPEDLPSGKATLAWTWINTIGNR
EFYMACAPVEITGDGGSESALAALPDMVIANIPSIGGTCATEEGKYYEYP
NPGKSVETIPGWTDLVPLQGECGAASGVSGSGGNASSATPAAGAAPTPAV
RGRRPTWNA

[0321] The polynucleotide (SEQ ID NO:55) and amino acid (SEQ ID NO:56) sequences of a *M. thermophila* GH61m are provided below. The signal sequence is shown underlined in SEQ ID NO:56. SEQ ID NO:57 provides the sequence of this GH61m without the signal sequence.

(SEQ ID NO: 55)

(SEQ ID NO: 56)

MKLATLLAALTLGVADQLSVGSRKFGVYEHIRKNTNYNSPVTDLSDTNLR

CNVGGGSGTSTTVLDVKAGDSFTFFSDVAVYHQGPISLCVDRTSAESMDG

REPDMRCRTGSQAGYLAVTDYDGSGDCFKIYDWGPTFNGGQASWPTRNSY

EYSILKCIRDGEYLLRIQSLAIHNPGALPQFYISCAQVNVTGGGTVTPRS

RRPILIYFNFHSYIVPGPAVFKC

(SEQ ID NO: 57)

DQLSVGSRKFGVYEHIRKNTNYNSPVTDLSDTNLRCNVGGGSGTSTTVLD
VKAGDSFTFFSDVAVYHQGPISLCVDRTSAESMDGREPDMRCRTGSQAGY
LAVTDYDGSGDCFKIYDWGPTFNGGQASWPTRNSYEYSILKCIRDGEYLL
RIQSLAIHNPGALPQFYISCAQVNVTGGGTVTPRSRRPILIYFNFHSYIV
PGPAVFKC

[0322] The polynucleotide (SEQ ID NO:58) and amino acid (SEQ ID NO:59) sequences of an alternative *M. thermophila* GH61m are provided below. The signal sequence is shown underlined in SEQ ID NO:59. SEQ ID NO:60 provides the sequence of this GH61m without the signal sequence.

(SEQ ID NO: 58)

-continued

GGGGACCGACGTTCAACGGGGGCCAGGCGTCGTGGCCGACGAGGAATTCG
TACGAGTACAGCATCCTCAAGTGCATCAGGGACGGCGAATACCTACTGCG
GATTCAGTCCCTGGCCATCCATAACCCAGGTGCCCTTCCGCAGTTCTACA
TCAGCTGCGCCCAGGTGAATGTGACGGGCGGAGGCACCATCTATTTCAAC
TTCCACTCGTATATCGTCCCTGGGCCGGCAGTGTTCAAGTGC

(SEQ ID NO: 59)

MKLATLLAALTLGLSVGSRKFGVYEHIRKNTNYNSPVTDLSDTNLRCNVG

GGSGTSTTVLDVKAGDSFTFFSDVAVYHQGPISLCVDRTSAESMDGREPD

MRCRTGSQAGYLAVTVMTVTDYDGSGDCFKIYDWGPTFNGGQASWPTRNS

YEYSILKCIRDGEYLLRIQSLAIHNPGALPQFYISCAQVNVTGGGTIYFN

FHSYTVPGPAVFKC

(SEQ ID NO: 60)
RKFGVYEHIRKNTNYNSPVTDLSDTNLRCNVGGGSGTSTTVLDVKAGDSF
TFFSDVAVYHQGPISLCVDRTSAESMDGREPDMRCRTGSQAGYLAVTVMT
VTDYDGSGDCFKIYDWGPTFNGGQASWPTRNSYEYSILKCIRDGEYLLRI
OSLAIHNPGALPOFYISCAOVNVTGGGTIYFNFHSYIVPGPAVFKC

[0323] The polynucleotide (SEQ ID NO:61) and amino acid (SEQ ID NO:62) sequences of a *M. thermophila* GH61n are provided below.

(SEQ ID NO: 61)
ATGACCAAGAATGCGCAGAGCAAGCAGGCGTTGAGAACCCAACAAGCGG
CGACATCCGCTGCTACACCTCGCAGACGGCGGCCAACGTCGTGACCGTGC
CGGCCGGCTCGACCATTCACTACATCTCGACCCAGCAGATCAACCACCCC
GGCCCGACTCAGTACTACCTGGCCAAGGTACCCCCGGGCTCGTCGGCCAA
GACCTTTGACGGGTCCGGCGCCGTCTGGTTCAAGATCTCGACCACGATGC
CTACCGTGGACAGCAACAAGCAGATGTTCTGGCCAGGGCAGAACACTTAT
GAGACCTCAAACACCACCATTCCCGCCAACACCCCGGACGGCGAGTACCT
CCTTCGCGTCAAGCAGATCGCCCTCCACATGGCGTCTCAGCCCAACAAGG
TCCAGTTCTACCTCGCCTGCACCCAGATCAAGATCACCGGTGGTCGCAAC
GGCACCCCCAGCCCGTTGGTCGCGCTGCCCGGAGCCTACAAGAGCACCGA
CCCCGGCATCCTGGTCGACATCTACTCCATGAAGCCCGAATCGTACCAGC
CTCCCGGGCCGCCGTTTGGCGGCGGCTAA

(SEQ ID NO: 62)
MTKNAQSKQGVENPTSGDIRCYTSQTAANVVTVPAGSTIHYISTQQINHP
GPTQYYLAKVPPGSSAKTFDGSGAVWFKISTTMPTVDSNKQMFWPGQNTY
ETSNTTIPANTPDGEYLLRVKQIALHMASQPNKVQFYLACTQIKITGGRN
GTPSPLVALPGAYKSTDPGILVDIYSMKPESYQPPGPPVWRG

[0324] The polynucleotide (SEQ ID NO:63) and amino acid (SEQ ID NO:64) sequences of an alternative *M. thermophila* GH61n are provided below. The signal sequence is shown underlined in SEQ ID NO:64. SEQ ID NO:65 provides the sequence of this GH61n without the signal sequence.

CGGGCCGCCGTCTGGCGCGGC

(SEQ ID NO: 63)
ATGAGGCTTCTCGCAAGCTTGTTGCTCGCAGCTACGGCTGTTCAAGCTCA
CTTTGTTAACGGACAGCCCGAAGAGAGTGACTGGTCAGCCACGCGCATGA
CCAAGAATGCGCAGAGCAAGCAGGGCGTTGAGAACCCAACAAGCGGCGAC
ATCCGCTGCTACACCTCGCAGACGGCGGCCAACGTCGTGACCGTGCCGGC
CGGCTCGACCATTCACTACATCTCGACCCAGCAGATCAACCACCCCGGCC
CGACTCAGTACTACCTGGCCAAGGTACCCCCGGCTCGTCGGCCAAGACC
TTTGACGGGTCCGGCGCGTCTGGTTCAAGATCTCGACCACGATGCCTAC
CGTGGACAGCAACAAGCAGATGTTCTGGCCAGGGCAGAACACTTATGAGA
CCTCAAACACCACCATTCCCGCCAACACCCCGGACGGCGAGTACCTCCTT
CGCGTCAAGCAGATCGCCCTCCACATGGCGTCTCAGCCCAACAAGGTCCA
GTTCTACCTCGCCTGCACCCAGATCAAGATCACCGGTGGTCGCAACGGCA
CCCCCAGCCCGCTGGTCGGCCTGCCCGGAGCCTACAAGAGCACCCCGG

(SEQ ID NO: 64)

MRLLASLLLAATAVQAHFVNGQPEESDWSATRMTKNAQSKQGVENPTSGD

IRCYTSQTAANVVTVPAGSTIHYISTQQINHPGPTQYYLAKVPPGSSAKT

FDGSGAVWFKISTTMPTVDSNKQMFWPGQNTYETSNTTIPANTPDGEYLL

RVKQIALHMASQPNKVQFYLACTQIKITGGRNGTPSPLVALPGAYKSTDP

GILVDIYSMKPESYQPPGPPVWRG

(SEQ ID NO: 65)
HFVNGQPEESDWSATRMTKNAQSKQGVENPTSGDIRCYTSQTAANVVTVP
AGSTIHYISTQQINHPGPTQYYLAKVPPGSSAKTFDGSGAVWFKISTTMP
TVDSNKQMFWPGQNTYETSNTTIPANTPDGEYLLRVKQIALHMASQPNKV
QFYLACTQIKITGGRNGTPSPLVALPGAYKSTDPGILVDIYSMKPESYQP
PGPPVWRG

[0325] The polynucleotide (SEQ ID NO:66) and amino acid (SEQ ID NO:67) sequences of an alternative *M. thermophila* GH610 are provided below. The signal sequence is shown underlined in SEQ ID NO:67. SEQ ID NO:68 provides the sequence of this GH610 without the signal sequence.

-continued
GCGTGTGGGCCTACCTGATGCGCTCGACGTCGACCTGCACAGCGCC
TCGAGCCCCGGCGGCGCCCAGTTCTACATGGGCTGTGCACAGATCGAAGT
CACTGGCTCCGGCACCAACTCGGGCTCCGACTTTGTCTCGTTCCCCGGCG
CCTACTCGGCCAACGACCCGGGCATCTTGCTGAGCATCTACGACAGCTCG
GGCAAGCCCAACAATGGCGGGCGCTCGTACCCGATCCCCGGCCCC
CATCTCCTGCTCCGGCAGCGGCGGCGGCGACAACAACGGCGGCGACGCG
GCGACGACAACAACGGTGGTGGCAACAACAACGGCGGCGGCGCCCC
CTGTACGGGCAGTGCGGCGCGCACCACCTGTGC
CCAGGGAACTTGCAAGGTGTCGAACGAATACTACAGCCAGTGCCTCCCC

(SEQ ID NO: 67)

MKPFSLVALATAVSGHAIFQRVSVNGQDQGQLKGVRAPSSNSPIQNVNDA

NMACNANIVYHDNTIIKVPAGARVGAWWQHVIGGPQGANDPDNPIAASHK

GPIQVYLAKVDNAATASPSGLKWFKVAERGLNNGVWAYLMRVELLALHSA

SSPGGAQFYMGCAQIEVTGSGTNSGSDFVSPPGAYSANDPGILLSIYDSS

GKPNNGGRSYPIPGPRPISCSGSGGGGNNGGDGGDDNNGGGNNNGGGSVP

LYGQCGGIGYTGPTTCAQGTCKVSNEYYSQCLP

(SEQ ID NO: 68)
HAIFQRVSVNGQDQGQLKGVRAPSSNSPIQNVNDANMACNANIVYHDNTI
IKVPAGARVGAWWQHVIGGPQGANDPDNPIAASHKGPIQVYLAKVDNAAT
ASPSGLKWFKVAERGLNNGVWAYLMRVELLALHSASSPGGAQFYMGCAQI
EVTGSGTNSGSDFVSFPGAYSANDPGILLSIYDSSGKPNNGGRSYPIPGP
RPISCSGSGGGGNNGGDGGDDNNGGGNNNGGGSVPLYGQCGGIGYTGPTT
CAQGTCKVSNEYYSQCLP

[0326] The polynucleotide (SEQ ID NO:69) and amino acid (SEQ ID NO:70) sequences of a *M. thermophila* GH61p are provided below. The signal sequence is shown underlined in SEQ ID NO:70. SEQ ID NO:71 provides the sequence of this GH61p without the signal sequence.

(SEQ ID NO: 69)
ATGAAGCTCACCTCGTCCCTCGCTGTCCTGGCCGCTGCCGGCGCCCAGGC
TCACTATACCTTCCCTAGGGCCGCACTGGTGTTCGCTCTCTGGCGAGT
GGGAGGTGGTCCGCATGACCGAGAACCATTACTCGCACGGCCCGGTCACC
GATGTCACCAGCCCCGAGATGACCTGCTATCAGTCCGGCGTGCAGGGTGC
GCCCCAGACCGTCCAGGTCAAGGCGGCCTCCCAATTCACCTTCAGCGTGG
ATCCCTCCATCGGCCACCCCGGCCCTCTCCAGTTCTACATGGCTAAGGTG
CCGTCGGGCCAGACGGCCCCCCTCCCAGTCTACATGCTTACCT
CAAGATCTACCAAGACGGCCCGAACGGCCTCGGCACCGACAGCATTACCT
GGCCCAGCGCCGGCAAAACCGAGGTCTCGGTCACCATCCCCAGCTGCATC
GAGGATGGCGAGAAACCGAGGTCTCGGTCACCATCCCCAGCTGCATC

(SEQ ID NO: 70)

MKLTSSLAVLAAAGAQAHYTFPRAGTGGSLSGEWEVVRMTENHYSHGPVT

DVTSPEMTCYQSGVQGAPQTVQVKAGSQFTFSVDPSIGHPGPLQFYMAKV

PSGQTAATFDGTGAVWFKIYQDGPNGLGTDSITWPSAGKTEVSVTIPSCI

EDGEYLLRVEHTPLPTAPAAQNRARSSPSPAAYKATDPGILFQLYWPIPT

EYINPGPAPVSC

(SEQ ID NO: 71)
HYTFPRAGTGGSLSGEWEVVRMTENHYSHGPVTDVTSPEMTCYQSGVQGA
PQTVQVKAGSQFTFSVDPSIGHPGPLQFYMAKVPSGQTAATFDGTGAVWF
KIYQDGPNGLGTDSITWPSAGKTEVSVTIPSCIEDGEYLLRVEHTPLPTA
PAAQNRARSSPSPAAYKATDPGILFQLYWPIPTEYINPGPAPVSC

[0327] The polynucleotide (SEQ ID NO:72) and amino acid (SEQ ID NO:73) sequences of an alternative *M. thermophila* GH61p are provided below. The signal sequence is shown underlined in SEQ ID NO:73. SEQ ID NO:74 provides the sequence of this GH61p without the signal sequence.

(SEQ ID NO: 73)

MKLTSSLAVLAAAGAQAHYTFPRAGTGGSLSGEWEVVRMTENHYSHGPVT

DVTSPEMTCYQSGVQGAPQTVQVKAGSQFTFSVDPSIGHPGPLQFYMAKV

PSGQTAATFDGTGAVWFKIYQDGPNGLGTDSITWPSAGKTEVSVTIPSCI

EDGEYLLRVEHIALHSASSVGGAQFYIACAQLSVTGGSGTLNTGSLVSLP

GAYKATDPGILFQLYWPIPTEYINPGPAPVSC

-continued

(SEQ ID NO: 74)
HYTFPRAGTGGSLSGEWEVVRMTENHYSHGPVTDVTSPEMTCYQSGVQGA
PQTVQVKAGSQFTFSVDPSIGHPGPLQFYMAKVPSGQTAATFDGTGAVWF
KIYQDGPNGLGTDSITWPSAGKTEVSVTIPSCIEDGEYLLRVEHIALHSA
SSVGGAQFYIACAQLSVTGGSGTLNTGSLVSLPGAYKATDPGILFQLYWP
IPTEYINPGPAPVSC

[0328] The polynucleotide (SEQ ID NO:75) and amino acid (SEQ ID NO:76) sequences of an alternative *M. thermophila* GH61q are provided below. The signal sequence is shown underlined in SEQ ID NO:76. SEQ ID NO:77 provides the sequence of this GH61q without the signal sequence.

(SEQ ID NO: 75)
ATGCCGCCACCACGACTGAGCACCTCCTTCCCCTCCTAGCCTTAATAGC
CCCCACCGCCCTGGGGCACTCCCACCTCGGGTACATCATCAACGGCG
AGGTATACCAAGGATTCGACCCGCGGCCGGAGCAGGCGAACTCGCCGTTG
CGCGTGGGCTGGTCGACGGGGGAATCGACGGGTTCGTGGCGCCGGC
CAACTACTCGTCGCCCGACATCATCTGCCACATCGAGGGGGCCAGCCCGC
CGGCGCACGCCCCGTCCGGGCGGGCGACCGGGTGCACTGCAATGGAAC
GGCTGGCCGCTCGGACACGTGGGGCCGGTGCACTGGCGCCCTG
CGGCGGGGTGGACACGTGGGGCCGGTGCACAGCGGC
AGCTGCGGTGGACAAGGTGGAGCGCTGCCGGGGTGGACAAGCGGC

(SEQ ID NO: 76)

MPPPRLSTLLPLLALIAPTALGHSHLGYIIINGEVYQGFDPRPEQANSPL

RVGWSTGAIDDGFVAPANYSSPDIICHIEGASPPAHAPVRAGDRVHVQWN

GWPLGHVGPVLSYLAPCGGLEGSESGCAGVDKRQLRWTKVDDSLPAMEL

(SEQ ID NO: 77)

HSHLGYIIINGEVYQGFDPRPEQANSPLRVGWSTGAIDDGFVAPANYSSP DIICHIEGASPPAHAPVRAGDRVHVQWNGWPLGHVGPVLSYLAPCGGLEG SESGCAGVDKRQLRWTKVDDSLPAMEL

[0329] The polynucleotide (SEQ ID NO:78) and amino acid (SEQ ID NO:79) sequences of an alternative *M. thermophila* GH61q are provided below. The signal sequence is shown underlined in SEQ ID NO:79. SEQ ID NO:80 provides the sequence of this GH61q without the signal sequence.

(SEQ ID NO: 78)
ATGCCGCCACCACGACTGAGCACCTCCTTCCCCTCCTAGCCTTAATAGC
CCCCACCGCCCTGGGGCACTCCCACCTCGGGTACATCATCATCAACGGCG
AGGTATACCAAGGATTCGACCCGCGGCCGGAGCAGGCGAACTCGCCGTTG
CGCGTGGGCTGGTCGACGGGGGCAATCGACGGGTTCGTGGCGCCGGC
CAACTACTCGTCGCCCGACATCATCTGCCACATCGAGGGGGCCAGCCCGC
CGGCGCACGCGCCCGTCCGGGCGGGCGACCGGGTGCACTGCAATGGAAA

(SEQ ID NO: 81)

continued CGGCTGGCCGCTCGGACACGTGGGGCCGGTGCTGTCGTACCTGGCGCCCT GCGGCGGGCTGGAGGGGTCCGAGAGCGGGTGGACGACTCGCTGCCGGCGA TGGAGCTGGTCGGGGCCGCGGGGGGGCGGGGGGGGGGCGACGACGGCAGC $\tt GGCAGCGACGGCAGCGGCAGCGGCAGCGGACGCGTCGGCGTGCCCGG$ GCAGCGCTGGGCCACCGACGTGTTGATCGCGGCCAACAACAGCTGGCAGG ${\tt TCGAGATCCCGCGCGGGCTGCGGGACGGGCCGTACGTGCTGCGCCACGAG}$ GCTCTGCGTCAACCTGTGGGTCGAGGGCGGCGACGCAGCATGGAGCTGG ACCACTTCGACGCCACCCAGTTCTACCGGCCCGACGACCCGGGCATCCTG CTCAACGTGACGGCCGGCCTGCGCTCATACGCCGTGCCGGGCCCGACGCT GGCCGCGGGGCGACGCCGGTGCCGTACGCGCAGCAGAACATCAGCTCGG CGAGGGCGGATGGAACCCCCGTGATTGTCACCAGGAGCACGGAGACGGTG CCCTTCACCGCGGCACCCACGCCAGCCGAGACGCCAGAGCCAAAGGGGG GAGGTATGATGACCAAACCCGAACTAAAGACCTAAATGAACGCTTCTTTT ATAGTAGCCGGCCAGAACAGAAGAGGCTGACAGCGACCTCAAGAAGGGAA CTAGTTGATCATCGTACCCGGTACCTCTCCGTAGCTGTCTGCGCAGATTT CGGCGCTCATAAGGCAGCAGAAACCAACCACGAAGCTTTGAGAGGCGGCA

(SEQ ID NO: 79)

MPPPRLSTLLPLLALIAPTALGHSHLGYIIINGEVYQGFDPRPEQANSPL

RVGWSTGAIDDGFVAPANYSSPDIICHIEGASPPAHAPVRAGDRVHVQWK

RLAARTRGAGAVVPGALRRAGGVRERVDDSLPAMELVGAAGGAGGEDDGS

GSDGSGSGGSGRVGVPGQRWATDVLIAANNSWQVEIPRGLRDGPYVLRHE

IVALHYAAEPGGAQNYPLCVNLWVEGGDGSMELDHFDATQFYRPDDPGIL

LNVTAGLRSYAVPGPTLAAGATPVPYAQQNISSARADGTPVIVTRSTETV

PFTAAPTPAETAEAKGGRYDDQTRTKDLNERFFYSSRPEQKRLTATSRRE

LVDHRTRYLSVAVCADFGAHKAAETNHEALRGGNKHHGGVSE

ATAAGCACCATGGCGGTGTTTCAGAG

(SEQ ID NO: 80)
HSHLGYIIINGEVYQGFDPRPEQANSPLRVGWSTGAIDDGFVAPANYSSP
DIICHIEGASPPAHAPVRAGDRVHVQWKRLAARTRGAGAVVPGALRRAGG
VRERVDDSLPAMELVGAAGGAGGEDDGSGSDGSGSGGSGRVGVPGQRWAT
DVLIAANNSWQVEIPRGLRDGPYVLRHEIVALHYAAEPGGAQNYPLCVNL
WVEGGDGSMELDHFDATQFYRPDDPGILLNVTAGLRSYAVPGPTLAAGAT
PVPYAQQNISSARADGTPVIVTRSTETVPFTAAPTPAETAEAKGGRYDDQ
TRTKDLNERFFYSSRPEQKRLTATSRRELVDHRTRYLSVAVCADFGAHKA
AETNHEALRGGNKHHGGVSE

[0330] The polynucleotide (SEQ ID NO:81) and amino acid (SEQ ID NO:82) sequences of an *M. thermophila* GH61r are provided below. The signal sequence is shown underlined in SEQ ID NO:82. SEQ ID NO:83 provides the sequence of this GH61r without the signal sequence.

(SEQ ID NO: 83)
HATFQQLWHGSSCVRLPASNSPVTNVGSRDFVCNAGTRPVSGKCPVKAGG
TVTIEMHQQPGDRSCNNEAIGGAHWGPVQVYLTKVQDAATADGSTGWFKI
FSDSWSKKPGGNLGDDDNWGTRDLNACCGKMD

[0331] The polynucleotide (SEQ ID NO:84) and amino acid (SEQ ID NO:85) sequences of an alternative *M. thermophila* GH61r are provided below. The signal sequence is shown underlined in SEQ ID NO:85. SEQ ID NO:86 provides the sequence of this GH61r without the signal sequence.

(SEO ID NO: 84) ATGAGGTCGACATTGGCCGGTGCCCTGGCAGCCATCGCTGCTCAGAAAGT AGCCGGCCACGTTTCAGCAGCTCTGGCACGGCTCCTCCTGTGTCC GCCTTCCGGCTAGCAACTCACCCGTCACCAATGTGGGAAGCAGAGACTTC GTCTGCAACGCTGGCACCCGCCCCGTCAGTGGCAAGTGCCCCGTGAAGGC TGGCGGCACCGTCACCATCGAGATGCACCAGCAACCCGGCGACCGCAGCT GCAACAACGAAGCCATCGGAGGGGCGCATTGGGGCCCCGTCCAGGTGTAC CTGACCAAGGTTCAGGACGCCGGCGACGGCCGACGGCTCGACGGCTGGTT CAAGATCTTCTCCGACTCGTGGTCCAAGAAGCCCGGGGGCAACTCGGGCG ACGACGACAACTGGGGCACGCGCGACCTGAACGCCTGCTGCGGGAAGATG ${\tt GACGTGGCCATCCCGGCCGACATCGCGTCGGGCGACTACCTGCTGCGGGC}$ $\tt CGAGGCGCTGGCCCTGCACACGGCCGGACAGGCCGGCGGCGCCCAGTTCT$ ACATGAGCTGCTACCAGATGACGGTCGAGGGCGGCTCCGGGACCGCCAAC CCGCCCACCGTCAAGTTCCCGGGCGCCTACAGCGCCCAACGACCCGGGCAT CCTCGTCAACATCCACGCCCCCCTTTCCAGCTACACCGCGCCCGGCCCGG CCGTCTACGCGGCGCACCATCCGCGAGGCCGGCTCCGCCTGCACCGGC TGCGCGCAGACCTGCAAGGTCGGGTCGTCCCCGAGCGCCGTTGCCCCCGG CAGCGGCGCGGCAACGGCGGCGGGTTCCAACCCCGA

(SEQ ID NO: 85)

MRSTLAGALAAIAAQKVAGHATFQQLWHGSSCVRLPASNSPVTNVGSRDF

VCNAGTRPVSGKCPVKAGGTVTIEMHQQPGDRSCNNEAIGGAHWGPVQVY

LTKVQDAATADGSTGWFKIFSDSWSKKPGGNSGDDDNWGTRDLNACCGKM

DVAIPADIASGDYLLRAEALALHTAGQAGGAQFYMSCYQMTVEGGSGTAN

PPTVKFPGAYSANDPGILVNIHAPLSSYTAPGPAVYAGGTIREAGSACTG

CAOTCKVGSSPSAVAPGSGAGNGGGFOPR

(SEQ ID NO: 86)
HATFQQLWHGSSCVRLPASNSPVTNVGSRDFVCNAGTRPVSGKCPVKAGG
TVTIEMHQQPGDRSCNNEAIGGAHWGPVQVYLTKVQDAATADGSTGWFKI
FSDSWSKKPGGNSGDDDNWGTRDLNACCGKMDVAIPADIASGDYLLRAEA
LALHTAGQAGGAQFYMSCYQMTVEGGSGTANPPTVKFPGAYSANDPGILV
NIHAPLSSYTAPGPAVYAGGTIREAGSACTGCAQTCKVGSSPSAVAPGSG
AGNGGGFQPR

[0332] The polynucleotide (SEQ ID NO:87) and amino acid (SEQ ID NO:88) sequences of an *M. thermophila* GH61s are provided below. The signal sequence is shown underlined in SEQ ID NO:88. SEQ ID NO:89 provides the sequence of this GH61s without the signal sequence.

(SEQ ID NO: 87) ATGCTCCTCACCCTAGCCACACTCGTCACCCTCCTGGCGCGCCACGT CTCGGCTCACGCCCGGCTGTTCCGCGTCTCTGTCGACGGGAAAGACCAGG GCGACGGGCTGAACAAGTACATCCGCTCGCCGGCGACCAACGACCCCGTG CGCGACCTCTCGAGCGCCGCCATCGTGTGCAACACCCAGGGGTCCAAGGC CGCCCGGACTTCGTCAGGGCCGCGGCCGGCGACAAGCTGACCTTCCTCT GGGCGCACGACAACCCGGACGACCCGGTCGACTACGTCCTCGACCCGTCC CACAAGGGCGCCATCCTGACCTACGTCGCCGCCTACCCCTCCGGGGACCC GACCGGCCCCATCTGGAGCAAGCTTGCCGAGGAAGGATTCACCGGCGGGC AGTGGGCGACCATCAAGATGATCGACAACGGCGGCAAGGTCGACGTGACG CTGCCCGAGGCCCTTGCGCCGGGAAAGTACCTGATCCGCCAGGAGCTGCT GGCCCTGCACCGGGCCGACTTTGCCTGCGACGACCCGGCCCACCCCAACC GCGGCGCCGAGTCGTACCCCAACTGCGTCCAGGTGGAGGTGTCGGGCAGC $\tt GGCGACAAGAAGCCGGACCAGAACTTTGACTTCAACAAGGGCTATACCTG$ CGATAACAAAGGACTCCACTTTAAGATCTACATCGGTCAGGACAGCCAGT ATGTGGCCCCGGGGCCGCGCCTTGGAATGGGAGC

(SEQ ID NO: 88)

MLLLTLATLVTLLARHVSAHARLFRVSVDGKDQGDGLNKYIRSPATNDPV

RDLSSAAIVCNTQGSKAAPDFVRAAAGDKLTFLWAHDNPDDPVDYVLDPS

HKGAILTYVAAYPSGDPTGPIWSKLAEEGFTGGQWATIKMIDNGGKVDVT

LPEALAPGKYLIRQELLALHRADFACDDPAHPNRGAESYPNCVQVEVSGS

GDKKPDQNFDFNKGYTCDNKGLHFKIYIGQDSQYVAPGPRPWNGS

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(SEQ ID NO: 89)
HARLFRVSVDGKDQGDGLNKYIRSPATNDPVRDLSSAAIVCNTQGSKAAP
DFVRAAAGDKLTFLWAHDNPDDPVDYVLDPSHKGAILTYVAAYPSGDPTG
PIWSKLAEEGFTGGQWATIKMIDNGGKVDVTLPEALAPGKYLIRQELLAL
HRADFACDDPAHPNRGAESYPNCVQVEVSGSGDKKPDQNFDFNKGYTCDN
KGLHFKIYIGQDSQYVAPGPRPWNGS

[0333] The polynucleotide (SEQ ID NO:90) and amino acid (SEQ ID NO:91) sequences of an M. thermophila GH61t are provided below.

(SEQ ID NO: 90)
ATGTTCACTTCGCTTTGCATCACAGATCATTGGAGGACTCTTAGCAGCCA
CTCTGGGCCAGTCATGAACTATCTCGCCCATTGCACCAATGACGACTGCA
AGTCTTTCAAGGGCGACAGCGGCAACGTCTGGGTCAAGATCGAGCAGCTC
GCGTACAACCCGTCAGCCAACCCCCCCTGGGCGTCTTGCCCCGGCG
AATATCTGCTGCGGCACGAGATCCTGGGTTGCACGTCGTGCACGGGGAACCGTG
ATGGGCGCCCAGTTCTACCCCGGCTGCACCCAGATCAGGGTCACCGAAGG
CGGGAGCACGAGCTGCCCTCGGGTATTGCGCTCCCAGGGCTTACGGCC
CACAAGACGAGGGTACTTGGTCGACTTGTGGAGGGTTAACCAGGGCCAG
GTCAACTACACGGCGCCTGGAGGACCCGTTTGGAGCGAAGCCTGGGACAC
CGAGTTTGGCGGGTCCAACACGACCGAGTGCCCACCATGCTCGACGACC
TGCTCGACTACATGCGGGCCAACGACGAGTGGATCGGCTGGACGGCCTAG
(SEQ ID NO: 91)

MFTSLCITDHWRTLSSHSGPVMNYLAHCTNDDCKSFKGDSGNVWVKIEQL
AYNPSANPPWASDLLREHGAKWKVTIPPSLVPGEYLLRHEILGLHVAGTV
MGAQFYPGCTQIRVTEGGSTQLPSGIALPGAYGPQDEGILVDLWRVNQGQ
VNYTAPGGPVWSEAWDTEFGGSNTTECATMLDDLLDYMAANDEWIGWTA

[0334] The polynucleotide (SEQ ID NO:92) and amino acid (SEQ ID NO:93) sequences of an alternative M. thermophila GH61t are provided below.

(SEQ ID NO: 92)
ATGAACTATCTCGCCCATTGCACCAATGACGACTGCAAGTCTTTCAAGGG
CGACAGCGGCAACGTCTGGGTCAAGATCGAGCACGTCGCGTACAACCCGT
CAGCCAACCCCCCTGGGCGTCTGACCTCCTCCGTGAGCACGGTGCCAAG
TGGAAGGTGACGATCCCGCCCAGTCTTGTCCCCGGCGAATATCTGCTGCG
GCACGAGATCCTGGGGTTGCACGTCGCAGGAACCGTGATGGGCGCCCAGT
TCTACCCCGGCTGCACCCAGATCAGGGTCACCGAAGGCGGGAGCACGCAG
CTGCCCTCGGGTATTGCGCTCCCAGGCGCTTACGGCCCACAAGACGAGGG
TATCTTGGTCGACTTGTGGAGGGTTAACCAGGGCCAGGTCAACTACACGG
CGCCTGGAGGACCCGTTTGGAGCGAAGCGTGGACACCGAGTTTGGCGGG
TCCAACACGACCGAGTGCGCCACCATGCTCGACGACCTGCTCGACTACAT

(SEO ID NO: 97)

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GGCGGCCAACGACGACCCATGCTGCACCGACCAGAACCAGTTCGGGAGTC
TCGAGCCGGGGAGCAAGGCGGCCGGCGGCTCGCCGAGCCTGTACGATACC
GTCTTGGTCCCCGTTCTCCAGAAGAAAGTGCCGACAAAGCTGCAGTGGAG
CGGACCGGCGAGCGTCAACGGGGATGAGTTGACAGAGAGGCCC

(SEQ ID NO: 93)
MNYLAHCTNDDCKSFKGDSGNVWVKIEQLAYNPSANPPWASDLLREHGAK
WKVTIPPSLVPGEYLLRHEILGLHVAGTVMGAQFYPGCTQIRVTEGGSTQ
LPSGIALPGAYGPQDEGILVDLWRVNQGQVNYTAPGGPVWSEAWDTEFGG
SNTTECATMLDDLLDYMAANDDPCCTDQNQFGSLEPGSKAAGGSPSLYDT
VLVPVLQKKVPTKLQWSGPASVNGDELTERP

[0335] The polynucleotide (SEQ ID NO:94) and amino acid (SEQ ID NO:95) sequences of an *M. thermophila* GH61u are provided below. The signal sequence is shown underlined in SEQ ID NO:95. SEQ ID NO:96 provides the sequence of this GH61u without the signal sequence.

(SEQ ID NO: 95)

MKLSAAIAVLAAALAEGHYTFPSIANTADWQYVRITTNFQSNGPVTDVNS

DQIRCYERNPGTGAPGIYNVTAGTTINYNAKSSISHPGPMAFYIAKVPAG

QSAATWDGKGAVWSKIHQEMPHFGTSLTWDSNGRTSMPVTIPRCLQDGEY

LLRAEHIALHSAGSPGGAQFYISCAQLSVTGGSGTWNPRNKVSFPGAYKA

TDPGILINIYYPVPTSYTPAGPPVDTC

GC

(SEQ ID NO: 96)
HYTFPSIANTADWQYVRITTNFQSNGPVTDVNSDQIRCYERNPGTGAPGI
YNVTAGTTINYNAKSSISHPGPMAFYIAKVPAGQSAATWDGKGAVWSKIH

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QEMPHFGTSLTWDSNGRTSMPVTIPRCLQDGEYLLRAEHIALHSAGSPGG AQFYISCAQLSVTGGSGTWNPRNKVSFPGAYKATDPGILINIYYPVPTSY TPAGPPVDTC

[0336] The polynucleotide (SEQ ID NO:97) and amino acid (SEQ ID NO:98) sequences of an *M. thermophila* GH61v are provided below. The signal sequence is shown underlined in SEQ ID NO:98. SEQ ID NO:99 provides the sequence of this GH61v without the signal sequence.

CCACGGCGCCGTGACCAGCTACAACATTGCGGGCAAGGACTACCCTGGAT ${\tt ACTCGGGCTTCGCCCCTACCGGCCAGGATGTCATCCAGTGGCAATGGCCC}$ GACTATAACCCCGTGCTGTCCGCCAGCGACCCCAAGCTCCGCTGCAACGG CGGCACCGGGGCGCTGTATGCCGAGGCGGCCCCCGGCGACACCATCA CGGCCACCTGGGCCCAGTGGACGCACTCCCAGGGCCCGATCCTGGTGTGG ATGTACAAGTGCCCCGGCGACTTCAGCTCCTGCGACGGCTCCGGCGCGGG TTGGTTCAAGATCGACGAGGCCGGCTTCCACGGCGACGGCACGACCGTCT TCCTCGACACCGAGACCCCCTCGGGCTGGGACATTGCCAAGCTGGTCGGC GGCAACAAGTCGTGGAGCAGCAAGATCCCTGACGGCCTCGCCCCGGGCAA TTACCTGGTCCGCCACGAGCTCATCGCCCTGCACCAGGCCAACAACCCGC AATTCTACCCCGAGTGCGCCCAGATCAAGGTCACCGGCTCTGGCACCGCC GAGCCCGCCGCCTCCTACAAGGCCGCCATCCCCGGCTACTGCCAGCAGAG ${\tt ACAAGATCCCCGGTCCCCCGGTCTTCAAGGGCACCGCCTCCGCCAAGGCT}$ CGCGCTTTCCAGGCC

(SEQ ID NO: 98)

MYRTLGSIALLAGGAAAHGAVTSYNIAGKDYPGYSGFAPTGQDVIQWQWP

DYNPVLSASDPKLRCNGGTGAALYAEAAPGDTITATWAQWTHSQGPILVW

MYKCPGDFSSCDGSGAGWFKIDEAGFHGDGTTVFLDTETPSGWDIAKLVG

GNKSWSSKIPDGLAPGNYLVRHELIALHQANNPQFYPECAQIKVTGSGTA

EPAASYKAAIPGYCQQSDPNISFNINDHSLPQEYKIPGPPVFKGTASAKA

RAFQA

(SEQ ID NO: 99)
AVTSYNIAGKDYPGYSGFAPTGQDVIQWQWPDYNPVLSASDPKLRCNGGT
GAALYAEAAPGDTITATWAQWTHSQGPILVWMYKCPGDFSSCDGSGAGWF
KIDEAGFHGDGTTVFLDTETPSGWDIAKLVGGNKSWSSKIPDGLAPGNYL
VRHELIALHQANNPQFYPECAQIKVTGSGTAEPAASYKAAIPGYCQQSDP
NISFNINDHSLPQEYKIPGPPVFKGTASAKARAFQA

[0337] The polynucleotide (SEQ ID NO:100) and amino acid (SEQ ID NO:101) sequences of an *M. thermophila* GH61w are provided below. The signal sequence is shown underlined in SEQ ID NO:101. SEQ ID NO:102 provides the sequence of this GH61w without the signal sequence.

(SEQ ID NO: 100)

(SEQ ID NO: 101)

MLTTTFALLTAALGVSAHYTLPRVGTGSDWQHVRRADNWQNNGFVGDVNS
EQIRCFQATPAGAQDVYTVQAGSTVTYHANPSIYHPGPMQFYLARVPDGQ
DVKSWTGEGAVWFKVYEEQPQFGAQLTWPSNGKSSFEVPIPSCIRAGNYL
LRAEHIALHVAQSQGGAQFYISCAQLQVTGGGSTEPSQKVSFPGAYKSTD
PGILININYPVPTSYONPGPAVFRC

(SEQ ID NO: 102)

HYTLPRVGTGSDWQHVRRADNWQNNGFVGDVNSEQIRCFQATPAGAQDVY
TVQAGSTVTYHANPSIYHPGPMQFYLARVPDGQDVKSWTGEGAVWFKVYE
EQPQFGAQLTWPSNGKSSFEVPIPSCIRAGNYLLRAEHIALHVAQSQGGA
QFYISCAQLQVTGGGSTEPSQKVSFPGAYKSTDPGILININYPVPTSYQN
PGPAVFRC

[0338] The polynucleotide (SEQ ID NO:103) and amino acid (SEQ ID NO:104) sequences of a *M. thermophila* GH61x are provided below. The signal sequence is shown underlined in SEQ ID NO:104. SEQ ID NO:105 provides the sequence of this GH61x without the signal sequence.

(SEQ ID NO: 103)

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AAGATCCCCGAGTGCATCGCCCCCGGCCAGTACCTCCTCCGCGCCGAGAT
GCTTGCCCTGCACGAGCTTCCAACTACCCCGGCGCTCAGTTCTACATGG
AGTGCGCCCAGCTCAATATCGTCGGCGGCACCGGCAGCAAGACGCCGTCC
ACCGTCAGCTTCCCGGGCGCTTACAAGGGTACCGACCCCGGAGTCAAGAT
CAACATCTACTGGCCCCCCGTCACCAGCTACCAGATTCCCGGCCCCGGCG
TGTTCACCTGC

(SEQ ID NO: 104)

 $\underline{\mathsf{MKVLAPLILAGAASA}} \text{HTIFSSLEVGGVNQGIGQGVRVPSYNGPIEDVTSN}$ SIACNGPPNPTTPTNKVITVRAGETVTAVWRYMLSTTGSAPNDIMDSSHK GPTMAYLKKVDNATTDSGVGGGWFKIQEDGLTNGVWGTERVINGQGRHNI KIPECIAPGQYLLRAEMLALHGASNYPGAQFYMECAQLNIVGGTGSKTPS TVSFPGAYKGTDPGVKINIYWPPVTSYQIPGPGVFTC

(SEQ ID NO: 105)

HTIFSSLEVGGVNQGIGQGVRVPSYNGPIEDVTSNSIACNGPPNPTTPTN

KVITVRAGETVTAVWRYMLSTTGSAPNDIMDSSHKGPTMAYLKKVDNATT

DSGVGGGWFKIQEDGLTNGVWGTERVINGQGRHNIKIPECIAPGQYLLRA

EMLALHGASNYPGAQFYMECAQLNIVGGTGSKTPSTVSFPGAYKGTDPGV

KINIYWPPVTSYQIPGPGVFTC

[0339] The polynucleotide (SEQ ID NO:106) and amino acid (SEQ ID NO:107) sequences of an *M. thermophila* GH61y are provided below. The signal sequence is underlined in SEQ ID NO:107. SEQ ID NO:108 provides the sequence of GH61y, without the signal sequence.

(SEQ ID NO: 106)

(SEQ ID NO: 107)

 ${\tt MIDNLPDDSLQPACLRPGHYLVRHEIIALHSAWAEGEAQFYPFPLFPFFP}$

SLLLSGNYTIPGPAIWKCPEAQQNE

PEAQQNE

(SEQ ID NO: 108)

HYLVRHEIIALHSAWAEGEAQFYPFPLFPFFPSLLLSGNYTIPGPAIWKC

[0340] Additional enzymes (i.e., non-GH61 enzymes) that find us in the present invention include, but are not limited to the following enzymes.

[0341] Wild-type EG1b cDNA (SEQ ID NO:109) and amino acid (SEQ ID NO:110) sequences are provided below. The signal sequence is underlined in SEQ ID NO:110. SEQ ID NO:111 provides the sequence of EG1b, without the signal sequence.

(SEQ ID NO: 109)

ATGGGGCAGAAGACTCTCCAGGGGCTGGTGGCGGCGGCGCGCACTGGCAGC CTCGGTGGCGAACGCGCAGCAACCGGGCACCTTCACGCCCGAGGTGCATC CGACGCTGCCGACGTGGAAGTGCACGACGAGCGGCGGGTGCGTCCAGCAG GACACGTCGGTGGTGCTCGACTGGAACTACCGCTGGTTCCACACCGAGGA CGGTAGCAAGTCGTGCATCACCTCTAGCGGCGTCGACCGGACCCTGTGCC CGGACGAGGCGACGTGCCCAAGAACTGCTTCGTCGAGGGCGTCAACTAC ACGAGCAGCGGGGTCGAGACGTCCGGCAGCTCCCTCACCCTCCGCCAGTT CTTCAAGGGCTCCGACGGCGCCATCAACAGCGTCTCCCCGCGCGTCTACC TGCTCGGGGGAGACGGCAACTATGTCGTGCTCAAGCTCCTCGGCCAGGAG CTGAGCTTCGACGTGGACGTATCGTCGCTCCCGTGCGGCGAGAACGCGGC CCTGTACCTGTCCGAGATGGACGCGACGGGAGGACGGAACGAGTACAACA CGGGCGGGCCGAGTACGGGTCGGGCTACTGTGACGCCCAGTGCCCCGTG CAGAACTGGAACAACGGGACGCTCAACACGGGCCGGGTGGGCTCGTGCTG CAACGAGATGGACATCCTCGAGGCCAACTCCAAGGCCGAGGCCTTCACGC CGCACCCTGCATCGGCAACTCGTGCGACAAGAGCGGGTGCGGCTTCAAC GCGTACGCGCGCGGTTACCACAACTACTGGGCCCCCGGCGCACGCTCGA CACGTCCCGGCCTTTCACCATGATCACCCGCTTCGTCACCGACGACGCCA CCACCTCGGGCAAGCTCGCCCGCATCGAGCGCGTCTACGTCCAGGACGGC AAGAAGGTGCCCAGCGCGCGCCCCGGGGGGGACGTCATCACGGCCGACGG GTGCACCTCCGCGCAGCCCTACGGCGGCCTTTCCGGCATGGGCGACGCCC ${\tt TCGGCCGCGGCATGGTCCTGGCCCTGAGCATCTGGAACGACGCGTCCGGG}$ TACATGAACTGGCTCGACGCCGGCAGCAACGGCCCCTGCAGCGACACCGA GGGTAACCCGTCCAACATCCTGGCCAACCACCCGGACGCCCACGTCGTGC TCTCCAACATCCGCTGGGGCGACATCGGCTCCACCGTCGACACCGGCGAT GGCGACAACAACGGCGGCGGCCCCAACCCGTCATCCACCACCACCGCTAC CGCTACCACCACCTCCTCCGGCCCGGCCGAGCCTACCCAGACCCACTACG GCCAGTGTGGAGGGAAAGGATGGACGGCCCTACCCGCTGCGAGACGCCC ${\tt TACACCTGCAAGTACCAGAACGACTGGTACTCGCAGTGCCTGTAG}$

(SEQ ID NO: 110)

MGQKTLQGLVAAAALAASVANAQQPGTFTPEVHPTLPTWKCTTSGGCVQQ

DTSVVLDWNYRWFHTEDGSKSCITSSGVDRTLCPDEATCAKNCFVEGVNY

TSSGVETSGSSLTLRQFFKGSDGAINSVSPRVYLLGGDGNYVVLKLLGQE

LSFDVDVSSLPCGENAALYLSEMDATGGRNEYNTGGAEYGSGYCDAQCPV

QNWNNGTLNTGRVGSCCNEMDILEANSKAEAFTPHPCIGNSCDKSGCGFN

AYARGYHNYWAPGGTLDTSRPFTMITRFVTDDGTTSGKLARIERVYVQDG

KKVPSAAPGGDVITADGCTSAQPYGGLSGMGDALGRGMVLALSIWNDASG

YMNWLDAGSNGPCSDTEGNPSNILANHPDAHVVLSNIRWGDIGSTVDTGD

GDNNGGGPNPSSTTTATATTTSSGPAEPTQTHYGQCGGKGWTGPTRCETP

YTCKYQNDWYSQCL

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QQPGTFTPEVHPTLPTWKCTTSGGCVQQDTSVVLDWNYRWFHTEDGSKSC

ITSSGVDRTLCPDEATCAKNCFVEGVNYTSSGVETSGSSLTLRQFFKGSD

GAINSVSPRVYLLGGDGNYVVLKLLGGELSFDVDVSSLPCGENAALVLSE

(SEO ID NO: 111)

(SEQ ID NO: 112)

GAINSVSPRVYLLGGDGNYVVLKLLGQELSFDVDVSSLPCGENAALYLSE
MDATGGRNEYNTGGAEYGSGYCDAQCPVQNWNNGTLNTGRVGSCCNEMDI
LEANSKAEAFTPHPCIGNSCDKSGCGFNAYARGYHNYWAPGGTLDTSRPF
TMITRFVTDDGTTSGKLARIERVYVQDGKKVPSAAPGGDVITADGCTSAQ
PYGGLSGMGDALGRGMVLALSIWNDASGYMNWLDAGSNGPCSDTEGNPSN
ILANHPDAHVVLSNIRWGDIGSTVDTGDGDNNGGGPNPSSTTTATATTTS

SGPAEPTOTHYGOCGGKGWTGPTRCETPYTCKYONDWYSOCL

[0342] Wild-type *M. thermophila* EG2 polynucleotide (SEQ ID NO:112) and amino acid (SEQ ID NO:113) sequences are provided below. The signal sequence is underlined in SEQ ID NO:113. SEQ ID NO:114 provides the sequence of EG2, without the signal sequence.

ATGAAGTCCTCCATCCTCGCCAGCGTCTTCGCCACGGGCGCCGTGGCTCA AAGTGGTCCGTGGCAGCAATGTGGTGGCATCGGATGGCAAGGATCGACCG ACTGTGTGTCGGGTTACCACTGCGTCTACCAGAACGATTGGTACAGCCAG ${\tt TGCGTGCCTGGCGCGGCGTCGACAACGCTCCAGACATCTACCACGTCCAG}$ GCCCACCGCCACCAGCACCGCCCCTCCGTCGTCCACCACCTCGCCTAGCA AGGGCAAGCTCAAGTGGCTCGGCAGCAACGAGTCGGGCGCCGAGTTCGGG GAGGGCAACTACCCCGGCCTCTGGGGCAAGCACTTCATCTTCCCGTCGAC TTCGGCGATTCAGACGCTCATCAATGATGGATACAACATCTTCCGGATCG ACTTCTCGATGGAGCGTCTGGTGCCCAACCAGTTGACGTCGTCCTTCGAC GAGGGCTACCTCCGCAACCTGACCGAGGTGGTCAACTTCGTGACGAACGC GGGCAAGTACGCCGTCCTGGACCCGCACAACTACGGCCGGTACTACGGCA ACGTCATCACGGACACGACGCGTTCCGGACCTTCTGGACCAACCTGGCC AAGCAGTTCGCCTCCAACTCGCTCGTCATCTTCGACACCAACAACGAGTA CAACACGATGGACCAGACCCTGGTGCTCAACCTCAACCAGGCCGCCATCG ACGGCATCCGGGCCGCCGCGCGCGCCCACTCCCAGTACATCTTCGTCGAGGGC AACGCGTGGAGCCGGGGCCTGGAGCTGGAACACGACCAACACCCAACATGGC CGCCCTGACGGACCCGCAGAACAAGATCGTGTACGAGATGCACCAGTACC ${\tt TCGACTCGGACAGCTCGGGCACCCACGCCGAGTGCGTCAGCAGCAACATC}$ GGCGCCCAGCGCGTCGTCGGAGCCACCCAGTGGCTCCGCGCCAACGGCAA $\tt GCTCGGCGTCCTCGGCGAGTTCGCCGGCGGCGCCCAACGCCGTCTGCCAGC$ AGGCCGTCACCGGCCTCCTCGACCACCTCCAGGACAACAGCGACGTCTGG CTGGGTGCCCTCTGGTGGGCCGCCGGTCCCTGGTGGGGCGACTACATGTA CTCGTTCGAGCCTCCTTCGGGCACCGGCTATGTCAACTACAACTCGATCC

TAAAGAAGTACTTGCCGTAA

(SEQ ID NO: 113)

MKSSILASVFATGAVAQSGPWQQCGGIGWQGSTDCVSGYHCVYQNDWYSQ

CVPGAASTTLQTSTTSRPTATSTAPPSSTTSPSKGKLKWLGSNESGAEFG

EGNYPGLWGKHFIFPSTSAIQTLINDGYNIFRIDFSMERLVPNQLTSSFD

EGYLRNLTEVVNFVTNAGKYAVLDPHNYGRYYGNVITDTNAFRTFWTNLA

KQFASNSLVIFDTNNEYNTMDQTLVLNLNQAAIDGIRAAGATSQYIFVEG

NAWSGAWSWNTTNTNMAALTDPQNKIVYEMHQYLDSDSSGTHAECVSSNI

GAQRVVGATQWLRANGKLGVLGEFAGGANAVCQQAVTGLLDHLQDNSEVW

LGALWWAAGPWWGDYMYSFEPPSGTGYVNYNSILKKYLP

(SEQ ID NO: 114)
QSGPWQQCGGIGWQGSTDCVSGYHCVYQNDWYSQCVPGAASTTLQTSTTS
RPTATSTAPPSSTTSPSKGKLKWLGSNESGAEFGEGNYPGLWGKHFIFPS
TSAIQTLINDGYNIFRIDFSMERLVPNQLTSSFDEGYLRNLTEVVNFVTN
AGKYAVLDPHNYGRYYGNVITDTNAFRTFWTNLAKQFASNSLVIFDTNNE
YNTMDQTLVLNLNQAAIDGIRAAGATSQYIFVEGNAWSGAWSWNTTNTNM
AALTDPQNKIVYEMHQYLDSDSSGTHAECVSSNIGAQRVVGATQWLRANG
KLGVLGEFAGGANAVCQQAVTGLLDHLQDNSEVWLGALWWAAGPWWGDYM
YSFEPPSGTGYVNYNSILKKYLP

[0343] The polynucleotide (SEQ ID NO:115) and amino acid (SEQ ID NO:116) sequences of a wild-type BGL are provided below. The signal sequence is underlined in SEQ ID NO:116. SEQ ID NO:117 provides the polypeptide sequence without the signal sequence.

(SEO ID NO: 115) ATGAAGGCTGCTGCGCTTTCCTGCCTCTTCGGCAGTACCCTTGCCGTTGC AGGCGCCATTGAATCGAGAAAGGTTCACCAGAAGCCCCTCGCGAGATCTG AACCTTTTTACCCGTCGCCATGGATGAATCCCAACGCCGACGGCTGGGCG GAGGCCTATGCCCAGGCCAAGTCCTTTGTCTCCCAAATGACTCTGCTAGA GAAGGTCAACTTGACCACGGGAGTCGGCTGGGGGGCTGAGCAGTGCGTCG GACTCCCCTCTCGGCATCCGAGGAGCCGACTACAACTCAGCGTTCCCCTC TGGCCAGACCGTTGCTGCTACCTGGGATCGCGGTCTGATGTACCGTCGCG GCTACGCAATGGGCCAGGAGGCCAAAGGCAAGGGCATCAATGTCCTTCTC GGACCAGTCGCCGGCCCCTTGGCCGCATGCCCGAGGGCGGTCGTAACTG GGAAGGCTTCGCTCCGGATCCCGTCCTTACCGGCATCGGCATGTCCGAGA CGATCAAGGGCATTCAGGATGCTGGCGTCATCGCTTGTGCGAAGCACTTT ATTGGAAACGAGCAGGAGCACTTCAGACAGGTGCCAGAAGCCCAGGGATA CGGTTACAACATCAGCGAAACCCTCTCCTCCAACATTGACGACAAGACCA TGCACGAGCTCTACCTTTGGCCGTTTGCCGATGCCGTCCGGGCCGGCGTC GGCTCTGTCATGTGCTCGTACCAGCAGGTCAACAACTCGTACGCCTGCCA GAACTCGAAGCTGCTGAACGACCTCCTCAAGAACGAGCTTGGGTTTCAGG

-continued

GCTTCGTCATGAGCGACTGGCAGGCACACACTGGCGCAGCAAGCGCC GTGGCTGGTCTCGATATGTCCATGCCGGGCGACACCCAGTTCAACACTGG $\tt CGTCAGTTTCTGGGGGCGCCAATCTCACCCTCGCCGTCCTCAACGGCACAG$ TCCCTGCCTACCGTCTCGACGACATGGCCATGCGCATCATGGCCGCCCTC TTCAAGGTCACCAAGACCACCGACCTGGAACCGATCAACTTCTCCTTCTG GACCGACGACACTTATGGCCCGATCCACTGGGCCGCCAAGCAGGGCTACC ${\tt AGGAGATTAATTCCCACGTTGACGTCCGCGCCGACCACGGCAACCTCATC}$ CGGGAGATTGCCGCCAAGGGTACGGTGCTGCTGAAGAATACCGGCTCTCT ACCCCTGAACAAGCCAAAGTTCGTGGCCGTCATCGGCGAGGATGCTGGGT CGAGCCCCAACGGCCCAACGGCTGCAGCGACCGCGGCTGTAACGAAGGC ACGCTCGCCATGGGCTGGGGATCCGGCACAGCCAACTATCCGTACCTCGT TTCCCCGACGCCGCTCCAGGCCCGGGCCATCCAGGACGCACGAGGT ACGAGAGCGTCCTGTCCAACTACGCCGAGGAAAAGACAAAGGCTCTGGTC TCGCAGGCCAATGCAACCGCCATCGTCTTCGTCAATGCCGACTCAGGCGA GGGCTACATCAACGTGGACGGTAACGAGGGCGACCGTAAGAACCTGACTC TCTGGAACAACGGTGATACTCTGGTCAAGAACGTCTCGAGCTGGTGCAGC AACACCATCGTCGTCATCCACTCGGTCGGCCCGGTCCTCCTGACCGATTG GTACGACAACCCCAACATCACGGCCATTCTCTGGGCTGGTCTTCCGGGCC AGGAGTCGGGCAACTCCATCACCGACGTGCTTTACGGCAAGGTCAACCCC GCCGCCCGCTCGCCCTTCACTTGGGGCAAGACCCGCGAAAGCTATGGCGC GGACGTCCTGTACAAGCCGAATAATGGCAATGGTGCGCCCCAACAGGACT TCACCGAGGGCGTCTTCATCGACTACCGCTACTTCGACAAGGTTGACGAT GACTCGGTCATCTACGAGTTCGGCCACGGCCTGAGCTACACCACCTTCGA GTACAGCAACATCCGCGTCGTCAAGTCCAACGTCAGCGAGTACCGGCCCA CGACGGCCACGGCCCAGGCCCCGACGTTTGGCAACTTCTCCACCGAC CTCGAGGACTATCTCTTCCCCAAGGACGAGTTCCCCTACATCTACCAGTA CATCTACCCGTACCTCAACACGACCGACCCCCGGAGGGCCTCGGCCGATC CCCACTACGGCCAGACCGCCGAGGAGTTCCTCCCGCCCCACGCCACCGAT GACGACCCCAGCCGCTCCTCCGGTCCTCGGGCGGAAACTCCCCCGGCGG CAACCGCCAGCTGTACGACATTGTCTACACAATCACGGCCGACATCACGA ATACGGGCTCCGTTGTAGGCGAGGGGGGGGCTCCACGTCTCGCTG GGCGGTCCCGAGGATCCCAAGGTGCAGCTGCGCGACTTTGACAGGATGCG GATCGAACCCGGCGAGACGAGGCAGTTCACCGGCCGCCTGACGCGCAGAG ATCTGAGCAACTGGGACGTCACGGTGCAGGACTGGGTCATCAGCAGGTAT CCCAAGACGCATATGTTGGGAGGAGCAGCCGGAAGTTGGATCTCAAGAT TGAGCTTCCTTGA

(SEQ ID NO: 116)

MKAAALSCLFGSTLAVAGA
IESRKVHQKPLARSEPFYPSPWMNPNADGWA

EAYAQAKSFVSQMTLLEKVNLTTGVGWGAEQCVGQVGAIPRLGLRSLCMH

DSPLGIRGADYNSAFPSGQTVAATWDRGLMYRRGYAMGQEAKGKGINVLL GPVAGPLGRMPEGGRNWEGFAPDPVLTGIGMSETIKGIQDAGVIACAKHF IGNEOEHFROVPEAOGYGYNISETLSSNIDDKTMHELYLWPFADAVRAGV GSVMCSYQQVNNSYACQNSKLLNDLLKNELGFQGFVMSDWQAQHTGAASA VAGLDMSMPGDTQFNTGVSFWGANLTLAVLNGTVPAYRLDDMAMRIMAAL FKVTKTTDLEPINFSFWTDDTYGPIHWAAKQGYQEINSHVDVRADHGNLI REIAAKGTVLLKNTGSLPLNKPKFVAVIGEDAGSSPNGPNGCSDRGCNEG TLAMGWGSGTANYPYLVSPDAALOARAIODGTRYESVLSNYAEEKTKALV SOANATAIVFVNADSGEGYINVDGNEGDRKNLTLWNNGDTLVKNVSSWCS NTTVVTHSVGPVLLTDWYDNPNTTATLWAGLPGOESGNSTTDVLYGKVNP AARSPFTWGKTRESYGADVLYKPNNGNGAPQQDFTEGVFIDYRYFDKVDD DSVIYEFGHGLSYTTFEYSNIRVVKSNVSEYRPTTGTTAOAPTFGNFSTD LEDYLFPKDEFPYIYOYIYPYLNTTDPRRASADPHYGOTAEEFLPPHATD DDPOPLLRSSGGNSPGGNROLYDIVYTITADITNTGSVVGEEVPOLYVSL ${\tt GGPEDPKVQLRDFDRMRIEPGETRQFTGRLTRRDLSNWDVTVQDWVISRY}$ PKTAYVGRSSRKLDLKIELP

(SEQ ID NO: 117) ${\tt IESRKVHQKPLARSEPFYPSPWMNPNADGWAEAYAQAKSFVSQMTLLEKV}$ NLTTGVGWGAEQCVGQVGAIPRLGLRSLCMHDSPLGIRGADYNSAFPSGQ TVAATWDRGLMYRRGYAMGOEAKGKGINVLLGPVAGPLGRMPEGGRNWEG ${\tt FAPDPVLTGIGMSETIKGIQDAGVIACAKHFIGNEQEHFRQVPEAQGYGY}$ NISETLSSNIDDKTMHELYLWPFADAVRAGVGSVMCSYQQVNNSYACQNS KLLNDLLKNELGFQGFVMSDWQAQHTGAASAVAGLDMSMPGDTQFNTGVS FWGANLTLAVLNGTVPAYRLDDMAMRIMAALFKVTKTTDLEPINFSFWTD DTYGPIHWAAKOGYOEINSHVDVRADHGNLIREIAAKGTVLLKNTGSLPL NKPKFVAVIGEDAGSSPNGPNGCSDRGCNEGTLAMGWGSGTANYPYLVSP DAALOARAIODGTRYESVLSNYAEEKTKALVSOANATAIVFVNADSGEGY INVDGNEGDRKNLTLWNNGDTLVKNVSSWCSNTIVVIHSVGPVLLTDWYD NPNITATLWAGLPGOESGNSTTDVLYGKVNPAARSPFTWGKTRESYGADV $\verb|LYKPNNGNGAPQQDFTEGVFIDYRYFDKVDDDSVIYEFGHGLSYTTFEYS|$ NIRVVKSNVSEYRPTTGTTAQAPTFGNFSTDLEDYLFPKDEFPYIYQYIY PYLNTTDPRRASADPHYGQTAEEFLPPHATDDDPQPLLRSSGGNSPGGNR OLYDIVYTITADITNTGSVVGEEVPOLYVSLGGPEDPKVOLRDFDRMRIE PGETROFTGRLTRRDLSNWDVTVODWVISRYPKTAYVGRSSRKLDLKIEL

[0344] The polynucleotide (SEQ ID NO:118) and amino acid (SEQ ID NO:119) sequences of a BGL variant ("Variant 883") are provided below. The signal sequence is underlined in SEQ ID NO:119. SEQ ID NO:120 provides the sequence of this BGL variant, without the signal sequence.

P

(SEQ ID NO: 118) ATGAAGGCTGCTGCGCTTTCCTGCCTCTTCGGCAGTACCCTTGCCGTTGC AGGCGCCATTGAATCGAGAAAGGTTCACCAGAAGCCCCTCGCGAGATCTG $\verb|AACCTTTTTACCCGTCGCCATGGATGAATCCCAACGCCGACGGCTGGGCG|$ GAGGCCTATGCCCAGGCCAAGTCCTTTGTCTCCCAAATGACTCTGCTAGA GAAGGTCAACTTGACCACGGGAGTCGGCTGGGGGGGCTGAGCAGTGCGTCG GACTCCCCTCTCGGCATCCGAGGAGCCGACTACAACTCAGCGTTCCCCTC TGGCCAGACCGTTGCTGCTACCTGGGATCGCGGTCTGATGTACCGTCGCG GCTACGCAATGGGCCAGGAGGCCAAAGGCAAGGGCATCAATGTCCTTCTC GGACCAGTCGCCGGCCCCCTTGGCCGCATGCCCGAGGGCGGTCGTAACTG GGAAGGCTTCGCTCCGGATCCCGTCCTTACCGGCATCGGCATGTCCGAGA CGATCAAGGGCATTCAGGATGCTGGCGTCATCGCTTGTGCGAAGCACTTT ATTGGAAACGAGCAGGAGCACTTCAGACAGGTGCCAGAAGCCCAGGGATA CGGTTACAACATCAGCGAAACCCTCTCCTCCAACATTGACGACAAGACCA TGCACGAGCTCTACCTTTGGCCGTTTGCCGATGCCGTCCGGGCCGGCGTC $\tt GGCTCTGTCATGTGCTCGTACAACCAGGTCAACAACTCGTACGCCTGCCA$ ${\tt GAACTCGAAGCTGCTGAACGACCTCCTCAAGAACGAGCTTGGGTTTCAGG}$ GCTTCGTCATGAGCGACTGGTGGGCACAGCACCTGGCGCAGCAAGCGCC GTGGCTGGTCTCGATATGTCCATGCCGGGCGACACCATGTTCAACACTGG $\tt CGTCAGTTTCTGGGGCGCCAATCTCACCCTCGCCGTCCTCAACGGCACAG$ ${\tt TCCCTGCCTACCGTCTCGACGACATGGCCATGCGCATCATGGCCGCCCTC}$ TTCAAGGTCACCAAGACCACCGACCTGGAACCGATCAACTTCTCCTTCTG GACCCGCGACACTTATGGCCCGATCCACTGGGCCGCCAAGCAGGGCTACC AGGAGATTAATTCCCACGTTGACGTCCGCGCCGACCACGGCAACCTCATC CGGAACATTGCCGCCAAGGGTACGGTGCTGCTGAAGAATACCGGCTCTCT CGAGCCCCAACGGCCCAACGGCTGCAGCGACCGCGGCTGTAACGAAGGC ACGCTCGCCATGGGCTGGGGATCCGGCACAGCCAACTATCCGTACCTCGT TTCCCCCGACGCCGCGCTCCAGTTGCGGGCCATCCAGGACGGCACGAGGT ACGAGAGCGTCCTGTCCAACTACGCCGAGGAAAATACAAAGGCTCTGGTC TCGCAGGCCAATGCAACCGCCATCGTCTTCGTCAATGCCGACTCAGGCGA GGGCTACATCAACGTGGACGGTAACGAGGGCGACCGTAAGAACCTGACTC TCTGGAACAACGGTGATACTCTGGTCAAGAACGTCTCGAGCTGGTGCAGC AACACCATCGTCGTCATCCACTCGGTCGGCCCGGTCCTCCTGACCGATTG GTACGACACCCCAACATCACGCCCATTCTCTGGGCTGGTCTTCCGGGCC AGGAGTCGGGCAACTCCATCACCGACGTGCTTTACGGCAAGGTCAACCCC $\tt GCCGCCCGCTCGCCCTTCACTTGGGGCAAGACCCGCGAAAGCTATGGCGC$ $\tt GGACGTCCTGTACAAGCCGAATAATGGCAATTGGGCGCCCCAACAGGACT$ TCACCGAGGGCGTCTTCATCGACTACCGCTACTTCGACAAGGTTGACGAT

GACTCGGTCATCTACGAGTTCGGCCACGGCCTGAGCTACACCACCTTCGA
GTACAGCAACATCCGCGTCGTCAAGTCCAACGTCAGCGAGTACCGGCCCA
CGACGGGCACCACGATTCAGGCCCCGACGTTTGGCAACTTCTCCACCGAC
CTCGAGGACTATCTCTCCCCAAGGACGACTCCCGGAGGGCCTCGGCCGAGTA
CATCTACCCGTACCTCAACACGACCGACCCCGGAGGGCCTCGGCCGATC
CCCACTACGGCCAGACCGCCGAGGAGTTCCTCCCGCCCCACGCCACCGAT
GACGACCCCCAGCCGCTCCTCCGGTCCTCGGGCGGAAACTCCCCCGGCGG
CAACCGCCAGCTGTACGACATTGTCTACACAATCACGGCCGACATCACGA
ATACGGGCTCCGTTGTAGGCGAGGAGGTACCGCAGCTCTACGTCTCGCTG
GGCGGTCCCGAGGATCCCAAGGTGCAGCTCTACGTCTTCGCTG
GATCGAACCCGGCGAGACCGAGGAGTTCACCGGCCGCAGAGA
ATCTGAGCAACTGGGACGTCACGGTGCAGGTTCACGCAGGTAT
CCCAAGACCGCCATTGTTGGGAGGAGCAGCCGGAAGTTGGATCTCAAGAT
TGAGCTTCCTTGA

(SEQ ID NO: 119) $\underline{\mathsf{MKAAALSCLFGSTLAVAGA}} \mathtt{IESRKVHQKPLARSEPFYPSPWMNPNADGWA}$ EAYAQAKSFVSQMTLLEKVNLTTGVGWGAEQCVGQVGAIPRLGLRSLCMH DSPLGIRGADYNSAFPSGQTVAATWDRGLMYRRGYAMGQEAKGKGINVLL ${\tt GPVAGPLGRMPEGGRNWEGFAPDPVLTGIGMSETIKGIQDAGVIACAKHF}$ ${\tt IGNEQEHFRQVPEAQGYGYNISETLSSNIDDKTMHELYLWPFADAVRAGV}$ ${\tt GSVMCSYNQVNNSYACQNSKLLNDLLKNELGFQGFVMSDWWAQHTGAASA}$ VAGLDMSMPGDTMFNTGVSFWGANLTLAVLNGTVPAYRLDDMAMRIMAAL FKVTKTTDLEPINFSFWTRDTYGPIHWAAKQGYQEINSHVDVRADHGNLI RNIAAKGTVLLKNTGSLPLNKPKFVAVIGEDAGPSPNGPNGCSDRGCNEG TLAMGWGSGTANYPYLVSPDAALOLRAIODGTRYESVLSNYAEENTKALV SQANATAIVFVNADSGEGYINVDGNEGDRKNLTLWNNGDTLVKNVSSWCS NTIVVIHSVGPVLLTDWYDNPNITAILWAGLPGQESGNSITDVLYGKVNP AARSPFTWGKTRESYGADVLYKPNNGNWAPQQDFTEGVFIDYRYFDKVDD DSVIYEFGHGLSYTTFEYSNIRVVKSNVSEYRPTTGTTIQAPTFGNFSTD LEDYLFPKDEFPYIPOYIYPYLNTTDPRRASADPHYGOTAEEFLPPHATD DDPOPLLRSSGGNSPGGNROLYDIVYTITADITNTGSVVGEEVPOLYVSL GGPEDPKVQLRDFDRMRIEPGETRQFTGRLTRRDLSNWDVTVQDWVISRY

(SEQ ID NO: 120)
IESRKVHQKPLARSEPFYPSPWMNPNADGWAEAYAQAKSFVSQMTLLEKV
NLTTGVGWGAEQCVGQVGAIPRLGLRSLCMHDSPLGIRGADYNSAFPSGQ
TVAATWDRGLMYRRGYAMGQEAKGKGINVLLGPVAGPLGRMPEGGRNWEG
FAPDPVLTGIGMSETIKGIQDAGVIACAKHFIGNEQEHFRQVPEAQGYGY
NISETLSSNIDDKTMHELYLWPFADAVRAGVGSVMCSYNQVNNSYACQNS
KLLNDLLKNELGFQGFVMSDWWAQHTGAASAVAGLDMSMPGDTMFNTGVS

PKTAYVGRSSRKLDLKTELP

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FWGANLTLAVLNGTVPAYRLDDMAMRIMAALFKVTKTTDLEPINFSFWTR
DTYGPIHWAAKQGYQEINSHVDVRADHGNLIRNIAAKGTVLLKNTGSLPL
NKPKFVAVIGEDAGPSPNGPNGCSDRGCNEGTLAMGWGSGTANYPYLVSP
DAALQLRAIQDGTRYESVLSNYAEENTKALVSQANATAIVFVNADSGEGY
INVDGNEGDRKNLTLWNNGDTLVKNVSSWCSNTIVVIHSVGPVLLTDWYD
NPNITAILWAGLPGQESGNSITDVLYGKVNPAARSPFTWGKTRESYGADV
LYKPNNGNWAPQQDFTEGVFIDYRYFDKVDDDSVIYEFGHGLSYTTFEYS
NIRVVKSNVSEYRPTTGTTIQAPTFGNFSTDLEDYLFPKDEFPYIPQYIY
PYLNTTDPRRASADPHYGQTAEEFLPPHATDDDPQPLLRSSGGNSPGGNR
QLYDIVYTITADITNTGSVVGEEVPQLYVSLGGPEDPKVQLRDFDRMRIE
PGETRQFTGRLTRRDLSNWDVTVQDWVISRYPKTAYVGRSSRKLDLKIEL

[0345] The polynucleotide (SEQ ID NO:121) and amino acid (SEQ ID NO:122) sequences of a BGL variant ("Variant 900") are provided below. The signal sequence is underlined in SEQ ID NO:122. SEQ ID NO:123 provides the sequence of this BGL variant, without the signal sequence.

(SEQ ID NO: 121) ATGAAGGCTGCTGCGCTTTCCTGCCTCTTCGGCAGTACCCTTGCCGTTGC AGGCGCCATTGAATCGAGAAAGGTTCACCAGAAGCCCCTCGCGAGATCTG ${\tt AACCTTTTTACCCGTCGCCATGGATGAATCCCAACGCCATCGGCTGGGCG}$ GAGGCCTATGCCCAGGCCAAGTCCTTTGTCTCCCAAATGACTCTGCTAGA GAAGGTCAACTTGACCACGGGAGTCGGCTGGGGGGGAGGAGCAGTGCGTCG GACTCCCCTCTCGGCGTGCGAGGAACCGACTACAACTCAGCGTTCCCCTC TGGCCAGACCGTTGCTGCTACCTGGGATCGCGGTCTGATGTACCGTCGCG GCTACGCAATGGGCCAGGAGGCCAAAGGCAAGGGCATCAATGTCCTTCTC GGACCAGTCGCCGCCCCCTTGGCCGCATGCCCGAGGGCGGTCGTAACTG GGAAGGCTTCGCTCCGGATCCCGTCCTTACCGGCATCGGCATGTCCGAGA CGATCAAGGGCATTCAGGATGCTGGCGTCATCGCTTGTGCGAAGCACTTT $\tt ATTGGAAACGAGCAGGAGCACTTCAGACAGGTGCCAGAAGCCCAGGGATA$ CGGTTACAACATCAGCGAAACCCTCTCCTCCAACATTGACGACAAGACCA TGCACGAGCTCTACCTTTGGCCGTTTGCCGATGCCGTCCGGGCCGGCGTC $\tt GGCTCTGTCATGTGCTCGTACAACCAGGGCAACAACTCGTACGCCTGCCA$ GAACTCGAAGCTGCTGAACGACCTCCTCAAGAACGAGCTTGGGTTTCAGG $\tt GCTTCGTCATGAGCGACTGGTGGGCACACACTGGCGCAGCAAGCGCC$ GTGGCTGGTCTCGATATGTCCATGCCGGGCGACACCATGGTCAACACTGG CGTCAGTTTCTGGGGCGCCAATCTCACCCTCGCCGTCCTCAACGGCACAG TCCCTGCCTACCGTCTCGACGACATGTGCATGCGCATCATGGCCGCCCTC ${\tt TTCAAGGTCACCAAGACCACCGACCTGGAACCGATCAACTTCTCCTTCTG}$

GACCCGCGACACTTATGGCCCGATCCACTGGGCCGCCAAGCAGGGCTACC AGGAGATTAATTCCCACGTTGACGTCCGCGCCGACCACGGCAACCTCATC $\tt CGGAACATTGCCGCCAAGGGTACGGTGCTGCTGAAGAATACCGGCTCTCT$ ACCCCTGAACAAGCCAAAGTTCGTGGCCGTCATCGGCGAGGATGCTGGGC CGAGCCCCAACGGCCCAACGGCTGCAGCGACCGCGGCTGTAACGAAGGC ACGCTCGCCATGGGCTGGGGATCCGGCACAGCCAACTATCCGTACCTCGT TTCCCCCGACGCCGCGCTCCAGGCGCGGGCCATCCAGGACGCACGAGGT ACGAGAGCGTCCTGTCCAACTACGCCGAGGAAAATACAAAGGCTCTGGTC TCGCAGGCCAATGCAACCGCCATCGTCTTCGTCAATGCCGACTCAGGCGA GGGCTACATCAACGTGGACGGTAACGAGGGCGACCGTAAGAACCTGACTC TCTGGAACAACGGTGATACTCTGGTCAAGAACGTCTCGAGCTGGTGCAGC AACACCATCGTCGTCATCCACTCGGTCGGCCCGGTCCTCCTGACCGATTG GTACGACAACCCCAACATCACGGCCATTCTCTGGGCTGGTCTTCCGGGCC AGGAGTCGGGCAACTCCATCACCGACGTGCTTTACGGCAAGGTCAACCCC GCCGCCCGCTCGCCCTTCACTTGGGGCAAGACCCGCGAAAGCTATGGCGC GGACGTCCTGTACAAGCCGAATAATGGCAATTGGGCGCCCCAACAGGACT ${\tt TCACCGAGGGCGTCTTCATCGACTACCGCTACTTCGACAAGGTTGACGAT}$ GACTCGGTCATCTACGAGTTCGGCCACGGCCTGAGCTACACCACCTTCGA GTACAGCAACATCCGCGTCGTCAAGTCCAACGTCAGCGAGTACCGGCCCA CGACGGCCACCACTTCAGGCCCCGACGTTTGGCAACTTCTCCACCGAC CTCGAGGACTATCTCTTCCCCAAGGACGAGTTCCCCTACATCCCGCAGTA CATCTACCCGTACCTCAACACGACCGACCCCCGGAGGGCCTCGGGCGATC CCCACTACGGCCAGACCGCCGAGGAGTTCCTCCCGCCCCACGCCACCGAT GACGACCCCAGCCGCTCCTCCGGTCCTCGGGCGGAAACTCCCCCGGCGG CAACCGCCAGCTGTACGACATTGTCTACACAATCACGGCCGACATCACGA ATACGGGCTCCGTTGTAGGCGAGGAGGTACCGCAGCTCTACGTCTCGCTG GGCGGTCCCGAGGATCCCAAGGTGCAGCTGCGCGACTTTGACAGGATGCG GATCGAACCCGGCGAGACGAGGCAGTTCACCGGCCGCCTGACGCGCAGAG ATCTGAGCAACTGGGACGTCACGGTGCAGGACTGGGTCATCAGCAGGTAT CCCAAGACGGCATATGTTGGGAGGAGCAGCCGGAAGTTGGATCTCAAGAT TGAGCTTCCTTGA

(SEQ ID NO: 122)

MKAAALSCLFGSTLAVAGA
IESRKVHQKPLARSEPFYPSPWMNPNAIGWA

EAYAQAKSFVSQMTLLEKVNLTTGVGWGEEQCVGNVGAIPRLGLRSLCMH

DSPLGVRGTDYNSAFPSGQTVAATWDRGLMYRRGYAMGQEAKGKGINVLL

GPVAGPLGRMPEGGRNWEGFAPDPVLTGIGMSETIKGIQDAGVIACAKHF

IGNEQEHFRQVPEAQGYGYNISETLSSNIDDKTMHELYLWPFADAVRAGV

GSVMCSYNQGNNSYACQNSKLLNDLLKNELGFQGFVMSDWWAQHTGAASA

VAGLDMSMPGDTMVNTGVSFWGANLTLAVLNGTVPAYRLDDMCMRIMAAL

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FKVTKTTDLEPINFSFWTRDTYGPIHWAAKQGYQEINSHVDVRADHGNLI
RNIAAKGTVLLKNTGSLPLNKPKFVAVIGEDAGPSPNGPNGCSDRGCNEG
TLAMGWGSGTANYPYLVSPDAALQARAIQDGTRYESVLSNYAEENTKALV
SQANATAIVFVNADSGEGYINVDGNEGDRKNLTLWNNGDTLVKNVSSWCS
NTIVVIHSVGPVLLTDWYDNPNITAILWAGLPGQESGNSITDVLYGKVNP
AARSPFTWGKTRESYGADVLYKPNNGNWAPQQDFTEGVFIDYRYFDKVDD
DSVIYEFGHGLSYTTFEYSNIRVVKSNVSEYRPTTGTTIQAPTFGNFSTD
LEDYLFPKDEFPYIPQYIYPYLNTTDPRRASGDPHYGQTAEEFLPPHATD
DDPQPLLRSSGGNSPGGNRQLYDIVYTITADITNTGSVVGEEVPQLYVSL
GGPEDPKVQLRDFDRMRIEPGETRQFTGRLTRRDLSNWDVTVQDWVISRY
PKTAYYGRSSRKLDLKIELP

(SEO ID NO: 123) IESRKVHQKPLARSEPFYPSPWMNPNAIGWAEAYAQAKSFVSQMTLLEKV ${\tt NLTTGVGWGEEQCVGNVGAIPRLGLRSLCMHDSPLGVRGTDYNSAFPSGQ}$ TVAATWDRGLMYRRGYAMGQEAKGKGINVLLGPVAGPLGRMPEGGRNWEG ${\tt FAPDPVLTGIGMSETIKGIQDAGVIACAKHFIGNEQEHFRQVPEAQGYGY}$ ${\tt NISETLSSNIDDKTMHELYLWPFADAVRAGVGSVMCSYNQGNNSYACQNS}$ $\verb|KLLNDLLKNELGFQGFVMSDWWAQHTGAASAVAGLDMSMPGDTMVNTGVS|\\$ FWGANLTLAVLNGTVPAYRLDDMCMRIMAALFKVTKTTDLEPINFSFWTR DTYGPIHWAAKQGYQEINSHVDVRADHGNLIRNIAAKGTVLLKNTGSLPL ${\tt NKPKFVAVIGEDAGPSPNGPNGCSDRGCNEGTLAMGWGSGTANYPYLVSP}$ DAALQARAIQDGTRYESVLSNYAEENTKALVSQANATAIVFVNADSGEGY INVDGNEGDRKNLTLWNNGDTLVKNVSSWCSNTIVVIHSVGPVLLTDWYD NPNITAILWAGLPGOESGNSITDVLYGKVNPAARSPFTWGKTRESYGADV $\verb|LYKPNNGNWAPQQDFTEGVFIDYRYFDKVDDDSVIYEFGHGLSYTTFEYS|$ NIRVVKSNVSEYRPTTGTTIQAPTFGNFSTDLEDYLFPKDEFPYIPQYIY ${\tt PYLNTTDPRRASGDPHYGQTAEEFLPPHATDDDPQPLLRSSGGNSPGGNR}$ QLYDIVYTITADITNTGSVVGEEVPQLYVSLGGPEDPKVQLRDFDRMRIE PGETRQFTGRLTRRDLSNWDVTVQDWVISRYPKTAYVGRSSRKLDLKIEL P

[0346] The polynucleotide (SEQ ID NO:124) and amino acid (SEQ ID NO:125) sequences of wild-type *Talaromyces emersonii* CBH1 are provided below. The signal sequence is shown underlined in SEQ ID NO:125. SEQ ID NO:126 provides the sequence of this CBH1, without the signal sequence.

(SEQ ID NO: 124)
ATGCTTCGACGGGCTCTTCTTCTATCCTCTTCCGCCATCCTTGCTGTCAA

GGCACAGCAGGCCGGCACGGCGACGAGAAACCACCCGCCCCTGACAT

GGCAGGAATGCACCGCCCCTGGGAGCTGCACCACCCAGAACGGGGCGGTC

GTTCTTGATGCGAACTGGCGTTGGGTGCACGATGTGAACGGATACACCAA

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CTGCTACACGGGCAATACCTGGGACCCCACGTACTGCCCTGACGACGAAA
CCTGCGCCCAGAACTGTGCGCTGGACGGCGCGGATTACGAGGGCACCTAC

GGCGTGACTTCGTCGGGCAGCTCCTTGAAACTCAATTTCGTCACCGGGTC GAACGTCGGATCCCGTCTCTACCTGCTGCAGGACGACTCGACCTATCAGA ${\tt TCTTCAAGCTTCTGAACCGCGAGTTCAGCTTTGACGTCGATGTCTCCAAT}$ CTTCCGTGCGGATTGAACGGCGCTCTGTACTTTGTCGCCATGGACGCCGA CGGCGGCGTGTCCAAGTACCCGAACAACAAGGCTGGTGCCAAGTACGGAA CCGGGTATTGCGACTCCCAATGCCCACGGGACCTCAAGTTCATCGACGGC GAGGCCAACGTCGAGGGCTGGCAGCCGTCTTCGAACAACGCCAACACCGG AATTGGCGACCACGGCTCCTGCTGTGCGGAGATGGATGTCTGGGAAGCAA ACAGCATCTCCAATGCGGTCACTCCGCACCCGTGCGACACGCCAGGCCAG ACGATGTGCTCTGGAGATGACTGCGGTGGCACATACTCTAACGATCGCTA CGCGGGAACCTGCGATCCTGACGGCTGTGACTTCAACCCTTACCGCATGG GCAACACTTCTTTCTACGGGCCTGGCAAGATCATCGATACCACCAAGCCC TTCACTGTCGTGACGCAGTTCCTCACTGATGATGGTACGGATACTGGAAC TCTCAGCGAGATCAAGCGCTTCTACATCCAGAACAGCAACGTCATTCCGC AGCCCAACTCGGACATCAGTGGCGTGACCGGCAACTCGATCACGACGGAG $\tt GCACGGTGGCCTGGCCAAGATGGGAGCGGCCATGCAGCAGGGTATGGTCC$ $\tt TGGTGATGAGTTTGTGGGACGACTACGCCGCGCAGATGCTGTGGTTGGAT$ ${\tt TCCGACTACCCGACGGATGCGGACCCCACGACCCCTGGTATTGCCCGTGG}$ AACGTGTCCGACGGACTCGGGCGTCCCATCGGATGTCGAGTCGCAGAGCC

(SEQ ID NO: 125)

MLRRALLLSSSAILAVKAQQAGTATAENHPPLTWQECTAPGSCTTQNGAV
VLDANWRWVHDVNGYTNCYTGNTWDPTYCPDDETCAQNCALDGADYEGTY
GVTSSGSSLKLNFVTGSNVGSRLYLLQDDSTYQIFKLLNREFSFDVDVSN
LPCGLNGALYFVAMDADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDG
EANVEGWQPSSNNANTGIGDHGSCCAEMDVWEANSISNAVTPHPCDTPGQ
TMCSGDDCGGTYSNDRYAGTCDPDGCDFNPYRMGNTSFYGPGKIIDTTKP
FTVVTQFLTDDGTDTGTLSEIKRFYIQNSNVIPQPNSDISGVTGNSITTE
FCTAQKQAFGDTDDFSQHGGLAKMGAAMQQGMVLVMSLWDDYAAQMLWLD
SDYPTDADPTTPGIARGTCPTDSGVPSDVESQSPNSYVTYSNIKFGPINS

CCAACTCCTACGTGACCTACTCGAACATTAAGTTTGGTCCGATCAACTCG

ACCTTCACCGCTTCGTGA

 $(SEQ\ ID\ NO:\ 126)$ QQAGTATAENHPPLTWQECTAPGSCTTQNGAVVLDANWRWVHDVNGYTNC YTGNTWDPTYCPDDETCAQNCALDGADYEGTYGVTSSGSSLKLNFVTGSN

VGSRLYLLQDDSTYQIFKLLNREFSFDVDVSNLPCGLNGALYFVAMDADG GVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPSSNNANTGI GDHGSCCAEMDVWEANSISNAVTPHPCDTPGQTMCSGDDCGGTYSNDRYA GTCDPDGCDFNPYRMGNTSFYGPGKIIDTTKPFTVVTQFLTDDGTDTGTL SEIKRFYIQNSNVIPQPNSDISGVTGNSITTEFCTAQKQAFGDTDDFSQH

 ${\tt GGLAKMGAAMQQGMVLVMSLWDDYAAQMLWLDSDYPTDADPTTPGIARGT}$

continued

CPTDSGVPSDVESQSPNSYVTYSNIKFGPINSTFTAS

[0347] The polynucleotide (SEQ ID NO:127) and amino acid (SEQ ID NO:128) sequences of wild-type *M. thermophila* CBH1a are provided below. The signal sequence is shown underlined in SEQ ID NO:128. SEQ ID NO:129 provides the sequence of this CBH1a, without the signal sequence.

(SEQ ID NO: 127)

TCAGAACGCCTGCACTCTGACCGCTGAGAACCACCCCTCGCTGACGTGGT $\tt CCAAGTGCACGTCTGGCGGCAGCTGCACCAGCGTCCAGGGTTCCATCACC$ ATCGACGCCAACTGGCGGTGGACTCACCGGACCGATAGCGCCACCAACTG CTACGAGGGCAACAAGTGGGATACTTCGTACTGCAGCGATGGTCCTTCTT GCGCCTCCAAGTGCTGCATCGACGGCGCTGACTACTCGAGCACCTATGGC ATCACCACGAGCGGTAACTCCCTGAACCTCAAGTTCGTCACCAAGGGCCA GTACTCGACCAACATCGGCTCGCGTACCTACCTGATGGAGAGCGACACCA AGTACCAGATGTTCCAGCTCCTCGGCAACGAGTTCACCTTCGATGTCGAC GTCTCCAACCTCGGCTGCGGCCTCAATGGCGCCCTCTACTTCGTGTCCAT GGATGCCGATGGTGGCATGTCCAAGTACTCGGGCAACAAGGCAGGTGCCA AGTACGGTACCGGCTACTGTGATTCTCAGTGCCCCCGCGACCTCAAGTTC ATCAACGGCGAGGCCAACGTAGAGAACTGGCAGAGCTCGACCAACGATGC CAACGCCGGCACGGCAAGTACGGCAGCTGCTCCCGAGATGGACGTCT GGGAGGCCAACAACATGGCCGCCGCCTTCACTCCCCACCCTTGCACCGTG ATCGGCCAGTCGCGCGCGAGGGCGACTCGTGCGGCGGTACCTACAGCAC CGACCGCTATGCCGCCATCTGCGACCCCGACGGATGCGACTTCAACTCGT ACCGCCAGGGCAACAAGACCTTCTACGGCAAGGGCATGACGGTCGACACG GCTCTCCGAGATCAAGCGGTTCTACGTCCAGAACGGCAAGGTCATCCCCA ACTCCGAGTCCACCATCCCGGGCGTCGAGGGCAACTCCATCACCCAGGAC TGGTGCGACCGCCAGAAGGCCGCCTTCGGCGACGTGACCGACTTCCAGGA ${\tt CAAGGGCGGCATGGTCCAGATGGGCAAGGCCCTCGCGGGGCCCATGGTCC}$ ${\tt TCGTCATGTCCATCTGGGACGACCACGCCGTCAACATGCTCTGGCTCGAC}$ TCCACCTGGCCCATCGACGGCGCCGGCAAGCCGGGCGCGAGCGCGGTGC CTGCCCCACCACCTCGGGCGTCCCCGCTGAGGTCGAGGCCGAGGCCCCCA $\verb|ACTCCAACGTCATCTTCTCCAACATCCGCTTCGGCCCCATCGGCTCCACC| \\$ $\tt CTCGTCCACCCCGGTCCCCTCCTCGTCCACCACATCCTCCGGTTCCTCCG$

GCCCGACTGGCGGCACGGGTGTCGCTAAGCACTATGAGCAATGCGGAGGA
ATCGGGTTCACTGGCCCTACCCAGTGCGAGAGCCCCTACACTTGCACCAA
GCTGAATGACTGGTACTCGCAGTGCCTGTAA

(SEQ ID NO: 128
MYAKFATLAALVAGAAAQNACTLTAENHPSLTYSKCTSGGSCTSVQGSIT
IDANWRWTHRTDSATNCYEGNKWDTSWCSDGPSCASKCCIDGADYSSTYG
ITTSGNSLNLKFVTKGQYSTNIGSRTYLMESDTKYQMFQLLGNEFTFDVD
VSNLGCGLNGALYFVSMDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKF
INGEANVENWQSSTNDANAGTGKYGSCCSEMDVWEANNMAAAFTPHPCTV
IGQSRCEGDSCGGTYSTDRYAGICDPDGCDFNSYRQGNKTFYGKGMTVDT
TKKITVVTQFLKNSAGELSEIKRFYVQNGKVIPNSESTIPGVEGNSITQD
WCDRQKAAFGDVTDFQDKGGMVQMGKALAGPMVLVMSIWDDHAVNMLWLD
STWPIDGAGKPGAERGACPTTSGVPAEVEAEAPNSNVIFSNIRFGPIGST
VSGLPDGGSGNPNPPVSSSTPVPSSSTTSSGSSGPTGGTGVAKHYEQCGG
IGFTGPTOCESPYTCTKLNDWYSOCL

(SEQ ID NO: 129)
QNACTLTAENHPSLTYSKCTSGGSCTSVQGSITIDANWRWTHRTDSATNC
YEGNKWDTSWCSDGPSCASKCCIDGADYSSTYGITTSGNSLNLKFVTKGQ
YSTNIGSRTYLMESDTKYQMFQLLGNEFTFDVDVSNLGCGLNGALYFVSM
DADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFINGEANVENWQSSTNDA
NAGTGKYGSCCSEMDVWEANNMAAAFTPHPCTVIGQSRCEGDSCGGTYST
DRYAGICDPDGCDFNSYRQGNKTFYGKGMTVDTTKKITVVTQFLKNSAGE
LSEIKRFYVQNGKVIPNSESTIPGVEGNSITQDWCDRQKAAFGDVTDFQD
KGGMVQMGKALAGPMVLVMSIWDDHAVNMLWLDSTWPIDGAGKPGAERGA
CPTTSGVPAEVEAEAPNSNVIFSNIRFGPIGSTVSGLPDGGSGNPNPPVS
SSTPVPSSSTTSSGSSGPTGGTGVAKHYEQCGGIGFTGPTQCESPYTCTK
LNDWYSQCL

[0348] The polynucleotide (SEQ ID NO:130) and amino acid (SEQ ID NO:131) sequences of a *M. thermophila* CBH1a variant ("Variant 145") are provided below. The signal sequence is shown underlined in SEQ ID NO:131. SEQ ID NO:132 provides the sequence of this CBH1a, without the signal sequence.

continued GTACTCGACCAACATCGGCTCGCGTACCTGATGGAGAGCGACACCA AGTACCAGATGTTCCAGCTCCTCGGCAACGAGTTCACCTTCGATGTCGAC GTCTCCAACCTCGGCTGCGGCCTCAATGGCGCCCTCTACTTCGTGTCCAT $\tt GGATGCCGATGGTGGCATGTCCAAGTACTCGGGCAACAAGGCAGGTGCCA$ ${\tt AGTACGGTACCGGCTACTGTGATTCTCAGTGCCCCCGCGACCTCAAGTTC}$ ATCAACGGCGAGGCCAACGTAGAGAACTGGCAGAGCTCGACCAACGATGC CAACGCCGGCACGGCAAGTACGGCAGCTGCTCCCGAGATGGACGTCT GGGAGGCCAACAACATGGCCGCCGCCTTCACTCCCCACCCTTGCACCGTG ATCGGCCAGTCGCGCGGGGGGGGGGGCGACTCGTGCGGCGGTACCTACAGCAC CGACCGCTATGCCGCCATCTGCGACCCCGACGGATGCGACTTCAACTCGT ACCGCCAGGGCAACAAGACCTTCTACGGCAAGGGCATGACGGTCGACACG GCTCTCCGAGATCAAGCGGTTCTACGTCCAGAACGGCAAGGTCATCCCCA ACTCCGAGTCCACCATCCCGGGCGTCGAGGGCAACTCCATCACCCAGGAC TGGTGCGACCGCCAGAAGGCCGCCTTCGGCGACGTGACCGACTTCCAGGA CAAGGGCGGCATGGTCCAGATGGGCAAGGCCCTCGCGGGGCCCATGGTCC TCGTCATGTCCATCTGGGACGACCACGCCGTCAACATGCTCTGGCTCGAC ${\tt TCCACCTGGCCCATCGACGGCGCCGGCCCGAGCGCGGTGC}$ $\tt CTGCCCACCACCTCGGGCGTCCCCGCTGAGGTCGAGGCCGAGGCCCCCA$ ${\tt ACTCCAACGTCATCTTCTCCAACATCCGCTTCGGCCCCATCGGCTCCACC}$ $\tt GTCTCCGGCCTGCCCGACGGCGGCAGCGGCAACCCCAACCCGCCGTCAG$ CTCGTCCACCCCGGTCCCCTCCTCGTCCACCACATCCTCCGGTTCCTCCG GCCCGACTGGCGGCACGGGTGTCGCTAAGCACTATGAGCAATGCGGAGGA ATCGGGTTCACTGGCCCTACCCAGTGCGAGAGCCCCTACACTTGCACCAA

(SEQ ID NO: 131)

MYAKFATLAALVAGAAAQNACTLTAENHPSLTWSKCTSGGSCTSVQGSIT

IDANWRWTHRTDSATNCYEGNKWDTSWCSDGPSCASKCCIDGADYSSTYG

ITTSGNSLNLKFVTKGQYSTNIGSRTYLMESDTKYQMFQLLGNEFTFDVD

VSNLGCGLNGALYFVSMDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKF

INGEANVENWQSSTNDANAGTGKYGSCCSEMDVWEANNMAAAFTPHPCTV

IGQSRCEGDSCGGTYSTDRYAGICDPDGCDFNSYRQGNKTFYGKGMTVDT

TKKITVVTQFLKNSAGELSEIKRFYVQNGKVIPNSESTIPGVEGNSITQD

WCDRQKAAFGDVTDFQDKGGMVQMGKALAGPMVLVMSIWDDHAVNMLWLD

STWPIDGAGKPGAERGACPTTSGVPAEVEAEAPNSNVIFSNIRFGPIGST

VSGLPDGGSGNPNPPVSSSTPVPSSSTTSSGSSGPTGGTGVAKHYEQCGG

GCTGAATGACTGGTACTCGCAGTGCCTGTAA

(SEQ ID NO: 132)
QNACTLTAENHPSLTWSKCTSGGSCTSVQGSITIDANWRWTHRTDSATNC
YEGNKWDTSWCSDGPSCASKCCIDGADYSSTYGITTSGNSLNLKFVTKGQ

YSTNIGSRTYLMESDTKYQMFQLLGNEFTFDVDVSNLGCGLNGALYFVSM
DADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFINGEANVENWQSSTNDA
NAGTGKYGSCCSEMDVWEANNMAAAFTPHPCTVIGQSRCEGDSCGGTYST
DRYAGICDPDGCDFNSYRQGNKTFYGKGMTVDTTKKITVVTQFLKNSAGE
LSEIKRFYVQNGKVIPNSESTIPGVEGNSITQDWCDRQKAAFGDVTDFQD
KGGMVQMGKALAGPMVLVMSIWDDHAVNMLWLDSTWPIDGAGKPGAERGA
CPTTSGVPAEVEAEAPNSNVIFSNIRFGPIGSTVSGLPDGGSGNPNPPVS
SSTPVPSSSTTSSGSSGPTGGTGVAKHYEQCGGIGFTGPTQCESPYTCTK
LNDWYSOCL

[0349] The polynucleotide (SEQ ID NO:133) and amino acid (SEQ ID NO:134) sequences of a *M. thermophila* CBH1a variant ("Variant 983") are provided below. The signal sequence is shown underlined in SEQ ID NO:134. SEQ ID NO:135 provides the sequence of this CBH1a variant, without the signal sequence.

(SEQ ID NO: 133)

TCAGAACGCCTGCACTCTGAACGCTGAGAACCACCCCTCGCTGACGTGGT CCAAGTGCACGTCTGGCGGCAGCTGCACCAGCGTCCAGGGTTCCATCACC ${\tt ATCGACGCCAACTGGCGGTGGACTCACCGGACCGATAGCGCCACCAACTG}$ CTACGAGGGCAACAAGTGGGATACTTCGTACTGCAGCGATGGTCCTTCTT GCGCCTCCAAGTGCTGCATCGACGGCGCTGACTACTCGAGCACCTATGGC ATCACCACGAGCGGTAACTCCCTGAACCTCAAGTTCGTCACCAAGGGCCA GTACTCGACCAACATCGGCTCGCGTACCTACCTGATGGAGAGCGACACCA AGTACCAGATGTTCCAGCTCCTCGGCAACGAGTTCACCTTCGATGTCGAC GTCTCCAACCTCGGCTGCGGCCTCAATGGCGCCCTCTACTTCGTGTCCAT GGATGCCGATGGTGCCATGTCCAAGTACTCGGGCAACAAGGCAGGTGCCA AGTACGGTACCGGCTACTGTGATTCTCAGTGCCCCCGCGACCTCAAGTTC ATCAACGGCGAGGCCAACGTAGAGAACTGGCAGAGCTCGACCAACGATGC CAACGCCGGCACGGCAGTACGCCAGCTGCTCCCGAGATGGACGTCT $\tt GGGAGGCCAACAACATGGCCGCCGCCTTCACTCCCCACCCTTGCACCGTG$ CGACCGCTATGCCGGCATCTGCGACCCCGACGGATGCGACTTCAACTCGT ACCGCCAGGGCAACAAGACCTTCTACGGCAAGGGCATGACGGTCGACACG GCTCTCCGAGATCAAGCGGTTCTACGTCCAGAACGGCAAGGTCATCCCCA ACTCCGAGTCCACCATCCCGGGCGTCGAGGGCAACTCCATCACCCAGGAG ${\tt TACTGCGACCGCCAGAAGGCCGCCTTCGGCGACGTGACCGACTTCCAGGA}$ ${\tt CAAGGGCGGCATGGTCCAGATGGGCAAGGCCCTCGCGGGGCCCATGGTCC}$ TCGTCATGTCCATCTGGGACGACCACGCCGACAACATGCTCTGGCTCGAC

-continued

MYAKFATLAALVAGAAAQNACTLNAENHPSLTWSKCTSGGSCTSVQGSIT

IDANWRWTHRTDSATNCYEGNKWDTSYCSDGPSCASKCCIDGADYSSTYG

ITTSGNSLNLKFVTKGQYSTNIGSRTYLMESDTKYQMFQLLGNEFTFDVD

VSNLGCGLNGALYFVSMDADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKF

INGEANVENWQSSTNDANAGTGKYGSCCSEMDVWEANNMAAAFTPHPCTV

IGQSRCEGDSCGGTYSTDRYAGICDPDGCDFNSYRQGNKTFYGKGMTVDT

TKKITVVTQFLKNSAGELSEIKRFYVQNGKVIPNSESTIPGVEGNSITQE

YCDRQKAAFGDVTDFQDKGGMVQMGKALAGPMVLVMSIWDDHADNMLWLD

STWPIDGAGKPGAERGACPTTSGVPAEVEAEAPNSNVIFSNIRFGPIGST

VSGLPDGGSGNPNPPVSSSTTVPSSSTTSSGSSGPTGGTGVAKHYEQCGG

IGFTGPTQCESPYTCTKLNDWYSQCL

(SEQ ID NO: 135)
QNACTLNAENHPSLTWSKCTSGGSCTSVQGSITIDANWRWTHRTDSATNC
YEGNKWDTSYCSDGPSCASKCCIDGADYSSTYGITTSGNSLNLKFVTKGQ
YSTNIGSRTYLMESDTKYQMFQLLGNEFTFDVDVSNLGCGLNGALYFVSM
DADGGMSKYSGNKAGAKYGTGYCDSQCPRDLKFINGEANVENWQSSTNDA
NAGTGKYGSCCSEMDVWEANNMAAAFTPHPCTVIGQSRCEGDSCGGTYST
DRYAGICDPDGCDFNSYRQGNKTFYGKGMTVDTTKKITVVTQFLKNSAGE
LSEIKRFYVQNGKVIPNSESTIPGVEGNSITQEYCDRQKAAFGDVTDFQD
KGGMVQMGKALAGPMVLVMSIWDDHADNMLWLDSTWPIDGAGKPGAERGA
CPTTSGVPAEVEAEAPNSNVIFSNIRFGPIGSTVSGLPDGGSGNPNPPVS
SSTPVPSSSTTSSGSSGPTGGTGVAKHYEQCGGIGFTGPTQCESPYTCTK
LNDWYSOCL

[0350] The polynucleotide (SEQ ID NO:136) and amino acid (SEQ ID NO:137) sequences of wild-type *M. thermophila* CBH2b are provided below. The signal sequence is shown underlined in SEQ ID NO:137. SEQ ID NO:138 provides the sequence of this CBH2b, without the signal sequence.

(SEQ ID NO: 136)
ATGGCCAAGAAGCTTTTCATCACCGCCGCGCTTGCGGCTGCCGTGTTGGC
GGCCCCCGTCATTGAGGAGCGCCAGAACTGCGGCGCTGTGTGGACTCAAT
GCGGCGGTAACGGGTGGCAAGGTCCCACATGCTGCGCCTCGGGCTCGACC

TGCGTTGCGCAGAACGAGTGGTACTCTCAGTGCCTGCCCAACAGCCAGGT GACGAGTTCCACCACTCCGTCGTCGACTTCCACCTCGCAGCGCAGCACCA GCACCTCCAGCAGCACCACCAGGAGCGGCAGCTCCTCCTCCTCCTCCACC ${\tt ACGCCCCGCCCGTCTCCAGCCCCGTGACCAGCATTCCCGGCGGTGCGAC}$ $\tt CTCCACGGCGAGCTACTCTGGCAACCCCTTCTCGGGCGTCCGGCTCTTCG$ CCAACGACTACTACAGGTCCGAGGTCCACAATCTCGCCATTCCTAGCATG ACTGGTACTCTGGCGGCCAAGGCTTCCGCCGTCGCCGAAGTCCCTAGCTT CCAGTGGCTCGACCGGAACGTCACCATCGACACCCTGATGGTCCAGACTC TGTCCCAGGTCCGGGCTCTCAATAAGGCCGGTGCCAATCCTCCCTATGCT GCCCAACTCGTCGTCTACGACCTCCCCGACCGTGACTGTGCCGCCGCTGC GTCCAACGGCGAGTTTTCGATTGCAAACGGCGCGCCGCCAACTACAGGA GCTACATCGACGCTATCCGCAAGCACATCATTGAGTACTCGGACATCCGG ATCATCCTGGTTATCGAGCCCGACTCGATGGCCAACATGGTGACCAACAT GAACGTGGCCAAGTGCAGCAACGCCGCGTCGACGTACCACGAGTTGACCG TGTACGCGCTCAAGCAGCTGAACCTGCCCAACGTCGCCATGTATCTCGAC GCCGGCCACGCCGGCTGGCTCGGCTGGCCCGCCAACATCCAGCCCGCCGC CGAGCTGTTTGCCGGCATCTACAATGATGCCGGCAAGCCGGCTGCCGTCC $\tt GCGGCCTGGCCACTAACGTCGCCAACTACAACGCCTGGAGCATCGCTTCG$ GCCCCGTCGTACACGTCGCCTAACCTACCTACGACGAGAAGCACTACAT $\tt CGAGGCCTTCAGCCCGCTCTTGAACTCGGCCGGCTTCCCCGCACGCTTCA$ ${\tt TTGTCGACACTGGCCGCAACGGCAACAACCTACCGGCCAACAACAGTGG}$ GGTGACTGGTGCAATGTCAAGGGCACCGGCTTTGGCGTGCGCCCGACGGC CAACACGGGCCACGAGCTGGTCGATGCCTTTGTCTGGGTCAAGCCCGGCG GCGAGTCCGACGGCACAAGCGACACCAGCGCCGCCCGCTACGACTACCAC TGCGGCCTGTCCGATGCCCTGCAGCCTGCCCCCGAGGCTGGACAGTGGTT $\tt CCAGGCCTACTTCGAGCAGCTGCTCACCAACGCCAACCCGCCCTTCTAA$

(SEQ ID NO: 137)

MAKKLFITAALAAAVLAAPVIEERQNCGAVWTQCGGNGWQGPTCCASGST

CVAQNEWYSQCLPNSQVTSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSSST

TPPPVSSPVTSIPGGATSTASYSGNPFSGVRLFANDYYRSEVHNLAIPSM

TGTLAAKASAVAEVPSFQWLDRNVTIDTLMVQTLSQVRALNKAGANPPYA

AQLVVYDLPDRDCAAAASNGEFSIANGGAANYRSYIDAIRKHIIEYSDIR

IILVIEPDSMANMVTNMNVAKCSNAASTYHELTVYALKQLNLPNVAMYLD

AGHAGWLGWPANIQPAAELFAGIYNDAGKPAAVRGLATNVANYNAWSIAS

APSYTSPNPNYDEKHYIEAFSPLLNSAGFPARFIVDTGRNGKQPTGQQQW

GDWCNVKGTGFGVRPTANTGHELVDAFVWVKPGGESDGTSDTSAARYDYH

CGLSDALQPAPEAGQWFQAYFEQLLTNANPPF

(SEQ ID NO: 138)
APVIEERQNCGAVWTQCGGNGWQGPTCCASGSTCVAQNEWYSQCLPNSQV
TSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSSTTPPPVSSPVTSIPGGAT

-continued
STASYSGNPFSGVRLFANDYYRSEVHNLAIPSMTGTLAAKASAVAEVPSF
QWLDRNVTIDTLMVQTLSQVRALNKAGANPPYAAQLVVYDLPDRDCAAAA
SNGEFSIANGGAANYRSYIDAIRKHIIEYSDIRIILVIEPDSMANMVTNM
NVAKCSNAASTYHELTVYALKQLNLPNVAMYLDAGHAGWLGWPANIQPAA
ELFAGIYNDAGKPAAVRGLATNVANYNAWSIASAPSYTSPNPNYDEKHYI
EAFSPLLNSAGFPARFIVDTGRNGKQPTGQQQWGDWCNVKGTGFGVRPTA
NTGHELVDAFVWVKPGGESDGTSDTSAARYDYHCGLSDALQPAPEAGQWF
OAYFEOLLTNANPPF

[0351] The polynucleotide (SEQ ID NO:139) and amino acid (SEQ ID NO:140) sequences of a *M. thermophila* CBH2b variant ("Variant 196") are provided below. The signal sequence is shown underlined in SEQ ID NO:140. SEQ ID NO:141 provides the sequence of this CBH2b variant, without the signal sequence.

(SEQ ID NO: 139) ATGGCCAAGAAGCTTTTCATCACCGCCGCGCTTGCGGCTGCCGTGTTGGC GGCCCCGTCATTGAGGAGCGCCAGAACTGCGGCGCTGTGTGGACTCAAT GCGGCGGTAACGGGTGGCAAGGTCCCACATGCTGCGCCTCGGGCTCGACC TGCGTTGCGCAGAACGAGTGGTACTCTCAGTGCCTGCCCAACAGCCAGGT GACGAGTTCCACCACTCCGTCGTCGACTTCCACCTCGCAGCGCAGCACCA GCACCTCCAGCAGCACCACCAGGAGCGGCAGCTCCTCCTCCTCCACC ACGCCCACCCCGTCTCCAGCCCCGTGACCAGCATTCCCGGCGGTGCGAC CTCCACGGCGAGCTACTCTGGCAACCCCTTCTCGGGCGTCCGGCTCTTCG CCAACGACTACTACAGGTCCGAGGTCCACAATCTCGCCATTCCTAGCATG ACTGGTACTCTGGCGGCCAAGGCTTCCGCCGTCGCCGAAGTCCCTAGCTT CCAGTGGCTCGACCGGAACGTCACCATCGACACCCTGATGGTCCCGACTC TGTCCCGCGTCCGGGCTCTCAATAAGGCCGGTGCCAATCCTCCCTATGCT GCCCAACTCGTCGTCTACGACCTCCCCGACCGTGACTGTGCCGCCGCTGC GTCCAACGGCGAGTTTTCGATTGCAAACGGCGGCGCCCCCCAACTACAGGA GCTACATCGACGCTATCCGCAAGCACATCATTGAGTACTCGGACATCCGG ATCATCCTGGTTATCGAGCCCGACTCGATGGCCAACATGGTGACCAACAT GAACGTGGCCAAGTGCAGCAACGCCGCGTCGACGTACCACGAGTTGACCG TGTACGCGCTCAAGCAGCTGAACCTGCCCAACGTCGCCATGTATCTCGAC GCCGGCCACGCCGGCTGGCTCGGCTGGCCCAACATCCAGCCCGCCGC $\tt CGAGCTGTTTGCCGGCATCTACAATGATGCCGGCAAGCCGGCTGCCGTCC$ GCGGCCTGGCCACTAACGTCGCCAACTACAACGCCTGGAGCATCGCTTCG GCCCGTCGTACACGTCGCCTAACCCTAACTACGACGAGAAGCACTACAT CGAGGCCTTCAGCCCGCTCTTGAACTCGGCCGGCTTCCCCGCACGCTTCA TTGTCGACACTGGCCGCAACGGCAAACAACCTACCGGCCAACAACAGTGG GGTGACTGGTGCAATGTCAAGGGCACCGGCTTTGGCGTGCGCCCGACGGC

GCGAGTCCGACGGCACAAGCGACACCAGCGCCGCCGCTACGACTACCAC
TGCGGCCTGTCCGATGCCCTGCAGCCTGCCCCGAGGCTGGACAGTGGTT
CCAGGCCTACTTCGAGCAGCTGCTCACCAACGCCAACCCGCCCTTCTAA

(SEQ ID NO: 140)

MAKKLFITAALAAAVLAAPVIEERQNCGAVWTQCGGNGWQGPTCCASGST

CVAQNEWYSQCLPNSQVTSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSSST

TPTPVSSPVTSIPGGATSTASYSGNPFSGVRLFANDYYRSEVHNLAIPSM

TGTLAAKASAVAEVPSFQWLDRNVTIDTLMVPTLSRVRALNKAGANPPYA

AQLVVYDLPDRDCAAAASNGEFSIANGGAANYRSYIDAIRKHIIEYSDIR

IILVIEPDSMANMVTNMNVAKCSNAASTYHELTVYALKQLNLPNVAMYLD

AGHAGWLGWPANIQPAAELFAGIYNDAGKPAAVRGLATNVANYNAWSIAS

APSYTSPNPNYDEKHYIEAFSPLLNSAGFPARFIVDTGRNGKQPTGQQQW

GDWCNVKGTGFGVRPTANTGHELVDAFVWVKPGGESDGTSDTSAARYDYH

CGLSDALOPAPEAGOWFOAYFEOLLTNANPPF

(SEQ ID NO: 141)
APVIEERQNCGAVWTQCGGNGWQGPTCCASGSTCVAQNEWYSQCLPNSQV
TSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSSTTPTPVSSPVTSIPGGAT
STASYSGNPFSGVRLFANDYYRSEVHNLAIPSMTGTLAAKASAVAEVPSF
QWLDRNVTIDTLMVPTLSRVRALNKAGANPPYAAQLVVYDLPDRDCAAAA
SNGEFSIANGGAANYRSYIDAIRKHIIEYSDIRIILVIEPDSMANMVTNM
NVAKCSNAASTYHELTVYALKQLNLPNVAMYLDAGHAGWLGWPANIQPAA
ELFAGIYNDAGKPAAVRGLATNVANYNAWSIASAPSYTSPNPNYDEKHYI
EAFSPLLNSAGFPARFIVDTGRNGKQPTGQQQWGDWCNVKGTGFGVRPTA
NTGHELVDAFVWVKPGGESDGTSDTSAARYDYHCGLSDALQPAPEAGQWF
OAYFEOLLTNANPPF

[0352] The polynucleotide (SEQ ID NO:142) and amino acid (SEQ ID NO:143) sequences of a *M. thermophila* CBH2b variant ("Variant 287") are provided below. The signal sequence is shown underlined in SEQ ID NO:143. SEQ ID NO:144 provides the sequence of this CBH2b variant, without the signal sequence.

(SEQ ID NO: 142)
ATGGCCAAGAAGCTTTTCATCACCGCCGCGCTTGCGGCTGCCGTGTTGGC
GGCCCCCGTCATTGAGGAGCGCCAGAACTGCGGCGCTGTGTGGACTCAAT
GCGGCGGTAACGGGTGGCAAGGTCCCACATGCTGCGCCTCGGGCTCGACC
TGCGTTGCGCAGAACGAGTGGTACTCTCAGTGCCTGCCCAACAGCCAGGT
GACGAGTTCCACCACTCCGTCGTCGACTTCCACCTCGCAGCGCAGCACCA
GCACCTCCAGCAGCACCACAGGAGCGGCAGCTCCTCCTCCTCCACC
ACGCCCCCGCCCGTCTCCAGCCCCGTGACCAGCATTCCCGGCGGTGCGAC
CTCCACGGCGAGCTACTCTGGCAACCCCTTCTCGGGCGTCCGGCTCTTCG
CCAACGACTACTACAGGTCCGAGGTCCACAATCTCGCCATTCCTAGCATG

GGTGACTGGTGCAATGTCAAGGGCACCGGCTTTGGCGTGCGCCCGACGGC

CAACACGGGCCACGAGCTGGTCGATGCCTTTGTCTGGGTCAAGCCCGGCG

 $\tt GCGAGTCCGACGGCACAAGCGACACCAGCGCCGCCCGCTACGACTACCAC$

 $\tt TGCGGCCTGTCCGATGCCCTGCAGCCTGCCCCCGAGGCTGGACAGTGGTT$

 $\tt CCAGGCCTACTTCGAGCAGCTGCTCACCAACGCCAACCCGCCCTTCTAA$

continued

(SEQ ID NO: 143)

MAKKLFITAALAAAVLAAPVIEERQNCGAVWTQCGGNGWQGPTCCASGST

CVAQNEWYSQCLPNSQVTSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSSST

TPPPVSSPVTSIPGGATSTASYSGNPFSGVRLFANDYYRSEVHNLAIPSM

TGTLAAKASAVAEVPSFQWLDRNVTIDTLMVPTLSRVRALNKAGANPPYA

AQLVVYDLPDRDCAAAASNGEFSIANGGAANYRSYIDAIRKHIKEYSDIR

IILVIEPDSMANMVTNMNVAKCSNAASTYHELTVYALKQLNLPNVAMYLD

AGHAGWLGWPANIQPAAELFAGIYNDAGKPAAVRGLATNVANYNAWSIAS

APSYTSPNPNYDEKHYIEAFSPLLNDAGFPARFIVDTGRNGKQPTGQQQW

GDWCNVKGTGFGVRPTANTGHELVDAFVWVKPGGESDGTSDTSAARYDYH

CGLSDALOPAPEAGOWFOAYFEOLLTNANPPF

(SEQ ID NO: 144)
APVIEERQNCGAVWTQCGGNGWQGPTCCASGSTCVAQNEWYSQCLPNSQV
TSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSSTTPPPVSSPVTSIPGGAT
STASYSGNPFSGVRLFANDYYRSEVHNLAIPSMTGTLAAKASAVAEVPSF
QWLDRNVTIDTLMVPTLSRVRALNKAGANPPYAAQLVVYDLPDRDCAAAA
SNGEFSIANGGAANYRSYIDAIRKHIKEYSDIRIILVIEPDSMANMVTNM
NVAKCSNAASTYHELTVYALKQLNLPNVAMYLDAGHAGWLGWPANIQPAA
ELFAGIYNDAGKPAAVRGLATNVANYNAWSIASAPSYTSPNPNYDEKHYI
EAFSPLLNDAGFPARFIVDTGRNGKQPTGQQQWGDWCNVKGTGFGVRPTA

-continued
NTGHELVDAFVWVKPGGESDGTSDTSAARYDYHCGLSDALQPAPEAGQWF

[0353] The polynucleotide (SEQ ID NO:145) and amino acid (SEQ ID NO:146) sequences of a *M. thermophila* CBH2b variant ("Variant 962") are provided below. The signal sequence is shown underlined in SEQ ID NO:146. SEQ ID NO:147 provides the sequence of this CBH2b variant, without the signal sequence.

(SEQ ID NO: 145)

ATGGCCAAGAAGCTTTTCATCACCGCCGCGCTTTGCGGCTGCCGTGTTGGC GGCCCCGTCATTGAGGAGCGCCAGAACTGCGGCGCTGTGTGGACTCAAT GCGGCGGTAACGGGTGGCAAGGTCCCACATGCTGCGCCTCGGGCTCGACC TGCGTTGCGCAGAACGAGTGGTACTCTCAGTGCCTGCCCAACAGCCAGGT GACGAGTTCCACCACTCCGTCGTCGACTTCCACCTCGCAGCGCAGCACCA GCACCTCCAGCAGCACCACCAGGAGCGGCAGCTCCTCCTCCTCCACC ACGCCCACCCCGTCTCCAGCCCCGTGACCAGCATTCCCGGCGGTGCGAC CTCCACGGCGAGCTACTCTGGCAACCCCTTCTCGGGCGTCCGGCTCTTCG CCAACGACTACTACAGGTCCGAGGTCATGAATCTCGCCATTCCTAGCATG ACTGGTACTCTGGCGGCCAAGGCTTCCGCCGTCGCCGAAGTCCCTAGCTT CCAGTGGCTCGACCGGAACGTCACCATCGACACCCTGATGGTCACCACTC TGTCCCAGGTCCGGGCTCTCAATAAGGCCGGTGCCAATCCTCCCTATGCT GCCCAACTCGTCGTCTACGACCTCCCCGACCGTGACTGTGCCGCCGCTGC GTCCAACGGCGAGTTTTCGATTGCAAACGGCGGCAGCGCCAACTACAGGA GCTACATCGACGCTATCCGCAAGCACATCATTGAGTACTCGGACATCCGG ATCATCCTGGTTATCGAGCCCGACTCGATGGCCAACATGGTGACCAACAT GAACGTGGCCAAGTGCAGCAACGCCGCGTCGACGTACCACGAGTTGACCG TGTACGCGCTCAAGCAGCTGAACCTGCCCAACGTCGCCATGTATCTCGAC GCCGGCCACGCCGGCTGGCTCGGCTGGCCCGCCAACATCCAGCCCGCCGC CGAGCTGTTTGCCGGCATCTACAATGATGCCGGCAAGCCGGCTGCCGTCC GCGGCCTGGCCACTAACGTCGCCAACTACAACGCCTGGAGCATCGCTTCG GCCCCGTCGTACACGCAGCCTAACCTACGACGAGAAGCACTACAT CGAGGCCTTCAGCCCGCTCTTGAACTCGGCCGGCTTCCCCGCACGCTTCA TTGTCGACACTGGCCGCAACGGCAAACAACCTACCGGCCAACAACAGTGG GGTGACTGGTGCAATGTCAAGGGCACCGGCTTTGGCGTGCGCCCGACGGC GCGAGTCCGACGGCACAAGCGACACCAGCGCCGCCCGCTACGACTACCAC TGCGGCCTGTCCGATGCCCTGCAGCCTGCCCCCGAGGCTGGACAGTGGTT CCAGGCCTACTTCGAGCAGCTGCTCACCAACGCCAACCCGCCCTTCTAA

(SEQ ID NO: 146)

MAKKLFITAALAAAVLAAPVIEERQNCGAVWTQCGGNGWQGPTCCASGST

CVAQNEWYSQCLPNSQVTSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSST

-continued
TPTPVSSPVTSIPGGATSTASYSGNPFSGVRLFANDYYRSEVMNLAIPSM
TGTLAAKASAVAEVPSFQWLDRNVTIDTLMVTTLSQVRALNKAGANPPYA
AQLVVYDLPDRDCAAAASNGEFSIANGGSANYRSYIDAIRKHIIEYSDIR
IILVIEPDSMANMVTNMNVAKCSNAASTYHELTVYALKQLNLPNVAMYLD
AGHAGWLGWPANIQPAAELFAGIYNDAGKPAAVRGLATNVANYNAWSIAS
APSYTQPNPNYDEKHYIEAFSPLLNSAGFPARFIVDTGRNGKQPTGQQQW
GDWCNVKGTGFGVRPTANTGHELVDAFVWVKPGGESDGTSDTSAARYDYH
CGLSDALOPAPEAGOWFOAYFEOLLTNANPPF

(SEQ ID NO: 147)
APVIEERQNCGAVWTQCGGNGWQGPTCCASGSTCVAQNEWYSQCLPNSQV
TSSTTPSSTSTSQRSTSTSSSTTRSGSSSSSSTTPTPVSSPVTSIPGGAT
STASYSGNPFSGVRLFANDYYRSEVMNLAIPSMTGTLAAKASAVAEVPSF
QWLDRNVTIDTLMVTTLSQVRALNKAGANPPYAAQLVVYDLPDRDCAAAA
SNGEFSIANGGSANYRSYIDAIRKHIIEYSDIRIILVIEPDSMANMVTNM
NVAKCSNAASTYHELTVYALKQLNLPNVAMYLDAGHAGWLGWPANIQPAA
ELFAGIYNDAGKPAAVRGLATNVANYNAWSIASAPSYTQPNPNYDEKHYI
EAFSPLLNSAGFPARFIVDTGRNGKQPTGQQQWGDWCNVKGTGFGVRPTA
NTGHELVDAFVWVKPGGESDGTSDTSAARYDYHCGLSDALQPAPEAGQWF
QAYFEQLLTNANPPF

[0354] The polynucleotide (SEQ ID NO:148) and amino acid (SEQ ID NO:149) sequences of another wild-type *M. thermophila* xylanase ("Xyl3") are provided below. The signal sequence is shown underlined in SEQ ID NO:149. SEQ ID NO:150 provides the sequence of this xylanase without the signal sequence.

(SEO ID NO: 148) ATGCACTCCAAAGCTTTCTTGGCAGCGCTTCTTGCGCCTGCCGTCTCAGG GCAACTGAACGACCTCGCCGTCAGGGCTGGACTCAAGTACTTTGGTACTG CTCTTAGCGAGAGCGTCATCAACAGTGATACTCGGTATGCTGCCATCCTC AGCGACAAGAGCATGTTCGGCCAGCTCGTCCCCGAGAATGGCATGAAGTG GGATGCTACTGAGCCGTCCCGTGGCCAGTTCAACTACGCCTCGGGCGACA TCACGGCCAACACGGCCAAGAAGAATGGCCAGGGCATGCGTTGCCACACC $\tt ATGGTCTGGTACAGCCAGCTCCCGAGCTGGGTCTCCTCGGGCTCGTGGAC$ ${\tt CAGGGACTCGCTCACCTCGGTCATCGAGACGCACATGAACAACGTCATGG}$ $\tt GCCACTACAAGGGCCAATGCTACGCCTGGGATGTCATCAACGAGGCCATC$ $\verb|AATGACGACGGCAACTCCTGGCGCGACAACGTCTTTCTCCGGACCTTTGG|$ GACCGACTACTTCGCCCTGTCCTTCAACCTAGCCAAGAAGGCCGATCCCG ATACCAAGCTGTACTACAACGACTACAACCTCGAGTACAACCAGGCCAAG ACGGACCGCGCTGTTGAGCTCGTCAAGATGGTCCAGGCCGCCGCCGCCCCC CATCGACGGTGTCGGCTTCCAGGGCCACCTCATTGTCGGCTCGACCCCGA CGCGCTCGCAGCTGGCCACCGCCCTCCAGCGCTTCACCGCGCTCGGCCTC

CCTG

TCQKLNDWYWQCL

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GAGGTCGCCTACACCGAGCTCGACATCGCCACTCGAGCCTGCCGGCCTC
TTCGTCGGCGCCCCGGACCCAGGGCAACGACTTCGCCAACGTGGTCGGCT
CTTGCCTCGACACCGCCGGCTGCGTCGGCGTCACCGTCTGGGGCTTCACC
GATGCGCACTCGTGGATCCCGAACACGTTCCCCGGCCAGGGCGACGCCCT
GATCTACGACAGCAACTACAACAAGAAGCCCGCGTGGACCTCGATCTCGT
CCGTCCTGGCCGCCAAGGCCACCGGCGCCCCGCCTCCTCCCACC
ACCCTCGTCACCATCACCACCCCTCCGCCGGCATCCACCACCGCCTCCTC
CTCCTCCAGTGCCACGCCCACGAGCGTCCCGACGCAGAGGTGGGGAC
AGTGCGGCGGCATCGGATGGACGAGGCCCATGG
ACCTGCCAGAAGCTGAACGACTGGTACTGGCAGTG

(SEQ ID NO: 149)

MHSKAFLAALLAPAVSGQLNDLAVRAGLKYFGTALSESVINSDTRYAAIL

SDKSMFGQLVPENGMKWDATEPSRGQFNYASGDITANTAKKNGQGMRCHT

MVWYSQLPSWVSSGSWTRDSLTSVIETHMNNVMGHYKGQCYAWDVINEAI

NDDGNSWRDNVFLRTFGTDYFALSFNLAKKADPDTKLYYNDYNLEYNQAK

TDRAVELVKMVQAAGAPIDGVGFQGHLIVGSTPTRSQLATALQRFTALGL

EVAYTELDIRHSSLPASSSALATQGNDFANVVGSCLDTAGCVGVTVWGFT

DAHSWIPNTFPGQGDALIYDSNYNKKPAWTSISSVLAAKATGAPPASSST

TLVTITTPPPASTTASSSSSATPTSVPTQTRWGQCGGIGWTGPTQCESPW

(SEQ ID NO: 150)
QLNDLAVRAGLKYFGTALSESVINSDTRYAAILSDKSMFGQLVPENGMKW

DATEPSRGQFNYASGDITANTAKKNGQGMRCHTMVWYSQLPSWVSSGSWT
RDSLTSVIETHMNNVMGHYKGQCYAWDVINEAINDDGNSWRDNVFLRTFG
TDYFALSFNLAKKADPDTKLYYNDYNLEYNQAKTDRAVELVKMVQAAGAP
IDGVGFQGHLIVGSTPTRSQLATALQRFTALGLEVAYTELDIRHSSLPAS
SSALATQGNDFANVVGSCLDTAGCVGVTVWGFTDAHSWIPNTFPGQGDAL
IYDSNYNKKPAWTSISSVLAAKATGAPPASSSTTLVTITTPPPASTTASS
SSSATPTSVPTQTRWGQCGGIGWTGPTQCESPWTCQKLNDWYWQCL

[0355] The polynucleotide (SEQ ID NO:151) and amino acid (SEQ ID NO:152) sequences of a wild-type *M. thermophila* xylanase ("Xyl2") are provided below. The signal sequence is shown underlined in SEQ ID NO:152. SEQ ID NO:153 provides the sequence of this xylanase without the signal sequence.

(SEQ ID NO: 151)
ATGGTCTCGTTCACTCCTCCTCACGGTCATCGCCGCTGCGGTGACGAC
GGCCAGCCCTCTCGAGGTGGTCAAGCGCGGCATCCAGCCGGGCACGGCA
CCCACGAGGGGTACTTCTACTCGTTCTGGACCGACGGCCGTGGCTCGGTC
GACTTCAACCCCGGGCCCCGCGGCTCGTACAGCGTCACCTGGAACAACGT
CAACAACTGGGTTGGCGGCAAGGGCTGGAACCCGGGCCCGCCGCAAGA

-continued

(SEQ ID NO: 152)

MVSFTLLLTVIAAAVTTASPLEVVKRGIQPGTGTHEGYFYSFWTDGRGSV

DFNPGPRGSYSVTWNNVNNWVGGKGWNPGPPRKIAYNGTWNNYNVNSYLA

LYGWTRNPLVEYYIVEAYGTYNPSSGTARLGTIEDDGGVYDIYKTTRYNQ

PSIEGTSTFDQYWSVRRQKRVGGTIDTGKHFDEWKRQGNLQLGTWNYMIM

ATEGYOSSGSATIEVREA

(SEQ ID NO: 153)

MVSFTLLLTVIAAAVTTASPLEVVKRGIQPGTGTHEGYFYSFWTDGRGSV

DFNPGPRGSYSVTWNNVNNWVGGKGWNPGPPRKIAYNGTWNNYNVNSYLA

LYGWTRNPLVEYYIVEAYGTYNPSSGTARLGTIEDDGGVYDIYKTTRYNQ

PSIEGTSTFDQYWSVRRQKRVGGTIDTGKHFDEWKRQGNLQLGTWNYMIM

ATEGYQSSGSATIEVREA

[0356] The polynucleotide (SEQ ID NO:154) and amino acid (SEQ ID NO:155) sequences of another wild-type *M. thermophila* xylanase ("Xyl1") are provided below. The signal sequence is shown underlined in SEQ ID NO:155. SEQ ID NO:156 provides the sequence of this xylanase without the signal sequence.

(SEO ID NO: 155)

MRTLTFVLAAAPVAVLAQSPLWGQCGGQGWTGPTTCVSGAVCQFVNDWYS
QCVPGSSNPPTGTTSSTTGSTPAPTGGGGSGTGLHDKFKAKGKLYFGTEI
DHYHLNNNALTNIVKKDFGQVTHENSLKWDATEPSRNQFNFANADAVVNF
AQANGKLIRGHTLLWHSQLPQWVQNINDRNTLTQVIENHVTTLVTRYKGK
ILHWDVVNEIFAEDGSLRDSVFSRVLGEDFVGIAFRAARAADPNAKLYIN
DYNLDIANYAKVTRGMVEKVNKWIAQGIPIDGIGTQCHLAGPGGWNTAAG
VPDALKALAAANVKEIAITELDIAGASANDYLTVMNACLQVSKCVGITVW
GVSDKDSWRSSSNPLLFDSNYQPKAAYNALINAL

(SEQ ID NO: 156)

QSPLWGQCGGQGWTGPTTCVSGAVCQFVNDWYSQCVPGSSNPPTGTTSST

TGSTPAPTGGGGSGTGLHDKFKAKGKLYFGTEIDHYHLNNNALTNIVKKD

FGQVTHENSLKWDATEPSRNQFNFANADAVVNFAQANGKLIRGHTLLWHS

QLPQWVQNINDRNTLTQVIENHVTTLVTRYKGKILHWDVVNEIFAEDGSL

RDSVFSRVLGEDFVGIAFRAARAADPNAKLYINDYNLDIANYAKVTRGMV

EKVNKWIAQGIPIDGIGTQCHLAGPGGWNTAAGVPDALKALAAANVKEIA

ITELDIAGASANDYLTVMNACLQVSKCVGITVWGVSDKDSWRSSSNPLLF

DSNYQPKAAYNALINAL

[0357] The polynucleotide (SEQ ID NO:157) and amino acid (SEQ ID NO:158) sequences of another wild-type *M. thermophila* xylanase ("Xyl6") are provided below. The signal sequence is shown underlined in SEQ ID NO:158. SEQ ID NO:159 provides the sequence of this xylanase without the signal sequence.

(SEQ ID NO: 157)

-continued

(SEQ ID NO: 158)

MVSLKSLLLAAAATLTAVTARPFDFDDGNSTEALAKRQVTPNAQGYHSGY

FYSWWSDGGGQATFTLLEGSHYQVNWRNTGNFVGGKGWNPGTGRTINYGG

SFNPSGNGYLAVYGWTHNPLIEYYVVESYGTYNPGSQAQYKGSFQSDGGT

YNIYVSTRYNAPSIEGTRTFQQYWSIRTSKRVGGSVTMQNHFNAWAQHGM

PLGSHDYQIVATEGYQSSGSSDIYVQTH

(SEQ ID NO: 159)
RPFDFDDGNSTEALAKRQVTPNAQGYHSGYFYSWWSDGGGQATFTLLEGS
HYQVNWRNTGNFVGGKGWNPGTGRTINYGGSFNPSGNGYLAVYGWTHNPL
IEYYVVESYGTYNPGSQAQYKGSFQSDGGTYNIYVSTRYNAPSIEGTRTF
QQYWSIRTSKRVGGSVTMQNHFNAWAQHGMPLGSHDYQIVATEGYQSSGS
SDIYVQTH

[0358] The polynucleotide (SEQ ID NO:160) and amino acid (SEQ ID NO:161) sequences of another wild-type *M. thermophila* xylanase ("Xyl5") are provided below. The signal sequence is shown underlined in SEQ ID NO:161. SEQ ID NO:162 provides the sequence of this xylanase, without the signal sequence.

(SEQ ID NO: 161)

MVTLTRLAVAAAAMISSTGLAAPTPEAGPDLPDFELGVNNLARRALDYNQ

NYRTSGNVNYSPTDNGYSVSFSNAGDFVVGKGWRTGATRNITFSGSTQHT

SGTVLVSVYGWTRNPLIEYYVQEYTSNGAGSAQGEKLGTVESDGGTYEIW

RHQQVNQPSIEGTSTFWQYISNRVSGQRPNGGTVTLANHFAAWQKLGLNL

GQHDYQVLATEGWGNAGGSSQYTVSG

(SEQ ID NO: 162)
APTPEAGPDLPDFELGVNNLARRALDYNQNYRTSGNVNYSPTDNGYSVSF
SNAGDFVVGKGWRTGATRNITFSGSTQHTSGTVLVSVYGWTRNPLIEYYV
QEYTSNGAGSAQGEKLGTVESDGGTYEIWRHQQVNQPSIEGTSTFWQYIS
NRVSGQRPNGGTVTLANHFAAWQKLGLNLGQHDYQVLATEGWGNAGGSSQ

[0359] The polynucleotide (SEQ ID NO:163) and amino acid (SEQ ID NO:164) sequences of a wild-type *M. thermophila* beta-xylosidase are provided below. The signal sequence is shown underlined in SEQ ID NO:164. SEQ ID NO:165 provides the sequence of this xylanase without the signal sequence.

(SEO ID NO: 163)

ACCGGGACACGGGCGAATTCCACCTTCTACAACCCCATCTTCCCCGGCT TCTACCCCGATCCGAGCTGCATCTACGTGCCCGAGCGTGACCACACCTTC TTCTGTGCCTCGTCGAGCTTCAACGCCTTCCCGGGCATCCCGATTCATGC CAGCAAGGACCTGCAGAACTGGAAGTTGATCGGCCATGTGCTGAATCGCA AGGAACAGCTTCCCCGGCTCGCTGAGACCAACCGGTCGACCAGCGGCATC ACTAGTGGACGACCGCCGCCGCAGGAGGACGCTTCCAGATGGGACAATA TTATCTTCAAGGCAAAGAATCCGTATGATCCGAGGTCCTGGTCCAAGGCC GTCCACTTCAACTTCACTGGCTACGACACGGAGCCTTTCTGGGACGAAGA TGGAAAGGTGTACATCACCGGCGCCCCATGCTTGGCATGTTGGCCCCATACA TCCAGCAGGCCGAAGTCGATCTCGACACGGGGGCCGTCGGCGAGTGGCGC ATCATCTGGAACGGAACGGGCGGCATGGCTCCTGAAGGGCCGCACATCTA $\tt CCGCAAAGATGGGTGGTACTACTTGCTGGCTGCTGAAGGGGGGGACCGGCA$ TCGACCATATGGTGACCATGGCCCGGTCGAGAAAAATCTCCAGTCCTTAC GAGTCCAACCCAAACAACCCCGTGTTGACCAACGCCAACACGACCAGTTA GGTGGGCAGTCGCCCTCTCCACCCGCTCCGGTCCAGAATATCTTCACTAC CCCATGGGCCGCGAGACCGTCATGACAGCCGTGAGCTGGCCGAAGGACGA GTGGCCAACCTTCACCCCCATATCTGGCAAGATGAGCGGCTGGCCGATGC

CTCCTTCGCAGAAGGACATTCGCGGAGTCGGCCCCTACGTCAACTCCCCC

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MFFASLLLGLLAGVSASPGHGRNSTFYNPIFPGFYPDPSCIYVPERDHTF

FCASSSFNAFPGIPIHASKDLQNWKLIGHVLNRKEQLPRLAETNRSTSGI
WAPTLRFHDDTFWLVTTLVDDDRPQEDASRWDNIIFKAKNPYDPRSWSKA
VHFNFTGYDTEPFWDEDGKVYITGAHAWHVGPYIQQAEVDLDTGAVGEWR
IIWNGTGGMAPEGPHIYRKDGWYYLLAAEGGTGIDHMVTMARSRKISSPY
ESNPNNPVLTNANTTSYFQTVGHSDLFHDRHGNWWAVALSTRSGPEYLHY
PMGRETVMTAVSWPKDEWPTFTPISGKMSGWPMPPSQKDIRGVGPYVNSP
DPEHLTFPRSAPLPAHLTYWRYPNPSSYTPSPPGHPNTLRLTPSRLNLTA
LNGNYAGADQTFVSRRQQHTLFTYSVTLDYAPRTAGEEAGVTAFLTQNHH
LDLGVVLLPRGSATAPSLPGLSSSTTTTSSSSSRPDEEEEREAGEEEEEG
GQDLMIPHVRFRGESYVPVPAPVVYPIPRAWRGGKLVLEIRACNSTHFSF
RVGPDGRRSERTVVMEASNEAVSWGFTGTLLGIYATSNGGNGTTPAYFSD
WRYTPLEQFRD

(SEQ ID NO: 165)
SPGHGRNSTFYNPIFPGFYPDPSCIYVPERDHTFFCASSSFNAFPGIPIH
ASKDLQNWKLIGHVLNRKEQLPRLAETNRSTSGIWAPTLRFHDDTFWLVT
TLVDDDRPQEDASRWDNIIFKAKNPYDPRSWSKAVHFNFTGYDTEPFWDE
DGKVYITGAHAWHVGPYIQQAEVDLDTGAVGEWRIIWNGTGGMAPEGPHI
YRKDGWYYLLAAEGGTGIDHMVTMARSRKISSPYESNPNNPVLTNANTTS
YFQTVGHSDLFHDRHGNWWAVALSTRSGPEYLHYPMGRETVMTAVSWPKD
EWPTFTPISGKMSGWPMPPSQKDIRGVGPYVNSPDPEHLTFPRSAPLPAH
LTYWRYPNPSSYTPSPPGHPNTLRLTPSRLNLTALNGNYAGADQTFVSRR
QQHTLFTYSVTLDYAPRTAGEEAGVTAFLTQNHHLDLGVVLLPRGSATAP

SLPGLSSSTTTTSSSSSRPDEEEEREAGEEEEGGQDLMIPHVRFRGESY VPVPAPVVYPIPRAWRGGKLVLEIRACNSTHFSFRVGPDGRRSERTVVME ASNEAVSWGFTGTLLGIYATSNGGNGTTPAYFSDWRYTPLEQFRD

[0360] The polynucleotide (SEQ ID NO:166) and amino acid (SEQ ID NO:167) sequences of a wild-type *M. ther-mophila* acetylxylan esterase ("Axe3") are provided below. The signal sequence is shown underlined in SEQ ID NO:167. SEQ ID NO:168 provides the sequence of this acetylxylan esterase without the signal sequence.

(SEQ ID NO: 166) GCTGGCTGCCGCGCACCCGGTCTTCGACGAGCTGATGCGGCCGACGGCGC CGCTGGTGCGCCCGCGGGCGCCCTGCAGCAGGTGACCAACTTTGGCAGC AACCCGTCCAACACGAAGATGTTCATCTACGTGCCCGACAAGCTGGCCCC CAACCCGCCCATCATAGTGGCCATCCACTACTGCACCGGCACCGCCCAGG $\tt CCTACTACTCGGGCTCCCCTTACGCCCGCCTCGCCGACCAGAAGGGCTTC$ ATCGTCATCTACCCGGAGTCCCCCTACAGCGGCACCTGTTGGGACGTCTC GTCGCGCCCCCTGACCCACAACGGCGGCGGCGACAGCAACTCGATCG CCAACATGGTCACCTACACCCTCGAAAAGTACAATGGCGACGCCAGCAAG GTCTTTGTCACCGGCTCCTCGTCCGGCGCCATGATGACGAACGTGATGGC $\tt CGCCGCGTACCCGGAACTGTTCGCGGCAGGAATCGCCTACTCGGGCGTGC$ CCGCCGGCTGCTTCTACAGCCAGTCCGGAGGCACCAACGCGTGGAACAGC TCGTGCGCCAACGGGCAGATCAACTCGACGCCCCAGGTGTGGGCCAAGAT GGTCTTCGACATGTACCCGGAATACGACGGCCCGCGCCCCAAGATGCAGA TCTACCACGGCTCGGCCGACGCCACGCTCAGACCCAGCAACTACAACGAG ACCATCAAGCAGTGGTGCGGCGTCTTCGGCTTCGACTACACCCGCCCCGA CACCACCCAGGCCAACTCCCCGCAGGCCGGCTACACCACCTACACCTGGG GCGAGCAGCAGCTCGTCGGCATCTACGCCCAGGGCGTCGGACACACGGTC CCCATCCGCGGCAGCGACGACATGGCCTTCTTTGGCCTGTGA

(SEQ ID NO: 167)

MKLLGKLSAALALAGSRLAAAHPVFDELMRPTAPLVRPRAALQQVTNFGS

NPSNTKMFIYVPDKLAPNPPIIVAIHYCTGTAQAYYSGSPYARLADQKGF

IVIYPESPYSGTCWDVSSRAALTHNGGGDSNSIANMVTYTLEKYNGDASK

VFVTGSSSGAMMTNVMAAAYPELFAAGIAYSGVPAGCFYSQSGGTNAWNS

SCANGQINSTPQVWAKMVFDMYPEYDGPRPKMQIYHGSADGTLRPSNYNE

TIKQWCGVFGFDYTRPDTTQANSPQAGYTTYTWGEQQLVGIYAQGVGHTV

PIRGSDDMAFFGL

(SEQ ID NO: 168)
HPVFDELMRPTAPLVRPRAALQQVTNFGSNPSNTKMFIYVPDKLAPNPPI
IVAIHYCTGTAQAYYSGSPYARLADQKGFIVIYPESPYSGTCWDVSSRAA
LTHNGGGDSNSIANMVTYTLEKYNGDASKVFVTGSSSGAMMTNVMAAAYP

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ELFAAGIAYSGVPAGCFYSQSGGTNAWNSSCANGQINSTPQVWAKMVFDM

YPEYDGPRPKMQIYHGSADGTLRPSNYNETIKQWCGVFGFDYTRPDTTQA

NSPQAGYTTYTWGEQQLVGIYAQGVGHTVPIRGSDDMAFFGL

[0361] The polynucleotide (SEQ ID NO:169) and amino acid (SEQ ID NO:170) sequences of a wild-type *M. thermophila* ferulic acid esterase ("FAE") are provided below. The signal sequence is shown underlined in SEQ ID NO:170. SEQ ID NO:171 provides the sequence of this xylanase without the signal sequence

(SEQ ID NO: 169) ATGATCTCGGTTCCTGCTCTCGCTCTGGCCCTTCTGGCCGCCGTCCAGGT $\tt CGTCGAGTCTGCCTCGGCTGGCTGTGGCAAGGCGCCCCCTTCCTCGGGCA$ ${\tt CCAAGTCGATGACGGTCAACGGCAAGCAGCGCCAGTACATTCTCCAGCTG}$ CCCAACAACTACGACGCCAACAAGGCCCACAGGGTGGTGATCGGGTACCA $\tt CTGGCGCGACGGATCCATGAACGACGTGGCCAACGGCGGCTTCTACGATC$ TGCGGTCCCGGGCGGCGACAGCACCATCTTCGTTGCCCCCAACGGCCTC AATGCCGGATGGGCCAACGTGGGCGGCGAGGACATCACCTTTACGGACCA GATCGTAGACATGCTCAAGAACGACCTCTGCGTGGACGAGACCCAGTTCT TTGCTACGGGCTGGAGCTATGGCGGTGCCATGAGCCATAGCGTGGCTTGT TCTCGGCCAGACGTCTTCAAGGCCGTCGCGGTCATCGCCGGGGCCCAGCT $\tt GTCCGGCTGCGCCGGCGCACGACGCCCGTGGCGTACCTAGGCATCCACG$ GAGCCGCCGACAACGTCCTGCCCATCGACCTCGGCCGCCAGCTGCGCGAC AAGTGGCTGCAGACCAACGGCTGCAACTACCAGGGCGCCCAGGACCCCGC GCCGGGCCAGCAGCCCACATCAAGACCACCTACAGCTGCTCCCGCGCGC CCGTCACCTGGATCGGCCACGGGGGCGGCCACGTCCCCGACCCCACGGGC AACAACGGCGTCAAGTTTGCGCCCCAGGAGACCTGGGACTTCTTTGATGC CGCCGTCGGAGCGGCCGGCGCGCAGAGCCCGATGACATAA

(SEQ ID NO: 170)

MISVPALALALLAAVQVVESASAGCGKAPPSSGTKSMTVNGKQRQYILQL

PNNYDANKAHRVVIGYHWRDGSMNDVANGGFYDLRSRAGDSTIFVAPNGL

NAGWANVGGEDITFTDQIVDMLKNDLCVDETQFFATGWSYGGAMSHSVAC

SRPDVFKAVAVIAGAQLSGCAGGTTPVAYLGIHGAADNVLPIDLGRQLRD

KWLQTNGCNYQGAQDPAPGQQAHIKTTYSCSRAPVTWIGHGGGHVPDPTG

NNGVKFAPQETWDFFDAAVGAAGAQSPMT

(SEQ ID NO: 171)
ASAGCGKAPPSSGTKSMTVNGKQRQYILQLPNNYDANKAHRVVIGYHWRD
GSMNDVANGGFYDLRSRAGDSTIFVAPNGLNAGWANVGGEDITFTDQIVD
MLKNDLCVDETQFFATGWSYGGAMSHSVACSRPDVFKAVAVIAGAQLSGC
AGGTTPVAYLGIHGAADNVLPIDLGRQLRDKWLQTNGCNYQGAQDPAPGQ
QAHIKTTYSCSRAPVTWIGHGGGHVPDPTGNNGVKFAPQETWDFFDAAVG
AAGAQSPMT

Example 1

Gene Acquisition and Construction of Expression Vectors

[0362] A protein from a strain of *M. thermophila* having the amino acid sequence provided in SEQ ID NO:2 was previously identified as having GH61 activity. It was designated "GH61a". FIG. 1 shows the improvement in glucose yield resulting from having GH61a present in a reaction where crystalline cellulose undergoes saccharification by cellulase enzymes that are contained in culture broth from *M. thermophila* cells.

[0363] In this Example, the wild type GH61a gene from *M. thermophila* was isolated from the genome and the DNA sequence verified. The gene was cloned into a *Saccharomyces cerevisiae/M. thermophila* shuttle vector pYTDX60 using Pml1 cloning sites, using standard methods known in the art. The signal peptide and gene were under the control of a yeast transcription elongation factor 1 promoter (pTEF1). The vector contained the REP2, rep1 and protein D (partial) origin of replication for *S. cerevisiae* and a URA3 resistance marker.

[0364] The resulting plasmid (pYTDX60-GH61a) was transformed into *S. cerevisiae* INVSC1 strain and the transformed host cells were grown in Costar 96 deep well plates for GH61a protein production. The GH61a sequence from the transformants were verified as the wild type GH61a DNA sequence (SEQ ID NO:1) and the encoded polypeptide (SEQ ID NO:2).

Example 2

Shake Flask Procedure

[0365] A single colony of S. cerevisiae containing a plasmid with the GH61a gene was inoculated into 3 mL synthetic defined-uracil (SD-ura) broth (2 g/L synthetic dropout minus uracil without yeast nitrogen base (US Biological), 5 g/L ammonium sulfate, 0.1 g/L calcium chloride, 2 mg/L inositol, 0.5 g/L magnesium sulfate, 1 g/L potassium phosphate monobasic (KH·2PO4), 0.1 g/L sodium chloride) containing 6% glucose. Cells were grown overnight (at least 21 hrs) in an incubator at 30° C. with shaking at 250 rpm. Then, 500 µL of the overnight culture was diluted into either 50 mL SD-ura medium or modified galactose expression medium (30 g/L galactose, 6.7 g/L yeast nitrogen base without amino acids, 5 g/L ammonium sulfate, 24 g/L amino acid mix minus uracil, 10 g/L potassium phosphate monobasic (KH₂PO₄) and 0.38% vitamin mix) containing 2% glucose in a 250 mL baffled sterile shake flask and incubated at 37° C. (for SD-ura medium) or 30° C. (for modified galactose expression medium) for 48 hours. Cells were pelleted by centrifugation (4000 rpm, 15 min, 4° C.). The clear media supernatant containing the secreted GH61a enzyme was collected and stored at 4° C. until used.

Example 3

GH61 Activity Assays

[0366] In some experiments, GH61 activity was determined using a biomass assay. The substrate was wheat straw that had been pretreated under acidic conditions (hereinafter referred to as "pretreated wheat straw"). The reaction was carried out in a total volume of 77 μ L in the presence of 10

mg of pre-treated wheat straw, with 62 μ L of 1×-20× concentrated clear media supernatant ("broth") containing *S. cerevisiae*-produced *M. thermophila* GH61a enzyme and 15 μ L of sodium acetate buffer (pH 5.0), *M. thermophila*-produced cellobiohydrolase 1a (CBH1a), cellobiohydrolase 2b (CBH2b) and beta-glucosidase. The final concentration of sodium acetate was 150 mM and the enzyme loads of CBHs and beta-glucosidase were approximately 0.0025%-0.0125% (CBH1a and CBH2b in 1:1 ratio) and 0.01 to 0.02% with respect to substrate glucan mass in the biomass substrate, respectively.

[0367] Some experiments were also performed in the presence of inhibitors that may arise through the routine preparation or pre-treatment of a cellulose substrate. In this way, GH61 protein variants can be identified that are more resistant to the presence of such inhibitors, and therefore find use with a wider range of feedstocks and have wider applicability in the processing of biomass from different sources.

[0368] In some experiments, the pretreatment filtrate was obtained by washing pretreated substrate solids with water. The GH61 activity assay was carried out with 50 μL of GH61a containing supernatant, 12 μL of pretreatment filtrate, and 15 μL of sodium acetate buffer mixed with CBH1a, CBH2b and beta-glucosidase isolated from *M. thermophila*. Background negative controls were obtained by using media supernatant from cultures of cells without the GH61a gene in the plasmid. Thus, the negative controls represent activities of CBH1a, CBH2b and beta-glucosidase in the absence of GH61a. The reaction was incubated at 50 to 60° C. for 24 to 72 hours with shaking, and then quenched by adding 130 μL H₂O at room temperature.

[0369] Some experiments were carried out in a total volume of 360 μL in the presence of 10 mg of pre-treated wheat straw and 40 µL filtrate (11% total volume), with 262 μL of clear media supernatant containing S. cerevisiaeproduced M. thermophila GH61a enzyme and 48 µL of sodium acetate buffer (pH 5; supplemented with CuSO₄) mixed with M. thermophila-produced CBH1a, CBH2b and β -glucosidase. The final concentrations of sodium acetate and CuSO₄ were 128 mM and 15 μM, respectively, and the enzyme loads of CBH's and beta-glucosidase were 0.01% (CBH1a and CBH2b in 1:1 ratio) and 0.02% with respect to substrate glucan mass in the biomass substrate, respectively. Background negative controls were obtained by using media supernatant from cultures of S. cerevisiae cells without the GH61a gene in the plasmid. Thus, the negative controls represent glucose production by CBH1a, CBH2b and betaglucosidase in the absence of GH61a. The reaction was incubated at 55° C. for 72 hours with shaking.

[0370] The GH61 activity in the reaction mixture was measured by monitoring glucose production, as determined using an enzymatic glucose assay kit (K-GLUC, Megazyme). In a total volume of 200 μL , 20 μL of GH61a reaction mixture was added to 180 μL of 2× concentrated glucose determination reagent (GOPOD Reagent TM , supplied as part of the K-GLUC assay kit). The reaction was incubated at room temperature for 30 minutes and the absorbance of the solution was measured at 510 nm. The glucose oxidase enzyme in the GOPOD reagent reacts with glucose and produces hydrogen peroxide, which then reacts with the 4-aminoantipyrine in the reagent to produce a quinoneimine dye. The amount of quinoneimine dye was measured spectrophotometrically at 510 nm to calculate the total amount of

D-glucose in the reaction mixture. The total amount of glucose in the reaction mixture was also measured using an AGILENT® HPLC 1200 equipped with an AMINEXTM HPX-87H ion exclusion column (300 mm×7.8 mm+Bio-Rad) with 5 mM sulfuric acid in water as eluent at a flow rate of 0.6 mL/min at 65° C. The retention time of glucose was 9.5 minutes.

[0371] Detectable amounts of glucose, as a measure of GH61 activity, were observed under high throughput screening conditions (pH 5, 55° C.). GH61a specific activity in the reaction mixture (which also comprised CBH1a, CBH2b and beta-glucosidase) was determined by subtracting the amount of glucose in the negative control reaction (comprising CBH1a, CBH2b and BGL, but not GH61a) from the total glucose measurement.

Example 4

High Throughput Assays to Identify Improved GH61a Variants

[0372] Plasmid libraries containing variant GH61a genes were transformed into S. cerevisiae INVSC1 strain and plated on SD-ura agar plate containing 2% glucose. After incubation for at least 48 hours at 30° C., colonies were picked using a Q-bot® robotic colony picker (Genetix) into shallow, 96-well well microtiter plates containing 200 µL SD-ura media and 6% glucose. Cells were grown for at least 21 hours at 30° C. with shaking at 250 rpm and 85% humidity. Then, 20 µL of the overnight culture was transferred into 96-deep well microtiter plates containing 380 μL SD-ura medium with 2% glucose as described in Example 2. In some cases, 15 µL of the overnight culture was transferred into 96-deep well microtiter plates containing 285 µL modified galactose expression medium with 2% glucose as described in Example 2. The plates were incubated at 37° C. (for SD-ura medium) or 30° C. (for modified galactose expression medium) with shaking at 250 rpm and 85% humidity for 48 hours. The deep well plates were centrifuged at 4000 rpm for 15 minutes and the clear media supernatant containing the secreted GH61a enzyme was used for the high throughput biomass assay.

[0373] The GH61a libraries were screened for thermoactivity using a biomass-based high throughput method using the assays described in Example 3.

Example 5

Improved GH61 Activity of Engineered GH61a Variants

[0374] Improved GH61a variants were identified from the high throughput screening of various GH61a variant libraries as described in the previous Example. The screening was done by measuring thermoactivity of these variants compared with that of the parental GH61a enzyme (expressed from GH61a DNA; SEQ ID NO:1). The high throughput (HTP) saccharification reactions were conducted at pH 5, 55° C. for 24-72 hrs, using 50 g/L pretreated wheat straw, 0.0025-0.01% of mixture of CBH1a and CBH2b (1:1 ratio), and 0.01 to 0.02% of beta-glucosidase.

Example 6

Shake Flask Validation of Improved GH61a Variants

[0375] Improved GH61a variants identified in the high throughput screening (as described in the previous Example)

were prepared using the shake flask procedure described above. GH61 activities were determined using a biomass assay as described above, in which normalized concentrations of GH61a variants were used for direct comparison of the specific activities of the GH61a variants. Reactions were quenched at different time points between 24 to 72 hours and glucose levels measured for time-course analysis. FIG. 2 shows time course results for three GH61a variants. FIG. 2 also shows specific activities observed under the following assay conditions: pH 5.0, and 55° C., utilizing 50 g/L pretreated wheat straw, 0.0025%-0.0125% of mixture of CBH1a and CBH2b (1:1 ratio) and 0.01 to 0.02% of beta-galactosidase. The protein concentration was normalized in reactions. In this Figure, N=3; error bars represent ±1 standard deviation. GH61 activity is shown as the increase in glucose production by the enzyme combination [CBH1a+ CBH2b+BGL1] supplemented by the GH61 protein, minus the glucose production by the same enzyme combination in the absence of the GH61 protein.

[0376] The results show that Variants 5 and 9 (SEQ ID NOS:6 and 8) have a 2.0 to 2.9 fold improvement over the native GH61a (SEQ ID NO:2); and Variant 1 has a 3.0 to 3.9 fold improvement over GH61a (SEQ ID NO:2).

[0377] Substitutions improving GH61 activity are compiled in Table 6-1 below. This table shows GH61a variants derived from the native GH61a enzyme (SEQ ID NO:2) that were shown to have improved thermoactivity. Improvement in GH61 activity in relation to the parental GH61a protein (SEQ ID NO:2) is indicated with the following scale:

[0378] +=1.1 to 1.9 fold improvement compared with wild-type (SEQ ID NO:2)

[0379] ++=2.0 to 2.9 fold improvement compared with wild-type

[0380] +++=3.0 to 3.9 fold improvement compared with wild-type

TABLE 6-1

	GH61 Variants with Improved Activity											
Var. No.	Amino Acid Changes	Silent Nucleotide Changes	Improve- ment in GH61 Activity									
1	N35G/E104H/A168P	t60c/c573g	+++									
	(SEQ ID NO: 4)											
2	W42P/E104H/K167A	t60c/c573g/g1026a	++									
3	N35G/W42P/V97Q/A191N		++									
4	W42P/E104H	c573g	++									
5	E104H/K167A	t60c/c291a/c573g	++									
6	W42P/A191N	t60c/c291a	++									
7	N35G/W42P/A191N	t60c/c291a	++									
8	H20D		++									
9	V97Q/A191N		++									
10	N35G/E104H/A191N	t60c/c876t	++									
11	E104H		++									
12	E104Q		+									
13	H20D/E104D/Q190H/Y192H		+									
14	H20D/Q190E/Y192Q	a312g	+									
15	H20D/E104C		+									
16	H20D/P103H/E104C		+									
17	H20D/P103H	a312g	+									
18	N35G/E104H	t60c/c573g	+									
19	H20D/P103H/E104Q/Q190E		+									
20	H20D/P103H/E104C/Y192Q		+									
21	E104D	t60c	+									
22	N35G/W42P	t60c/c573g	+									
23	A137P		+									
24	H20D/P103H/E104Q		+									

TABLE 6-1-continued

TABLE 6-1-continued

	GH61 Variants with I	mproved Activity		GH61 Variants with Improved Activity						
Var. No.	Amino Acid Changes	Silent Nucleotide Changes	Improve- ment in GH61 Activity	Var. No.	Amino Acid Changes	Silent Nucleotide Changes	Improve- ment in GH61 Activity			
25	P103E/E104D	t60c	+	92	H175T		+			
26	N35G/F68Y/A191N	t379a/c380g/g381c	+	93	N187K/S330R	c597g	+			
27 28	W42P/A168P H20D/E104C/Q190E/Y192Q		+ +	94 95	H175R L166H		+			
29	A142W		+	96	I178L		+			
30	N35G		+	97	L173H		+			
31	H20C/Q190E		+	98	I177T		+			
32	W42P/A212P/T236P	160 (572	+	99	N170Y		+			
33	N35G/W42P/V97Q/K167A/ A168P	t60c/c573g	+	100 101	H175S K167T		+			
34	V97Q/A168P	c573g	+		L166R		+			
35	S232A	8	+	103	V172Y		+			
36	W42P/E104H/K167A/A168P/	c573g	+	'104			+			
	Q190E			105			+			
37	W42P/A168P/A212P/T236P		+	106	H175M		+			
38 39	N35G/V97Q/K167A N35G/V97Q		+	107 108			+			
40	N35G/A191N		+	109	W131K/H175Q	g1026a	+			
41	S127T/K167A/A191N		+		Y171A	· ·	+			
42	W42P		+		N170H		+			
43	W42P/E104C/K167A/A168P	t60c/c291a/c573g	+		P163R		+			
44 45	K167Q W131V		+	113 114	A168C G169T		+			
46	E176C		+	114	R174F		+			
47	K167I/P273S	c300t	+	116			+			
48	W42P/T87P		+	117	I134L		+			
49	W42P/A212P		+		I177V		+			
50	K133H		+	119			+			
51 52	D165N D165A		+	120	H175C W131I		+			
53	A168D		+	121	W42P/A143P		+			
54	K218T		+	123	I178G	c72t	+			
55	P45T		+	124	N170P		+			
56	Q44V		+	125	A179D/N317K	c732g/c843t/c882t/	+			
57	S164W		+	126	11 (2) /	c909t/c912g				
58 59	I177F A191N		+	126 127	I162V I178M		+			
60	I134P		+		V172A		+			
61	K133F		+	129	K167A/A191N	t60c/c291a	+			
62	I134D		+		F132A		+			
63	N35G/K167A	t60c/c291a/c573g	+	131			+			
64 65	I162R N35G/K167A	t204c/t379a/c380g/	+	132	F132M A179G		+			
0.5	N33G/K10/A	g381c/c385t	+	134			+			
66	D165W/A246T	800100000	+	135	K167A	g921a	+			
67	I162L		+	136		C	+			
68	S164M		+	137			+			
69	F132D/A244D		+	138	A179N	-7024	+			
70 71	H181Q I177G	g1026a	+	139 140	I134A K167E	c792t g972t	+			
72	L166W	510204	+	141	R174K	87720	+			
73	I162F		+	142	S164F		+			
74	I134V		+	143	V172L		+			
75	E176Q		+	144	A168H		+			
76 77	H181S I178A		+	145 146	I134T K167H		+			
78	K167A		+	140	L166A		+			
79	V172K		+	148	S164R		+			
80	I177H		+	149	R174C		+			
81	I134N		+	150	A179P	1000	+			
82	K133Y N25G/V130I		+	151		g1026a	+			
83 84	N35G/Y139L A168G		+	152 153	L173M D165K		+			
85	T12A/I162G	c246t	+		E176S		+			
86	D165E		+	155	F132L		+			
87	D165M		+	156	F132I/A179I		+			
88	I134M		+		F132P		+			
89	A168P		+	158	S164Q		+			
90 91	I177D S164P		+ +	159 160	V172Q W131D		+			
21	510-11		Τ-	100	11 13117		т			

TABLE 6-1-continued

TABLE 6-1-continued

	TABLE 0-1-			TABLE 0-1-continued							
	GH61 Variants with 1	Improved Activity		GH61 Variants with Improved Activity							
Var. No.	Amino Acid Changes	Silent Nucleotide Changes	Improve- ment in GH61 Activity	Var. No.	Amino Acid Changes	Silent Nucleotide Changes	Improve ment in GH61 Activity				
161	W131Q		+	229	L173F		+				
162	A179H		+	230	N170Q		+				
163	I134H/G270S		+	231	I177P		+				
164	N170G		+	232	R174N		+				
165	A168T		+	233	V172K/S215W		+				
166 167	A179C		+	234 235	D165R	o520o/o522a	+				
168	K133N K167L		+	235	G239D H175V	c520a/c522g	+				
169	L180M		+	237	H181R		+				
170	W131F		+	238	I134Y		+				
171	I134W	g1026a	+	239	V172F		+				
172	I178H		+	240	V172G		+				
173	N170A		+								
174	V172H		+								
175	A168H/S205N		+	[0381]	Table 6-2 shows GH6	1a variants deriv	ed from the				
176	I134H	g921a	+	GH61	a protein designated "V						
177	S164C		+		yed thermoactivity. The s						
178	S164K		+								
179 180	I177C I178Q		+		ed the alterations of Varian						
181	L180W		+		a (N35G/E104H/A168P),						
182	I177M		+	tions.]	Improvement in GH61 ac	tivity in relation	to Variant				
183	R174D		+	(SEQ	ID NO:4) is indicated in	Table 6-2 acco	rding to the				
184	V172M		+		ing scale:		C				
185	A179M		+		-	4					
186	H175Y		+		*=0.5 to 1.0 fold in	nprovement con	ipared wi				
187	I178P		+	Varian	t 1 (SEQ ID NO:4)						
107											
188	L173A		+	[0383]	+=1.1 to 1.9 fold in	nnrovement con	nared wi				
188 189	N170E		+		+=1.1 to 1.9 fold in	nprovement con	npared wi				
188 189 190	N170E N170F	4270 / 200 / 201 /	++	Varian	t 1;						
188 189	N170E	t379a/c380g/g381c/ c454a/c456a/c732t/	+	Varian [0384]	t 1; ++=2.0 to 2.9 fold in						
188 189 190 191	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R		+ + +	Varian	t 1; ++=2.0 to 2.9 fold in						
188 189 190	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R	c454a/c456a/c732t/	++	Varian [0384]	t 1; ++=2.0 to 2.9 fold in t 1	mprovement con	_				
188 189 190 191	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R	c454a/c456a/c732t/	+ + + +	Varian [0384]	t 1; ++=2.0 to 2.9 fold in	mprovement con					
188 189 190 191 192 193	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D165I	c454a/c456a/c732t/	+ + + + +	Varian [0384] Varian	t 1; ++=2.0 to 2.9 fold in t 1	mprovement con	npared wi				
188 189 190 191 192 193 194	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S	c454a/c456a/c732t/	+ + + + +	Varian [0384] Varian	t 1; ++=2.0 to 2.9 fold in t 1	mprovement con	npared wi				
188 189 190 191 192 193 194 195 196 197	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D165I I162M K167V A179S E176N	c454a/c456a/c732t/	+ + + + + +	Varian [0384] Varian	t 1; ++=2.0 to 2.9 fold in t 1	mprovement con	npared wi				
188 189 190 191 192 193 194 195 196 197 198	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D165I I162M K167V A179S E176N I134L/P322L	c454a/c456a/c732t/	+ + + + + + + + +	Varian [0384] Varian	t 1; ++=2.0 to 2.9 fold in t 1	mprovement con	npared wi				
188 189 190 191 192 193 194 195 196 197 198 199	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D165I I162M K167V A179S E176N II34L/P322L P163L	c454a/c456a/c732t/	+ + + + + + + + + +	Varian [0384] Varian G Variant	t 1; ++=2.0 to 2.9 fold in t 1	mprovement con 6-2 ctivity Compared to	npared wi Variant 1 GH61 Activity				
188 189 190 191 192 193 194 195 196 197 198 199 200	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D	c454a/c456a/c732t/	+ + + + + + + + +	Varian [0384] Varian G Varian Number	t 1; ++=2.0 to 2.9 fold in t 1 TABLE H61 Variants with Improved A	mprovement con E 6-2 ctivity Compared to Silent Nucleotide Changes	Nariant 1 GH61 Activity Improvement				
188 189 190 191 192 193 194 195 196 197 198 199 200 201	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S	c454a/c456a/c732t/	+ + + + + + + + + + + +	Varian [0384] Varian G Variant	t 1; ++=2.0 to 2.9 fold in t 1 TABLE H61 Variants with Improved A r Amino Acid Changes N35G/T40A/E104H/A168P/	mprovement con 6-2 ctivity Compared to Silent Nucleotide	npared wariant 1 GH61 Activity				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D165I I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G	c454a/c456a/c732t/	+ + + + + + + + + + + + +	Varian [0384] Varian G Variant Number	t 1;	mprovement con E 6-2 ctivity Compared to Silent Nucleotide Changes t60c/c573g	Nariant 1 GH61 Activity Improvement				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D165I I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R	c454a/c456a/c732t/	+ + + + + + + + + + + +	Varian [0384] Varian G Varian Number	t 1;	mprovement con E 6-2 ctivity Compared to Silent Nucleotide Changes	Nariant 1 GH61 Activity Improvement				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C	c454a/c456a/c732t/	+ + + + + + + + + + + + +	Varian [0384] Varian G Variant Number 241	t 1;	mprovement con E 6-2 ctivity Compared to Y Silent Nucleotide Changes t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D165I I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Number	t 1;	mprovement con E 6-2 ctivity Compared to Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g	Nariant 1 GH61 Activity Improvement				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Varian Number 241 242 243	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Number 241 242 243 244	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++				
188 189 190 191 192 193 194 195 196 197 198 200 201 202 203 204 205 206 207 208 209	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Number 241 242 243 244 245	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 207 208 209 210	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Number 241 242 243 244 245	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 200 201 202 203 204 205 207 207 208 209 210 211	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132P/Q190E/A191T F132Q I134C	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Variant Number 241 242 243 244 245 246 247	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 200 201 202 203 204 205 206 207 208 209 211 212	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Varian Numbe: 241 242 243 244 245 246	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 221 211 212 213	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Numbe: 241 242 243 244 245 246 247 248	t 1;	mprovement con 3 6-2 ctivity Compared to Y Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 200 201 202 203 204 205 206 207 208 209 210 221 211 212 213 214	N170E N170F N35G/A191N/T258L/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Varian Numbe: 241 242 243 244 245 246 247 248 249	t 1;	mprovement con E 6-2 ctivity Compared to Y Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 197 198 199 200 201 202 203 204 205 206 207 208 209 211 212 213 214 215	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Numbe: 241 242 243 244 245 246 247 248	t 1;	mprovement con 3 6-2 ctivity Compared to Y Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 200 201 202 203 204 205 206 207 208 209 211 212 213 214 215 216	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169K H181A	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Number 241 242 243 244 245 246 247 248 249 250	t 1;	mprovement con Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169K H181A I177L	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Varian Numbe: 241 242 243 244 245 246 247 248 249	t 1;	mprovement con 3 6-2 ctivity Compared to Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 200 201 202 203 204 205 206 207 208 209 210 211 212 212 213 214 215 216 217 218	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169K H181A I177L A168G	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Varian Number 241 242 243 244 245 246 247 248 249 250 251	t 1;	mprovement con 3 6-2 ctivity Compared to Y Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 211 212 213 214 215 216 217 217 218 219	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169K H181A I177L A168G A179R	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Varian Varian Varian Varian 241 242 243 244 245 246 247 248 249 250 251 252	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g	Nariant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 207 208 209 211 212 213 214 215 216 217 218 218	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169K H181A I177L A168G A179R D165T	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Varian Number 241 242 243 244 245 246 247 248 249 250 251	t 1;	mprovement con 3 6-2 ctivity Compared to Y Silent Nucleotide Changes t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g t60c/c573g	Variant 1 GH61 Activity Improvem ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 199 200 201 202 202 202 202 203 204 205 206 207 208 210 211 212 213 214 215 216 217 218 217 218	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169K H181A I177L A168G A179R	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Varian Varian Numbe 241 242 243 244 245 246 247 248 249 250 251 252 253	t 1;	mprovement con Silent Nucleotide Changes t60c/c573g	npared w Variant 1 GH61 Activity Improvem ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 200 201 202 203 204 205 206 211 212 213 214 215 216 217 218 219 220 221 222 222	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132P/ G169A G169A G169A G169K H181A I177L A168G A179R D165T K167R	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Numbe: 241 242 243 244 245 246 247 248 249 250 251 252 253 254	t 1;	mprovement con 3 6-2 ctivity Compared to Silent Nucleotide Changes t60c/c573g	variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 210 211 212 213 214 215 216 217 218 219 220 221 222 223	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169K H181A I177L A168G A179R D165T K167R L166V	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Varian Varian Numbe 241 242 243 244 245 246 247 248 249 250 251 252 253	t 1;	mprovement con Silent Nucleotide Changes t60c/c573g	Variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 207 208 209 211 212 213 214 215 216 227 228 229 220 221 221 221 222 223 224 225 226 227 228 229 229 220 220 221 221 221 221 221 221 222 223 224 225 226 227 227 228 229 229 220 220 221 221 221 221 221 221 221 221	N170E N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169A G169K H181A I177L A168G A179R D165T K167R L166V N170C	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Variant Numbe: 241 242 243 244 245 246 247 248 249 250 251 252 253 254	t 1;	mprovement con E 6-2 Silent Nucleotide Changes t60c/c573g	variant 1 GH61 Activity Improvement ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204	N170E N170F N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169K H181A I177L A168G A179R D165T K167R L166V N170C I178R	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian G Varian 10384 Varian Varian 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255	t 1;	mprovement con 3 6-2 ctivity Compared to Silent Nucleotide Changes t60c/c573g	Nariant 1 GH61 Activity Improvements ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++				
188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 219 210 210 210 211 211 212 213 214 215 216 217 218 219 219 210 210 210 210 210 210 210 210 210 210	N170E N170F N170F N35G/A191N/T258I/T323P/ G328A/C341R A168R D1651 I162M K167V A179S E176N I134L/P322L P163L H181D N170S R174G I177R K167C L166Q P163I S164L/L166I Y171R F132P/Q190E/A191T F132Q I134C I177A E176R G169A G169A G169K H181A I177L A168G A179R D165T K167R L166V N170C I178R R174H	c454a/c456a/c732t/	+ + + + + + + + + + + + + + + + + + +	Varian [0384] Varian Varian Varian Varian Variant Numbe: 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256	t 1;	mprovement con Silent Nucleotide Changes t60c/c573g	npared wi Variant 1 GH61 Activity Improveme ++ ++ + + + + + + + + + + + + + + +				

TABLE 6-2-continued

TABLE 6-2-continued

Vari-		C'I NTI I'I-	GH61	Vari-		Cilora No. 1 ci 1.	GH61
ant Number	Amino Acid Changes	Silent Nucleotide Changes	Activity Improvement	ant Number	Amino Acid Changes	Silent Nucleotide Changes	Activity Improvemen
259	N35G/E104H/A168P/P233V	t60c/c573g	+	314	N35G/E104H/A116Q/A168P	t60c/c573g	+
260	R34E/N35G/E104H/R145T/	t60c/c573g	+	315	N35G/E104H/A168P/S330C	t60c/c573g	+
	A168P			316	N35G/T40S/E101T/E104H/	t60c/c573g	+
261	S24Q/N35G/E104H/A168P/	t60c/c573g	+		A168P/P327M		
	V237I			317	N35G/E104H/A168P/A326Q	t60c/c573g	+
262	Y32S/N35G/E64S/E104H/	t60c/c573g	+	318	N35G/N46R/E104H/A168P	t60c/c573g	+
	A168P			319	N35G/P45K/E104H/A168P/	t60c/c573g	+
263	N35G/E104H/A168P/V333R	t60c/c573g	+		A219R/S232E		
264	N35G/E104H/G144S/A168P/	t60c/c573g	+	320	S24Q/N35G/E104H/A168P/	t60c/c573g	+
	V333Q				V237I/P303T		
265	V28H/N35G/P45K/E104H/	t60c/c573g	+	321	N35G/E104H/A168P/G203E/	t60c/c573g	+
	A168P				T281A		
266	N35G/E104H/A168P/P327K	t60c/c573g	+	322	N35G/A56N/E104H/A168P	t60c/c573g	+
267	N35G/N66Q/E104H/A168P	t60c/c573g	+	323	N35G/E104H/A168P/E336G	t60c/c573g	+
268	N35G/E104H/A168P/G203E	t60c/c573g	+	324	N35G/E104H/A168P/E336R	t60c/c573g	+
269	N35G/E104H/A168P/S339W	t60c/c573g	+	325	N35G/T40S/E104H/S128K/	t60c/c573g	+
270	N35G/P45K/N46E/E104H/	t60c/c573g	+	226	A142G/A168P	+CO-/-1094/-573-	
271	A150Y/A168P	+600/05720		326	N35G/Q44K/S67T/E104H/ A168P	t60c/c198t/c573g	+
271	N35G/E104H/R130S/A168P N35G/E104H/R145T/A168P	t60c/c573g	+	327		+60a/a572a	
272	N35G/E104H/A168P/S231K	t60c/c573g/g891a t60c/c573g	+	327	N35G/E104H/A168P/N317A N35G/E104H/G155N/A168P	t60c/c573g	+
273	N35G/T40A/E104H/A168P/	t60c/c573g	+	328		t60c/c573g t60c/c573g	+
2/4	D234E/P327M	100c/c3/3g	+	330	N35G/E104H/Q161E/A168P N35G/E104H/N118S/A168P	t60c/c573g	+
275	N35G/E104H/A168P/S231H	t60c/c573g	+	331	N35G/P45T/V97Q/E104H/	t60c/c573g	+ +
276	N35G/E104H/A168P/N317M	t60c/c573g	+	331	A168P/G267S	1000re373g	т-
277	N35G/E104H/A168P/S330Y	t60c/c573g	+	332	V28H/N35G/E104H/A168P	t60c/c573g	+
278	N35G/E104H/A168P/S329I	t60c/c573g	+	333	N35G/E104H/A168P/Q184L	t60c/c573g	+
279	N35G/E104H/A168P/S329R	t60c/c573g	+	334	N35G/E104H/A168P/N317V	t60c/c573g	+
280	N35G/N66D/E104H/A168P/	t60c/c573g	+	335	N35G/Q44L/E104H/A168P	t60c/c573g	+
	P322R/S329L		·	336	N35G/E104H/A168P/S330G	t60c/c573g	+
281	N35G/E104H/A168P/P327F	t60c/c288t/c573g	+	337	N35G/E104H/A168P/T320A/	t60c/c573g	+
282	N35G/P45D/E104H/A168P	t60c/c573g	+		V333W	O	
283	N35G/E104H/A168P/S332R	t60c/c573g	+	338	N35G/E104H/A168P/E336A	t60c/c573g	+
284	N35G/E104H/A116S/A168P	t60c/c573g	+	339	N35G/E104H/A168P/N335S	t60c/c573g	+
285	N35G/T40A/E104H/A168P/	t60c/c573g	+	340	N35G/N66M/E104H/A168P	t60c/c573g	+
	V230I/P327M			341	N35G/T54G/E104H/A168P	t60c/c573g	+
286	N35G/T49A/E104H/A168P	t60c/c573g	+	342	N35G/E104H/A168P/N317S	t60c/c573g	+
287	N35G/E104H/A168P/N317T	t60c/c573g	+	343	N35G/E64L/E104H/A168P	t60c/c573g	+
288	N35G/N46Y/E104H/A168P	t60c/c573g	+	344	N35G/E104H/S164E/A168P/	t60c/c573g	+
289	N35G/E104H/A168P/G203V	t60c/c573g	+		A271T		
290	N35G/E104H/A168P/S329L	t60c/c573g	+	345	N35G/N66A/E104H/A168P	t60c/c573g	+
291	N35G/E104H/R145N/A168P/	t60c/c573g	+	346	N35G/G83R/E104H/A168P	t60c/c573g	+
	S329H			347	N35G/E104H/A168P/N317Q/	t60c/c573g	+
292	N35G/A56S/E104H/A168P	t60c/c573g	+		T320A		
293	N35G/T40S/T49R/E104H/	t60c/c573g	+	348	N35G/E104H/K141A/A168P	t60c/c573g	+
	A168P/D234E/P327M			349	N35G/P71T/E104H/A168P	t60c/c573g	+
294	N35G/E104H/Q161R/A168P	t60c/c573g	+	350	N35G/P71S/E104H/A168P	t60c/c573g	+
295	N35G/E104H/A168P/S332F	t60c/c573g	+	351	N35G/E104H/R130G/A168P	t60c/c573g	+
296	N35G/P45R/T49A/E104H/	t60c/c573g	+	352	N35G/E104H/R145Q/A168P	t60c/c573g	+
207	A168P/N317R/T320A	+60a/a572 a		353	N35G/T70A/E104H/A168P	t60c/c573g	+
297 298	N35G/E104H/A168P/V237I	t60c/c573g	+	354	N35G/E104H/A168P/K218R	t60c/c573g	+
298	N35G/Q44K/T80V/E104H/	t60c/c573g	+	355 356	N35G/E104H/A168P/Q184E	t60c/c573g t60c/c573g	+
299	A168P	+60a/a572 a		356 357	N35G/E104H/R130K/A168P		+
300	N35G/E104H/A168P/E336S	t60c/c573g	+	358	N35G/Q58H/E104H/A168P Y32S/N35G/E104H/A168P	t60c/c573g	+
301	N35G/E104H/A168P/P233T N35G/E104H/A168P/S329Y	t60c/c573g t60c/c573g	+	359	N35G/E104H/A168P/S329T	t60c/c573g t60c/c573g	+
302	N35G/E104H/A168P/P327L	t60c/c573g	+	360	N35G/E104H/A168P/S330I	t60c/c573g	+
303	N35G/E104H/A168P/N317I	t60c/c573g	+	361	Y32S/N35G/P71A/E104H/	t60c/c573g	+
304	N35G/E104H/R130H/A168P	t60c/c573g	+	301	A168P	1000/03/3g	т-
305	N35G/Q44K/E104H/A168P	t60c/c573g	+	362	N35G/E104H/A168P/S330T	t60c/c573g	+
306	N35G/N66D/E104H/A168P	t60c/c573g	+	363	N35G/G82A/E104H/A168P	t60c/c573g	+
307	N35G/E104H/A168P/S329V	t60c/c573g	+	364	N35G/T80V/E104H/A168P	t60c/c573g	+
308	N35G/E104H/A168P/W337F	t60c/c573g	+	365	N35G/E104H/A168P/S295T	t60c/c573g	+
309	N35G/E104H/A168P/N317H	t60c/c573g	+	366	N35G/N66G/E104H/A168P	t60c/c573g	+
310	N35G/T40L/E104H/S128K/	t60c/c573g	+	367	N35G/E104H/R145L/A168P	t60c/c573g	+
	A168P	- 0		368	N35G/S67H/E104H/A168P/	t60c/c573g	+
311	N35G/E104H/A168P/A326V	t60c/c573g	+		V230M		•
312	N35G/T80V/E104H/A168P/	t60c/c573g	+	369	N35G/E104H/G136E/A168P	t60c/c573g	+
-	P303T	υ		370	N35G/T54S/E104H/A168P	t60c/c573g	+
313	N35G/E104H/A168P/S231A/	t60c/c573g	+	371	N35G/P45S/E104H/A168P	t60c/c573g	+
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TABLE 6-2-continued

TABLE 6-2-continued

G	H61 Variants with Improved Ac	tivity Compared to	Variant 1	GH61 Variants with Improved Activity Compared to Variant						
Vari-			GH61	Vari-			GH61			
ant	A ' A '1 O1	Silent Nucleotide	Activity	ant	A ' A '1 O1	Silent Nucleotide	Activity			
Number	Amino Acid Changes	Changes	Improvement	Number	· Amino Acid Changes	Changes	Improvement			
373	N35G/N66D/N95E/E104H/ S164E/A168P/G267D	t60c/c573g	+	427	N35G/Q44K/N66V/E104H/ A168P	t60c/c573g	+			
374	N35G/E104H/A168P/S332C	t60c/c573g	+	428	N35G/E104H/A137M/A168P	t60c/c573g	+			
375	N35G/E104H/S128L/A168P	t60c/c573g	+	429	N35G/E104H/A168P/P327C	t60c/c573g	+			
376	N35G/T54W/E104H/A168P	t60c/c573g	+	430	N35G/E104H/A168P/T236R	t60c/c573g	+			
377	N35G/E104H/A168P/G268A/	t60c/c573g	+	431	N35G/I51A/E104H/A168P	t60c/c573g	+			
	G269A/G270A	C		432	N35G/S67H/E104H/A168P	t60c/c573g	+			
378	N35G/Q44K/E104H/A168P/	t60c/c573g	+	433	N35G/E104H/A168P/A326C	t60c/c573g	+			
	S231T	, and the second		434	N35G/T49A/E104H/S128N/	t60c/c573g	+			
379	R34E/N35G/E104H/A168P/ A280D	t60c/c573g	+	435	A168P N35G/T49R/E104H/A168P/	t60c/c573g	+			
380	N35G/E104H/A168P/A297T	t60c/g399a/c573g	+	755	K218L/N317Q	100 0/0 3/3g	'			
381	N35G/E104H/K141P/R145Q/	t60c/c573g	+	436	N35G/E104H/A168P/P266S/	t60c/c573g	+			
301	A168P	100 0/0 3/3g	•	750	G267V	100 0/0 3/3g	'			
382	N35G/P45E/E104H/K141R/	t60c/c573g	+	437	N35G/E104H/A168P/V237I/	t60c/c573g	+			
202	A168P	160 (572		420	P303T	160 573				
383	N35G/N66T/E104H/A168P	t60c/c573g	+	438	N35G/T49E/E104H/A168P	t60c/c573g	+			
384	N35G/E104H/S164E/A168P/	t60c/c573g	+	439	N35G/P45R/E104H/A168P/	t60c/c573g	+			
205	S295D N35G/E104H/A168P/N317F	t60a/a572~	1	440	T320A	t60a/a572~				
385 386	N35G/E104H/A168P/N317Q	t60c/c573g t60c/c573g	+	440 441	N35G/N66L/E104H/A168P N35G/P45R/E104H/A168P/	t60c/c573g t60c/c573g	+			
387	N35G/T40G/T49R/S78C/	t60c/c573g	+ +	441	K218L/N317Q	100c/c3/3g	+			
367	E104H/A142G/A168P	100C/C3/3g	T-	442	N35G/E104H/R145V/A168P	t60c/c573g				
388	N35G/G82S/E104H/A168P	t60c/c573g	+	443	N35G/N66D/E104H/A168P/	t60c/c573g	+			
389	N35G/Q58P/E104H/A168P	t60c/c573g	+	773	R290K	100 c/c 3/3g	т			
390	N35G/N46R/E104H/A168P/	t60c/c573g	+	444	N35G/T80L/E104H/A168P	t60c/c573g	+			
570	G203E/A263V	1000/03/35	•	445	N35G/A55G/E104H/A168P	t60c/c573g	+			
391	N35G/P45R/E104H/A168P	t60c/c573g	+	446	N35G/E104H/A168P/S330A	t60c/c573g	+			
392	N35G/S67G/E104H/A168P	t60c/c573g	+	447	N35G/E104H/K141N/A168P/	t60c/c573g	+			
393	N35G/E104H/A168P/R199E	t60c/c573g	+		P266S					
394	N35G/G69T/E104H/A168P	t60c/c573g	+	448	N35G/E104H/A142S/A168P	t60c/c573g	+			
395	N35G/E104H/A168P/G203E/	t60c/c573g	+	449	N35G/E104H/A168P/Q184G	t60c/c573g	+			
	G268A/G269A/G270A	U		450	N35G/E104H/N118E/A168P	t60c/c573g	+			
396	N35G/E104H/A168P/P266S	t60c/c573g	+	451	N35G/E104H/A168P/A212M	t60c/c573g	+			
397	N35G/E104H/A168P/V324M	t60c/c573g	+	452	N35G/E104H/A168P/G267D	t60c/c573g	+			
398	N35G/E104H/A168P/G245A	t60c/c573g	+	453	N35G/K93N/E104H/R130Y/	t60c/c573g	+			
399	N35G/N66R/E104H/A168P	t60c/c573g	+		A168P					
400	N35G/E104H/A168P/T236E	t60c/c573g	+	454	N35G/P45R/T49Y/E104H/	t60c/c573g	+			
401	S24Q/N35G/Q44K/T80H/	t60c/c573g	+		A168P/N317D					
	E104H/A168P			455	N35G/E104H/A168P/S329Q	t60c/c573g	+			
402	N35G/E104H/S128D/A168P	t60c/c573g	+	456	N35G/E104H/A168P/V230Q	t60c/c573g	+			
403	N35G/N66D/S78D/E104H/	t60c/c573g	+	457	N35G/P45K/E104H/A168P/	t60c/c573g	+			
	A168P/S253D				A219R					
404	N35G/E104H/R130Y/A168P	t60c/c573g	+	458	N35G/E104H/A142G/A168P	t60c/c573g	+			
405	N35G/E104H/A168P/K310I	t60c/c573g	+	459	N35G/E104H/A168P/S205T	t60c/c573g	+			
406	N35G/E104H/R145E/A168P	t60c/c573g	+	460	N35G/S78D/E104H/S164E/	t60c/c573g	+			
407	N35G/N66D/E104H/S164E/	t60c/c573g	+	461	A168P	160 573				
400	A168P/S282D	+60a/a572=		461	N35G/E104H/R130E/A168P	t60c/c573g	+			
408	N35G/E104H/K141P/A168P	t60c/c573g	+	462	N35G/E104H/A168P/Q184H	t60c/c573g	+			
409	N35G/E104H/A168P/Q184R	t60c/c573g	+		N35G/E104H/A116P/A168P	t60c/c573g t60c/c573g	+			
410 411	N35G/E104H/A168P/S231T N35G/N66V/E104H/A168P	t60c/c573g t60c/c573g	+	464 465	N35G/E104H/A142D/A168P V28H/N35G/N46E/Q58H/	t60c/c573g	+			
411	N35G/E104H/A142L/A168P	t60c/c573g	+ +	+03	E104H/A168P	1000/03/3g	+			
413	N35G/E104H/R145H/A168P	t60c/c573g	+	466	N35G/E104H/A168P/A280T	t60c/c573g	+			
414	N35G/E104H/A168P/K218L	t60c/c573g	+	467	R34E/N35G/E104H/A168P/	t60c/c573g	+			
415	N35G/E104H/K141T/A168P	t60c/c573g	+	707	A280T		T			
416	N35G/E104H/A168P/P233F	t60c/c573g	+	468	N35G/E104H/A168P/E336L	t60c/c573g	+			
417	N35G/T40S/E104H/A168P/	t60c/c573g	+	469	N35G/T49D/E104H/A168P	t60c/c573g	+			
	P327M		•	470	N35G/E104H/A168P/A219T	t60c/c573g	+			
418	N35G/T54M/E104H/A168P	t60c/c573g	+	471	N35G/E104H/A142W/A168P	t60c/c573g	+			
419	S24T/N35G/E104H/S164E/	t60c/c573g	+	472	N35G/E104H/A168P/P303T/	t60c/c573g	+			
	A168P	-0			G305D	-0	•			
420	N35G/P45T/E104H/A168P	t60c/c573g	+	473	N35G/Q44V/E104H/A168P	t60c/c573g	+			
421	N35G/N66D/E104H/S164E/	t60c/c573g	+	474	N35G/E104H/A168P/N187D	t60c/c573g	+			
	A168P/S231T/S253T	Ü		475	N35G/E104H/G136H/A168P	t60c/c573g	+			
422	N35G/G69H/E104H/A168P	t60c/c573g	+	476	S24Q/N35G/Q44K/E104H/	t60c/c573g	+			
423	N35G/E104H/S128Y/A168P	t60c/c573g	+		A168P/P303T/S332D	Ž.				
424	N35G/T49Q/E104H/A168P	t60c/c573g	+	477	N35G/E104H/A168P/Q184N	t60c/c573g	+			
425	N35G/T49A/E104H/A168P/	t60c/c573g	+	478	N35G/E104H/A168P/S332L	t60c/c573g	+			
	Q184H			479	S24T/N35G/N66D/S78D/	t60c/c573g	+			
426	N35G/E104H/A168P/G203Y	t60c/c573g	+		E104H/A168P/S205T/S253T					
120	1.55 5,215 11211001/02051		•		210 11211100170203 1702331					

TABLE 6-2-continued

C	H61 Variants with Improved Ac	tivity Compared to	Variant 1
Vari- ant Numbe	r Amino Acid Changes	Silent Nucleotide Changes	GH61 Activity Improvement
480	N35G/E104H/A168P/P327A	t60c/c573g	+
481	N35G/T40A/T49Q/S78C/ E104H/A168P	t60c/c573g	+
482	N35G/T40L/E104H/A142G/ A168P	t60c/c573g	+
483	N35G/T49Y/E104H/A168P/ N317R	t60c/c573g	+
484	R34E/N35G/K93T/E104H/ R130E/R145T/A168P/R199E/ K218T/A280D	t60c/c573g	+

Example 7

Selection of Further GH61 Candidates for Strain Improvement

[0385] This example illustrates the selection of potential candidates to further improve whole cellulase broth activity of *M. thermophila* cultures on different types of pretreated substrates like pretreated corn stover and pretreated wheat straw

[0386] In this Example, *M. thermophila*-produced and purified GH61a, GH61p, GH61f, GH61n, CBH1a, CBH2b, AXE3, FAE, and Xyl3, were used to supplement the activity present in culture broths (i.e., "whole broth cellulase base") of the *M. thermophila* strain CF-416 prepared using standard methods known in the art. The broth cellulase base was fixed to 0.5% protein and the single purified enzyme was added at 0.4% (wt added protein/wt glucan) to the saccharification reactions. The whole cellulase broth base and individual enzymes were characterized by standard BCA assays for total protein quantification.

[0387] The saccharification reactions were carried out at 74 g/L glucan load of pretreated wheat straw (PWS) or pretreated corn stover (PCS) at pH 5.0, 55° C. at 950 rpm in the presence of 50 µM copper in high throughput (HTP) 96 deep well plates. Glucose analysis was carried out by the glucose oxidase assay as described above. In each case, the fold improvement was calculated using the formula Fold Improvement=[Total Glucose Production with addition of 0.4% single enzyme to the whole cellulase broth base]/ [glucose production from the 0.5% whole cellulase broth base]. The results are provided in Table 10-1. In this Table, the fold improvements were ranked from 0 to 3; fold improvements less than 1.2x are indicated by "0," fold improvements of >1.2 to <1.5 are indicated by "1," fold improvements of >1.5× to <1.7× improvements are indicated by "2," and fold improvements>1.7 are indicated by "3."

[0388] As indicated by the results in the Table, the greatest benefit was observed using GH61p on pre-treated corn stover (PCS), and GH61a on pre-treated wheatstraw (PWS), indicating that GH61 activity is increases the cellulolytic activity of the reaction mix. In addition to the enzymes listed in Table 10-1, EG1b, Xyl1, Xyl6, beta-xylosidase, and another xylanase were also tested, but did not show any improvement under the test conditions.

TABLE 7-1

	Fold Improvement								
	Fold Improvement Over Whole Cellulase Broth Tested on PCS	Fold Improvement Over Whole Cellulase Broth Tested on PWS							
Whole broth cellulases	1	1							
from CF-416									
CBH1a	1	3							
CBH2b	1	1							
GH61a	2	3							
GH61p	3	2							
GH61f	1	1							
GH61n	1	1							
AXE3	0	1							
FAE	1	1							
Xyl3	0	1							

Example 8

Improvement of GH61 Activity by Copper(II) Ions

[0389] This example illustrates the enhancement in GH61 activity with the addition of copper(II) ion to the saccharification reaction.

[0390] Purified M. thermophila-produced GH61a or S. cerevisiae supernatant containing M. thermophila-GH61a was pre-incubated with different amounts of copper(II) (CuSO₄) at concentrations of 0 to 100 µM at ambient temperature for 30 min. The biomass assay was then performed in a total volume of 300 µL, in the presence of 10 mg of pre-treated wheat straw, using 261 µL of copper-treated GH61 samples, 39 μ L of sodium acetate buffer (pH 5), M. thermophila-produced CBH1a, CBH2b and β-glucosidase. The final concentration of sodium acetate was 120 mM and the enzyme loads of CBHs and β-glucosidase (CBH1a and CBH2b in 1:1 ratio) were 0.01% and 0.02% with respect to substrate glucan mass in the biomass substrate, respectively. Background (negative) controls were obtained by using either water or media supernatant from cultures of S. cerevisiae cells without the GH61a gene in the plasmid. Thus, the negative controls represent activities of CBH1a, CBH2b and beta-glucosidase in the absence of GH61a. The reaction was incubated at 55° C. for 72 hours with shaking. The GH61a activity in the reaction mixture was measured by monitoring glucose production using a glucose oxidase/ peroxidase-based glucose assay.

[0391] Some experiments were also performed without pre-incubating GH61 with copper(II), but instead, by directly adding different amounts of copper(II) (CuSO₄) to the biomass assay reactions as described herein.

[0392] FIG. 3 shows activity of *M. thermophila*-GH61a pre-incubated with different amounts of copper(II) ion. Biomass assays were performed with (A) *S. cerevisiae*-produced *M. thermophila* GH61a Variant 5, and (B) *M. thermophila*-produced wild-type GH61a. Glucose production after 72 h incubation at pH 5, 55° C. was determined by the glucose assay. The data in this Figure indicate GH61a-only activity, in which the amount of glucose produced in control reaction containing CBH and β -glucosidase was subtracted from the total amount of glucose produced in the reactions with GH61a. In this Figure, N=4; and the error bars represent ± 1 standard deviation. Copper concentrations shown are with respect to the total reaction volume.

[0393] The results indicate that the activities of *M. thermophila*-produced GH61a and *S. cerevisiae* supernatant containing *M. thermophila*-GH61a are improved by preincubation with copper(II) ions under the conditions tested. Similar results were obtained when copper(II) was directly added to the biomass assay reactions.

Example 9

Further Evaluation of Copper Requirements in Saccharification Reactions

[0394] This Example describes experiments designed to determine the effects of added copper in saccharification reactions. The saccharification reactions were run in 30 g shake flasks (250 mL flasks) using 82 g/kg glucan of acid-pretreated corn stover and whole broth enzymes produced by M. thermophila strain CF-416 (produced using standard methods known in the art) at a 0.81% total enzyme load with respect to glucan. The reactions were conducted at pH 5.0 or pH 6.0, 55° C. and 250 rpm mixing, with supplementation of either 0 or 50 μM CuSO₄, copper(II) with respect to the total reaction volume. A pH trim was also performed using 2M NaOH at time intervals of 1, 4, 7, 22, 24 29, 46, 52, 70, 75 and 96 hrs, to maintain the pH at the desired value of pH 5.0 or pH 6.0. Samples were removed at 72 hours and the total amount of glucose in the reaction mixture was determined using standard HPLC methods and equipment as known in the art. The results indicated that under the conditions described herein, the effect of copper is dependent on saccharification pH. As shown in FIG. 4, Panel A, at a saccharification pH of pH 5.0, the addition of copper caused an increase in glucose yields by ~3.5% while this effect was not observed when the saccharification was carried out at pH 6.0. Also, the addition of copper may cause a decrease in the total amounts of C5 sugars that are produced as shown in FIG. 4, Panel B.

Example 10

Effect of Reducing Agents on the Cellulolytic Activity of GH61a

[0395] This Example provides experiments conducted to determine the effect of adding reducing agents (e.g., gallic acid and ascorbic acid) to saccharification reactions. In these experiments, enhancement of GH61 activity was tested using Variant 1 (SEQ ID NO:5) in the presence of reducing agents (specifically, ascorbic acid or gallic acid) and pretreatment filtrate, which contains various reducing agents from lignin degradation. Reactions were performed on cellulosic substrates, AVICEL® PH microcrystalline cellulose and phosphoric acid swollen cellulose (PASC), with purified M. thermophila-produced GH61 Variant 1 and beta-glucosidase at 0.3% and 0.08% respectively, with respect to substrate glucan mass, and 128 mM sodium acetate buffer (pH 5) supplemented with 30 μM CuSO₄. Thus, reactions were performed with 0.3% GH61a and 0.08% BGL, where % enzyme loads are with respect to substrate glucan mass (36 g/L AVICEL® cellulose and 5 g/L PASC). Background (negative) controls were beta-glucosidase-only reactions tested in the absence of GH61a. Glucose production after 48 h incubation at pH 5, 55° C. was determined by glucose oxidase/peroxidase-based or HPLC-based glucose assay glucose assay, using methods known in the art.

[0396] FIG. 5 shows the activity of *M. thermophila*-produced GH61a Variant 1 on cellulosic substrates in the presence of ascorbic acid, gallic acid and pretreatment filtrate. Panel A shows the results for AVICEL® PH microcrystalline cellulose and Panel B shows the results for PASC. GH61-only activity is also shown, these results were obtained by subtracting the amount of glucose produced in the beta-glucosidase-only control reaction from the total amount of glucose produced in the reaction that included GH61a. Filtrate dilutions are indicated in this Figure, where undiluted filtrate equals 72% of the total reaction volume. In this Figure, N=4; and the error bars represent ±1 standard deviation.

[0397] The results indicate that supplementing the GH61a reaction with gallic acid improved the GH61 activity in generating soluble sugars from AVICEL® cellulose and PASC, which were then hydrolyzed by beta-glucosidase to generate glucose monomers. The improvement was also observed with diluted pretreatment filtrate, which suggests that the filtrate may contain gallic acid or gallic acid-like reductants that can beneficially impact GH61 activity.

Example 11

Evaluation of Oxygen Limitation in Saccharification Reactions

[0398] This example describes experiments conducted to determine if oxygen is a limiting factor in saccharification reactions. To investigate the level of oxygen required in the overall saccharification efficiency, two shake flask reactions were performed, in which one was left closed throughout the 72 hour reaction, while the other was opened at 4 hrs and 24 hrs for 10 seconds to provide fresh air. The reactions were run in $30\,\mathrm{g}$ shake flasks ($250\,\mathrm{mL}$ flasks) using $87\,\mathrm{g/kg}$ glucan and M. thermophila CF-416 whole broth cellulases. The total protein content in each reaction was 0.81% total enzyme load with respect to glucan. The reactions were conducted at pH 5.0, 55° C. and 250 rpm mixing, with supplementation of 50 μM CuSO₄. Samples were removed at 72 hours and glucose yields were measured by monitoring glucose production using a glucose oxidase/peroxidasebased glucose assay. The results indicated that under the reaction conditions tested, oxygen was not a limiting factor as the two reactions (control vs. the reaction with air supplemented) yielded similar levels of glucose.

Example 12

Enhancement of Saccharification Efficiency by Addition of Surfactants

[0399] This example illustrates the enhancement of overall saccharification yield with the addition of surfactants such as TWEEN®-20 and PEG-4000. Experiments were designed to monitor the enhancement in cellulase activity with different concentrations of TWEEN®-20 or PEG-4000 in the biomass assay. The biomass assay was performed in a total volume of 90 μL , including 10 mg of pre-treated wheat straw, 64.8 μL (72% by volume) of filtrate (or H_2O for no filtrate conditions), and 11.6 μL of a mixture of sodium acetate buffer (pH 5.0, supplemented with CuSO₄), *M. thermophila*-produced cellobiohydrolase 1a (CBH1a), cellobiohydrolase 2b (CBH2b), beta-glucosidase (BGL), and glycoside hydrolase type 61 (GH61a). The final concentration of sodium acetate was 128 mM (with 30 μM CuSO₄)

and the enzyme loads of CBH1a, CBH2b, BGL and GH61a were 0.15%, 0.15%, 0.08% and 0.3% with respect to the substrate glucan mass in the biomass substrate, respectively. Water was used in place of the enzymes as a negative control. Herein, "1x filtrate" indicates 72% of filtrate (i.e., the filtered liquid portion of pre-treated substrate) in the total reaction volume. The amount of glucose in the filtrate background was subtracted from the test data (N=2; Error bars in the Figures represent ±1 standard deviation). The reaction was incubated at 55° C. for 72 hours at pH 5, with shaking at 950 rpm, then was quenched by adding 180 μL of water. The total cellulase activity in the reaction mixture was measured by monitoring glucose production using a glucose oxidase/peroxidase-based glucose assay as known in the art. The results indicate that the total glucose production in the saccharification reaction was enhanced with the addition of TWEEN®-20 or PEG-4000.

[0400] FIG. **6**, Panel A, shows enzymatic hydrolysis activity of the cellulase mixture in the presence of TWEEN®-20. Data shown are total glucose produced by a mixture of GH61a, CBH1a, CBH2b, and BGL at 0.3%, 0.15%, 0.15%,

and 0.08% with respect to the substrate glucan mass in the biomass substrate, respectively. In this Figure, TWEEN®-20 concentrations are expressed as % total reaction volume. [0401] FIG. 6, Panel B, shows enzymatic hydrolysis activity of the cellulase mixture in the presence of PEG-4000. In this Figure, PEG-4000 concentrations are expressed as % total reaction volume.

[0402] While the invention has been described with reference to the specific embodiments, various changes can be made and equivalents can be substituted to adapt to a particular situation, material, composition of matter, process, process step or steps, thereby achieving benefits of the invention without departing from the scope of what is claimed.

[0403] For all purposes in the United States of America, each and every publication and patent document cited in this disclosure is incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an indication that any such document is pertinent prior art, nor does it constitute an admission as to its contents or date.

SEQUENCE LISTING

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n Gly Val Tyr \$20\$ \$25\$ 30

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Thr Val Ile Gly Trp Thr Ala Ala Asp Gln Asp Asn Gly Phe Val Glu 50 55 60

Pro Asn Ser Phe Gly Thr Pro Asp Ile Ile Cys His Lys Ser Ala Thr 65 70 75 80

Pro Gly Gly Gly His Ala Thr Val Ala Ala Gly Asp Lys Ile Asn Ile 85 90 95

Val Trp Thr Pro Glu Trp Pro Glu Ser His Ile Gly Pro Val Ile Asp \$100\$

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n Gly Asp Cys Glu Thr Val Asp Lys Ser Ser 115 120 125

Leu Arg Trp Phe Lys Ile Asp Gly Ala Gly Tyr Asp Lys Ala Ala Gly 130 135 140

Arg Trp Ala Ala Asp Ala Leu Arg Ala Asn Gly Asn Ser Trp Leu Val

Gln Ile Pro Ser Asp Leu Lys Ala Gly Asn Tyr Val Leu Arg His Glu 165 170 175

Ile Ile Ala Leu His Gly Ala Gln Ser Pro Asn Gly Ala Gln Ala Tyr 180 185 190

Pro Gln Cys Ile Asn Leu Arg Val Thr Gly Gly Gly Ser Asn Leu Pro 195 200 205

Ser Gly Val Ala Gly Thr Ser Leu Tyr Lys Ala Thr Asp Pro Gly Ile 210 215 220

Leu Phe Asn Pro Tyr Val Ser Ser Pro Asp Tyr Thr Val Pro Gly Pro 225 230 235

Ala Leu Ile Ala Gly Ala Ala Ser Ser Ile Ala Gln Ser Thr Ser Val $245 \hspace{1.5cm} 250 \hspace{1.5cm} 255$

Pro Thr Ala Thr Thr Thr Ala Ala Thr Ser Ala Ala Pro Ser Thr Thr 275 280 285

Leu Arg Thr Thr Thr Thr Ser Ala Ala Gln Thr Thr Ala Pro Pro Ser 290 295 300

Gly Asp Val Gln Thr Lys Tyr Gly Gln Cys Gly Gly Asn Gly Trp Thr 305 310 315 320

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Pro Gly	Gly	Gly	His 85		Thr	Val	Ala	Ala 90		Asp	ГЛа	Ile	Asn 95			
Pro Gly Val Trp		-	85	Ala				90	Gly	_	-		95	Ile		
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Arg Trp Ala Ala Asp Ala Leu Arg Ala Asn Gly Asn Ser Trp Leu Val 145 $$ 150 $$ 155 $$ 160

atgtccaagg cetetgetet eetegetgge etgaegggeg eggeeetegt egetgeacae

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Ser	Gly 210	Val	Ala	Gly	Thr	Ser 215	Leu	Tyr	Lys	Ala	Thr 220	Asp	Pro	Gly	Ile
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Ala	Leu	Ile	Ala	Gly 245	Ala	Ala	Ser	Ser	Ile 250	Ala	Gln	Ser	Thr	Ser 255	Val
Ala	Thr	Ala	Thr 260	Gly	Thr	Ala	Thr	Val 265	Pro	Gly	Gly	Gly	Gly 270	Ala	Asn
Pro	Thr	Ala 275	Thr	Thr	Thr	Ala	Ala 280	Thr	Ser	Ala	Ala	Pro 285	Ser	Thr	Thr
Leu	Arg 290	Thr	Thr	Thr	Thr	Ser 295	Ala	Ala	Gln	Thr	Thr 300	Ala	Pro	Pro	Ser
Gly 305	Asp	Val	Gln	Thr	Lys 310	Tyr	Gly	Gln	Cys	Gly 315	Gly	Asn	Gly	Trp	Thr 320
Gly	Pro	Thr	Val	Сув 325	Ala	Pro	Gly	Ser	Ser 330	СЛв	Ser	Val	Leu	Asn 335	Glu
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Tyr	Asp	Pro		5					10	_		-	-	Arg 15 Val	-
Tyr Gly	_		Thr 20	5 Thr	Asp	Trp	Tyr	Gln 25	10 Pro	Asn	Pro	Pro	Thr	15 Val	Ile
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Gly Phe	Trp Gly 50 His	Thr 35 Thr	Thr 20 Ala Pro	5 Thr Ala Asp Val	Asp Ile Ala 70	Trp Gln Ile 55 Ala	Tyr Asp 40 Cys	Gln 25 Asn His	10 Pro Gly Lys	Asn Phe Ser Ile	Pro Val Ala 60 Asn	Pro Glu 45 Thr	Thr 30 Pro Pro	Val Asn Gly Trp	Ile Ser Gly Thr
Gly Phe Gly 65	Trp Gly 50 His	Thr 35 Thr Ala	Thr 20 Ala Pro Thr	5 Thr Ala Asp Val His 85	Asp Ile Ala 70 Ser	Trp Gln Ile 55 Ala His	Tyr Asp 40 Cys Gly	Gln 25 Asn His Asp	10 Pro Gly Lys Lys Pro 90	Asn Phe Ser Ile 75 Val	Pro Val Ala 60 Asn	Pro Glu 45 Thr Ile	Thr 30 Pro Pro Val	Val Asn Gly Trp Leu 95	Ile Ser Gly Thr 80 Ala
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Gly Phe Gly 65 Pro	Trp Gly 50 His Glu Cys	Thr 35 Thr Ala Trp Asn Ile 115	Thr 20 Ala Pro Thr Pro Gly 100 Asp	Thr Ala Asp Val His 85 Asp	Asp Ile Ala 70 Ser Cys	Trp Gln Ile 55 Ala His Glu Gly	Tyr Asp 40 Cys Gly Ile Thr Tyr 120	Gln 25 Asn His Asp Gly Val 105 Asp	10 Pro Gly Lys Lys Pro 90 Asp	Asn Phe Ser Ile 75 Val Lys	Pro Val Ala 60 Asn Ile Ser Ala	Pro Glu 45 Thr Ile Asp Ser Gly 125	Thr 30 Pro Val Tyr Leu 110	Val Asn Gly Trp Leu 95 Arg	Ile Ser Gly Thr 80 Ala Trp
Gly Phe Gly 65 Pro Ala Phe	Trp Gly 50 His Glu Cys Lys Asp 130	Thr 35 Thr Ala Trp Asn Ile 115 Ala	Thr 20 Ala Pro Thr Pro Gly 100 Asp	5 Thr Ala Asp Val His 85 Asp Gly	Asp Ile Ala 70 Ser Cys Ala Ala	Trp Gln Ile 55 Ala His Glu Gly Asn 135	Tyr Asp 40 Cys Gly Ile Thr Tyr 120 Gly	Gln 25 Asn His Asp Gly Val 105 Asp	10 Pro Gly Lys Lys Pro 90 Asp Lys	Asn Phe Ser Ile 75 Val Lys Ala Trp	Pro Val Ala 60 Asn Ile Ser Ala Leu 140	Pro Glu 45 Thr Ile Asp Ser Gly 125 Val	Thr 30 Pro Pro Val Tyr Leu 110 Arg	Val Asn Gly Trp Leu 95 Arg	Ile Ser Gly Thr 80 Ala Trp Ala

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Pro Tyr Val Ser Ser Pro Asp Tyr Thr Val Pro Gly Pro Ala Leu Ile 210 215 220	
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225 230 235 240 Thr Gly Thr Ala Thr Val Pro Gly Gly Gly Gly Ala Asn Pro Thr Ala	
245 250 255	
Thr Thr Thr Ala Ala Thr Ser Ala Ala Pro Ser Thr Thr Leu Arg Thr 260 265 270	
Thr Thr Thr Ser Ala Ala Gln Thr Thr Ala Pro Pro Ser Gly Asp Val 275 280 285	
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acgacagact ggtaccagcc caaccegcca acagtcateg gctggacggc agccgatcag 180	
gataatggct tcgttgaacc caacagcttt ggcacgccag atatcatctg ccacaagagc 240	
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geogocacet ecgeogocoo gagoaceaco etgaggaega coactacete ggoogogoag 900	
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1035

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Tyr Arg Asn Tyr Asp Pro Thr Thr Asp Trp Tyr Gln Pro Asn I 35 40 45	Pro Pro												
Thr Val Ile Gly Trp Thr Ala Ala Asp Gln Asp Asn Gly Phe V 50 55 60	Val Glu												
Pro Asn Ser Phe Gly Thr Pro Asp Ile Ile Cys His Lys Ser 265 70 75	Ala Thr 80												
Pro Gly Gly His Ala Thr Val Ala Ala Gly Asp Lys Ile 2	Asn Ile 95												
Val Trp Thr Pro Glu Trp Pro His Ser His Ile Gly Pro Val 1	Ile Asp												
Tyr Leu Ala Ala Cys Asn Gly Asp Cys Glu Thr Val Asp Lys s	Ser Ser												
Leu Arg Trp Phe Lys Ile Asp Gly Ala Gly Tyr Asp Lys Ala i 130 135 140	Ala Gly												
Arg Trp Ala Ala Asp Ala Leu Arg Ala Asn Gly Asn Ser Trp 1 145 150 155	Leu Val 160												
Gln Ile Pro Ser Asp Leu Ala Ala Gly Asn Tyr Val Leu Arg I 165 170	His Glu 175												
Ile Ile Ala Leu His Gly Ala Gln Ser Pro Asn Gly Ala Gln 2 180 185 190	Ala Tyr												
Pro Gln Cys Ile Asn Leu Arg Val Thr Gly Gly Gly Ser Asn 1 195 200 205	Leu Pro												
Ser Gly Val Ala Gly Thr Ser Leu Tyr Lys Ala Thr Asp Pro (210 215 220	Gly Ile												
Leu Phe Asn Pro Tyr Val Ser Ser Pro Asp Tyr Thr Val Pro (225 230 235	Gly Pro 240												
Ala Leu Ile Ala Gly Ala Ala Ser Ser Ile Ala Gln Ser Thr S 245 250	Ser Val 255												
Ala Thr Ala Thr Gly Thr Ala Thr Val Pro Gly Gly Gly Gly 260 265 270	Ala Asn												
Pro Thr Ala Thr Thr Thr Ala Ala Thr Ser Ala Ala Pro Ser 1 275 280 285	Thr Thr												
Leu Arg Thr Thr Thr Ser Ala Ala Gln Thr Thr Ala Pro I 290 295 300	Pro Ser												
Gly Asp Val Gln Thr Lys Tyr Gly Gln Cys Gly Gly Asn Gly 305 310 315	Trp Thr 320												
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<210> SEQ ID NO 9

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<212> TYPE: PRT

<213 > ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polypeptides.

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Gly Trp Thr Ala Ala Asp Gln Asp Asn Gly Phe Val Glu Pro Asn Ser 35 40 45

Phe Gly Thr Pro Asp Ile Ile Cys His Lys Ser Ala Thr Pro Gly Gly 50 60

Gly His Ala Thr Val Ala Ala Gly Asp Lys Ile Asn Ile Val Trp Thr 65 70 75 80

Pro Glu Trp Pro His Ser His Ile Gly Pro Val Ile Asp Tyr Leu Ala

Ala Cys Asn Gly Asp Cys Glu Thr Val Asp Lys Ser Ser Leu Arg Trp \$100\$ 105 110

Phe Lys Ile Asp Gly Ala Gly Tyr Asp Lys Ala Ala Gly Arg Trp Ala 115 120 125

Ala Asp Ala Leu Arg Ala Asn Gly Asn Ser Trp Leu Val Gln Ile Pro 130 135 140

Ser Asp Leu Ala Ala Gly Asn Tyr Val Leu Arg His Glu Ile Ile Ala 145 150 155 160

Leu His Gly Ala Gln Ser Pro Asn Gly Ala Gln Ala Tyr Pro Gln Cys \$165\$ \$170\$ \$175\$

Ile Asn Leu Arg Val Thr Gly Gly Gly Ser Asn Leu Pro Ser Gly Val

Ala Gly Thr Ser Leu Tyr Lys Ala Thr Asp Pro Gly Ile Leu Phe Asn 195 200 205

Pro Tyr Val Ser Ser Pro Asp Tyr Thr Val Pro Gly Pro Ala Leu Ile 210 215 220

Ala Gly Ala Ala Ser Ser Ile Ala Gln Ser Thr Ser Val Ala Thr Ala 225 230 235 240

Thr Thr Thr Ala Ala Thr Ser Ala Ala Pro Ser Thr Thr Leu Arg Thr
260 265 270

Thr Thr Thr Ser Ala Ala Gln Thr Thr Ala Pro Pro Ser Gly Asp Val 275 280 285

Gln Thr Lys Tyr Gly Gln Cys Gly Gly Asn Gly Trp Thr Gly Pro Thr $290 \hspace{1cm} 295 \hspace{1cm} 300 \hspace{1cm}$

Val Cys Ala Pro Gly Ser Ser Cys Ser Val Leu Asn Glu Trp Tyr Ser 305 310 315 320

Gln Cys Leu

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<211> LENGTH: 1035
<212> TYPE: DNA
<213 > ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: Synthetic polynucleotide.
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gataatggct tcgttgaacc caacagcttt ggcacgccag atatcatctg ccacaagagc
                                                                      240
                                                                      300
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                                                                      420
                                                                      480
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cgcgtcaccg gcggcggcag caacctgccc agcggcgtcg ccggcacctc gctgtacaag
                                                                      660
                                                                      720
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ggcccggccc tcattgccgg cgccgccagc tcgatcgccc agagcacgtc ggtcgccact
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gccaceggea eggeeacegt teeeggegge ggeggegeea accetacege caceaceace
                                                                      840
geogecacet cogeogecee gageaceace etgaggaega ceactacete ggeogegeag
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actaccgccc cgccctccgg cgatgtgcag accaagtacg gccagtgtgg tggcaacgga
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tggacgggcc cgacggtgtg cgcccccggc tcgagctgct ccgtcctcaa cgagtggtac
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptides.
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Tyr Arg Asn Tyr Asp Pro Thr Thr Asp Trp Tyr Gln Pro Asn Pro Pro
Thr Val Ile Gly Trp Thr Ala Ala Asp Gln Asp Asn Gly Phe Val Glu
Pro Asn Ser Phe Gly Thr Pro Asp Ile Ile Cys His Lys Ser Ala Thr
Pro Gly Gly Gly His Ala Thr Val Ala Ala Gly Asp Lys Ile Asn Ile
Gln Trp Thr Pro Glu Trp Pro Glu Ser His Ile Gly Pro Val Ile Asp
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Tyr Leu Ala Ala Cys Asn Gly Asp Cys Glu Thr Val Asp Lys Ser Ser
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Gln	Ile	Pro	Ser	Asp 165	Leu	Lys	Ala	Gly	Asn 170	Tyr	Val	Leu	Arg	His 175	Glu
Ile	Ile	Ala	Leu 180	His	Gly	Ala	Gln	Ser 185	Pro	Asn	Gly	Ala	Gln 190	Asn	Tyr
Pro	Gln	Сув 195	Ile	Asn	Leu	Arg	Val 200	Thr	Gly	Gly	Gly	Ser 205	Asn	Leu	Pro
Ser	Gly 210	Val	Ala	Gly	Thr	Ser 215	Leu	Tyr	TÀa	Ala	Thr 220	Asp	Pro	Gly	Ile
Leu 225	Phe	Asn	Pro		Val 230	Ser	Ser	Pro		Tyr 235	Thr	Val	Pro	Gly	Pro 240
Ala	Leu	Ile	Ala	Gly 245	Ala	Ala	Ser	Ser	Ile 250	Ala	Gln	Ser	Thr	Ser 255	Val
Ala	Thr	Ala	Thr 260	Gly	Thr	Ala	Thr	Val 265	Pro	Gly	Gly	Gly	Gly 270	Ala	Asn
Pro	Thr	Ala 275	Thr	Thr	Thr	Ala	Ala 280	Thr	Ser	Ala	Ala	Pro 285	Ser	Thr	Thr
Leu	Arg 290	Thr	Thr	Thr	Thr	Ser 295	Ala	Ala	Gln	Thr	Thr 300	Ala	Pro	Pro	Ser
Gly 305	Asp	Val	Gln	Thr	110 310	Tyr	Gly	Gln		Gly 315	Gly	Asn	Gly	Trp	Thr 320
Gly	Pro	Thr		Сув 325	Ala	Pro	Gly	Ser	Ser 330	Càa	Ser	Val	Leu	Asn 335	Glu
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Tyr	Arg	Asn 35	Tyr	Asp	Pro	Thr	Thr 40	Asp	Trp	Tyr	Gln	Pro 45	Asn	Pro	Pro
Thr	Val 50	Ile	Gly	Trp	Thr	Ala 55	Ala	Asp	Gln	Asp	Asn 60	Gly	Phe	Val	Glu
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Pro	Gly	Gly	Gly	His 85	Ala	Thr	Val	Ala	Ala 90	Gly	Asp	Lys	Ile	Asn 95	Ile
Gln	Trp	Thr	Pro 100	Glu	Trp	Pro	Glu	Ser 105	His	Ile	Gly	Pro	Val 110	Ile	Asp
Tyr	Leu	Ala	Ala	CÀa	Asn	Gly	Asp	Cya	Glu	Thr	Val	Asp	ГЛа	Ser	Ser

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Arg Trp Ala Ala Asp Ala Leu Arg Ala Asn Gly Asn Ser Trp Leu Val 145 150 155 160
Gln Ile Pro Ser Asp Leu Lys Ala Gly Asn Tyr Val Leu Arg His Glu 165 170 175
Ile Ile Ala Leu His Gly Ala Gln Ser Pro Asn Gly Ala Gln Asn Tyr 180 185 190
Pro Gln Cys Ile Asn Leu Arg Val Thr Gly Gly Gly Ser Asn Leu Pro 195 200 205
Ser Gly Val Ala Gly Thr Ser Leu Tyr Lys Ala Thr Asp Pro Gly Ile 210 215 220
Leu Phe Asn Pro Tyr Val Ser Ser Pro Asp Tyr Thr Val Pro Gly Pro 225 230 235 240
Ala Leu Ile Ala Gly Ala Ala Ser Ser Ile Ala Gln Ser Thr Ser Val 245 250 255
Ala Thr Ala Thr Gly Thr Ala Thr Val Pro Gly Gly Gly Ala Asn 260 265 270
Pro Thr Ala Thr Thr Ala Ala Thr Ser Ala Ala Pro Ser Thr Thr 275 280 285
Leu Arg Thr Thr Thr Ser Ala Ala Gln Thr Thr Ala Pro Pro Ser 290 295 300
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G	ly	Gln	Ala	Gln	Phe 165	Tyr	Gln	Ser	Cya	Ala 170	Gln	Ile	Asn	Val	Ser 175	Gly		
G	ly	Gly	Ser	Phe 180	Thr	Pro	Ala	Ser	Thr 185	Val	Ser	Phe	Pro	Gly 190	Ala	Tyr		
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С	cca	.acct	gg (cga	aaaa.	ct c1	ggta	accaa	a aaq	ggact	tcc	agc	gctg	gga (egget	ccccg	480	
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Met Ala Leu Gln Leu Leu Ala Ser Leu Ala Leu Leu Ser Val Pro Ala

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Pro Lys Gly Leu Lys Ser Gly Thr Tyr Ile Ile Arg His Glu Ile Leu 145 150 155 160	
Ser Leu His Val Ala Leu Lys Pro Gln Phe Tyr Pro Glu Cys Ala His 165 170 175	
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Gara Anna Anna Wall Alla Gara Anna Glas Glas Glas Glas Glas Glas Glas Gla	

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Asp	Ser	Gly 115	Val	Gly	Gly	Gly	Trp 120	Phe	Lys	Ile	Gln	Glu 125	Asp	Gly	Tyr
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His 145	Tyr	Ile	Asp	Ile	Pro 150	Ala	Cys	Ile	Pro	Glu 155	Gly	Gln	Tyr	Leu	Leu 160
Arg	Ala	Glu	Met	Ile 165	Ala	Leu	His	Ala	Ala 170	Gly	Ser	Pro	Gly	Gly 175	Ala
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Gly	Asp	Tyr	Leu	Val 165	Arg	Ala	Glu	Ala	Ile 170	Ala	Leu	His	Thr	Ala 175	Asn
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Ala	Tyr 210	Ser	Ala	Asn	Asp	Pro 215	Gly	Ile	Leu	Val	Asp 220	Ile	Trp	Gly	Asn
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Lys	Trp	Ile 275	Ala	Leu	Gln	Lys	Ile 280	Ser	Tyr	Val	Asp	Gln 285	Thr	Gly	Thr
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105

Ala Gln Phe Tyr Met Ser Cys Tyr Gln Val Arg Ile Ser Gly Gly Glu 165 170 175
Gly Gly Gln Leu Pro Ala Gly Val Lys Ile Pro Gly Ala Tyr Ser Ala 180 185 190
Asn Asp Pro Gly Ile Leu Val Asp Ile Trp Gly Asn Asp Phe Asn Asp
Pro Pro Gly His Ser Ala Arg His Ala Ile Ile Ile Ser Ser
210 215 220
Ser Asn Asn Ser Gly Ala Lys Met Thr Lys Lys Ile Gln Glu Pro Thr 225 230 235 240
Ile Thr Ser Val Thr Asp Leu Pro Thr Asp Glu Ala Lys Trp Ile Ala 245 250 255
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Thr Ser Val Thr Ser Gly Asp Met Thr Cys Asn Val Gly Gly Thr Lys

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Glu	Met	His	Ala	Gln 85	Pro	Gly	Asp	Arg	Ser 90	Cys	Ala	Asn	Glu	Ala 95	Ile
Gly	Gly	Asn	His 100	Phe	Gly	Pro	Val	Leu 105	Ile	Tyr	Met	Ser	Lys 110	Val	Asp
Asp	Ala	Ser 115	Thr	Ala	Asp	Gly	Ser 120	Gly	Asp	Trp	Phe	Lys 125	Val	Asp	Glu
Phe	Gly 130	Tyr	Asp	Ala	Ser	Thr 135	Lys	Thr	Trp	Gly	Thr 140	Asp	Lys	Leu	Asn
Glu 145	Asn	CÀa	Gly	ГÀа	Arg 150	Thr	Phe	Asn	Ile	Pro 155	Ser	His	Ile	Pro	Ala 160
Gly	Asp	Tyr	Leu	Val 165	Arg	Ala	Glu	Ala	Ile 170	Ala	Leu	His	Thr	Ala 175	Asn
Gln	Pro	Gly	Gly 180	Ala	Gln	Phe	Tyr	Met 185	Ser	Сув	Tyr	Gln	Val 190	Arg	Ile
Ser	Gly	Gly 195	Glu	Gly	Gly	Gln	Leu 200	Pro	Ala	Gly	Val	Lys 205	Ile	Pro	Gly
Ala	Tyr 210	Ser	Ala	Asn	Asp	Pro 215	Gly	Ile	Leu	Val	Asp 220	Ile	Trp	Gly	Asn
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Gly Gly Gln Leu Pro Ala Gly Val Lys Ile Pro Gly Ala Tyr Ser Ala 180 185 190 Asn Asp Pro Gly Ile Leu Val Asp Ile Trp Gly Asn Asp Phe Asn Glu 205 Tyr Val Ile Pro Gly Pro Pro Val Ile Asp Ser Ser Tyr Phe 210 215 220 <210 > SEQ ID NO 28 <211 > LENGTH: 969 <212 > TypE: DNA <213 > ORGANISM: Myceliophthora thermophila <4400 > SEQUENCE: 28 atgaagteet teacecteae cactetggee geettggetg geaacgeege egeteacgeg 60
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gegtecaact egeeggteac egaegtgace tecaaegega teegetgeaa egeeaaecee 180
tegecegete ggggcaagtg eeeggteaag geeggetega eegttaeggt egagatgeat 240
cagcaacceg gtgacegete gtgcagcage gaggegateg geggggegea etaeggeece 300
gtgatggtgt acatgtccaa ggtgtcggac gcggcgtcgg cggacgggtc gtcgggctgg 360
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agegecagee egeceacegt etectteeeg ggegeetaca aggecacega eeegggeate 660
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Val Thr Ser Asn Ala Ile Arg Cys Asn Ala Asn Pro Ser Pro Ala Arg 50 55 60
Gly Lys Cys Pro Val Lys Ala Gly Ser Thr Val Thr Val Glu Met His 65 70 75 80

Gln	Gln	Pro	Gly	Asp 85	Arg	Ser	Cys	Ser	Ser 90	Glu	Ala	Ile	Gly	Gly 95	Ala
His	Tyr	Gly	Pro 100	Val	Met	Val	Tyr	Met 105	Ser	Lys	Val	Ser	Asp 110	Ala	Ala
Ser	Ala	Asp 115	Gly	Ser	Ser	Gly	Trp 120	Phe	Lys	Val	Phe	Glu 125	Asp	Gly	Trp
Ala	Lys 130	Asn	Pro	Ser	Gly	Gly 135	Ser	Gly	Asp	Asp	Asp 140	Tyr	Trp	Gly	Thr
Lys 145	Asp	Leu	Asn	Ser	Cys 150	Cys	Gly	Lys	Met	Asn 155	Val	ГÀЗ	Ile	Pro	Ala 160
Asp	Leu	Pro	Ser	Gly 165	Asp	Tyr	Leu	Leu	Arg 170	Ala	Glu	Ala	Leu	Ala 175	Leu
His	Thr	Ala	Gly 180	Ser	Ala	Gly	Gly	Ala 185	Gln	Phe	Tyr	Met	Thr 190	CÀa	Tyr
Gln	Leu	Thr 195	Val	Thr	Gly	Ser	Gly 200	Ser	Ala	Ser	Pro	Pro 205	Thr	Val	Ser
Phe	Pro 210	Gly	Ala	Tyr	Lys	Ala 215	Thr	Asp	Pro	Gly	Ile 220	Leu	Val	Asn	Ile
His 225	Ala	Pro	Leu	Ser	Gly 230	Tyr	Thr	Val	Pro	Gly 235	Pro	Ala	Val	Tyr	Ser 240
Gly	Gly	Ser	Thr	Lys 245	Lys	Ala	Gly	Ser	Ala 250	Сув	Thr	Gly	Сув	Glu 255	Ser
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Gly	Ser	Thr 275	Ala	Thr	Ser	Ala	Pro 280	Gly	Gly	Gly	Gly	Gly 285	Сув	Thr	Val
Gln	Lys 290	Tyr	Gln	Gln	Сла	Gly 295	Gly	Gln	Gly	Tyr	Thr 300	Gly	Сув	Thr	Asn
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	L> LE 2> TY			05											
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Ser	Asn	Ala 35	Ile	Arg	CAa	Asn	Ala 40	Asn	Pro	Ser	Pro	Ala 45	Arg	Gly	Lys
Cya	Pro 50	Val	Lys	Ala	Gly	Ser 55	Thr	Val	Thr	Val	Glu 60	Met	His	Gln	Gln
Pro 65	Gly	Asp	Arg	Ser	Сув 70	Ser	Ser	Glu	Ala	Ile 75	Gly	Gly	Ala	His	Tyr 80
Gly	Pro	Val	Met	Val 85	Tyr	Met	Ser	Lys	Val 90	Ser	Asp	Ala	Ala	Ser 95	Ala
Asp	Gly	Ser	Ser 100	Gly	Trp	Phe	Lys	Val 105	Phe	Glu	Asp	Gly	Trp 110	Ala	Lys

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Leu Asn Ser Cys Cys Gly Lys Met Asn Val Lys Ile Pro Ala Asp Leu 130 135 140
Pro Ser Gly Asp Tyr Leu Leu Arg Ala Glu Ala Leu Ala Leu His Thr 145 150 155 160
Ala Gly Ser Ala Gly Gly Ala Gln Phe Tyr Met Thr Cys Tyr Gln Leu 165 170 175
Thr Val Thr Gly Ser Gly Ser Ala Ser Pro Pro Thr Val Ser Phe Pro
Gly Ala Tyr Lys Ala Thr Asp Pro Gly Ile Leu Val Asn Ile His Ala 195 200 205
Pro Leu Ser Gly Tyr Thr Val Pro Gly Pro Ala Val Tyr Ser Gly Gly
210 215 220 Ser Thr Lys Lys Ala Gly Ser Ala Cys Thr Gly Cys Glu Ser Thr Cys
225 230 235 240
Ala Val Gly Ser Gly Pro Thr Ala Thr Val Ser Gln Ser Pro Gly Ser 245 250 255
Thr Ala Thr Ser Ala Pro Gly Gly Gly Gly Cys Thr Val Gln Lys 260 265 270
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Gly Ala Gly Thr Asp Thr Val Thr Val Arg Ala Gly Asp Ser Phe Thr 65 70 75 80
Phe Thr Thr Asp Thr Pro Val Tyr His Gln Gly Pro Thr Ser Ile Tyr 85 90 95
Met Ser Lys Ala Pro Gly Ser Ala Ser Asp Tyr Asp Gly Ser Gly Gly
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Trp Phe Lys Ile Lys Asp Trp Ala Asp Tyr Thr Ala Thr Ile Pro Glu
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Cys Ile Pro Pro Gly Asp Tyr Leu Leu Arg Ile Gln Gln Leu Gly Ile
His Asn Pro Trp Pro Ala Gly Ile Pro Gln Phe Tyr Ile Ser Cys Ala
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Gln Ile Thr Val Thr Gly Gly Gly Ser Ala Asn Pro Gly Pro Thr Val
                                 170
Ser Ile Pro Gly Ala Phe Lys Glu Thr Asp Pro Gly Tyr Thr Val Asn
                               185
Ile Tyr Asn Asn Phe His Asn Tyr Thr Val Pro Gly Pro Ala Val Phe
Thr Cys Asn Gly Ser Gly Gly Asn Asn Gly Gly Gly Ser Asn Pro Val
        215
Thr Thr Thr Thr Thr Thr Thr Arg Pro Ser Thr Ser Thr Ala Gln
Ser Gln Pro Ser Ser Ser Pro Thr Ser Pro Ser Ser Cys Thr Val Ala $245$
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Ile	Lys	Asp	Trp 100	Ala	Asp	Tyr	Thr	Ala 105	Thr	Ile	Pro	Glu	Cys 110	Ile	Pro	
Pro	Gly	Asp 115	Tyr	Leu	Leu	Arg	Ile 120	Gln	Gln	Leu	Gly	Ile 125	His	Asn	Pro	
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Ser 225	Ser	Ser	Pro	Thr	Ser 230	Pro	Ser	Ser	Сув	Thr 235	Val	Ala	Lys	Trp	Gly 240	
Gln	Cys	Gly	Gly	Gln 245	Gly	Tyr	Ser	Gly	Сув 250	Thr	Val	Сув	Ala	Ala 255	Gly	
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Tyr Gln Tyr Ile Arg Lys Asn Thr Asn Tyr Asn Ser Pro Val Thr Asp 20 25 30

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Met Ser Lys A	ala Pro	Gly	Ser	Ala	Ser 105	Asp	Tyr	Asp	Gly	Ser	Gly	Gly	
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Thr	Asp 50	Thr	Val	Thr	Val	Arg 55	Ala	Gly	Asp	Ser	Phe 60	Thr	Phe	Thr	Thr	
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Phe	Lys	Glu	Thr 180	Asp	Pro	Gly	Tyr	Thr 185	Val	Asn	Ile	Tyr	Asn 190	Asn	Phe	
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Сув	Gln	Lys 275	Thr	Asn	Asp	Tyr	Tyr 280	Ser	Gln	Cys	Leu					
		EQ II ENGTH														
		PE:		Myzer	eliop	hth.	ora t	herr	onhi	ila						
		EQUE				,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	JIU (,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	юрп	LIG						
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Val	Leu	Arg	His 180	Glu	Ile	Ile	Ala	Leu 185	His	Ser	Ala	Gly	Gln 190	Gln	Asn	
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Gly Thr Gln Lys Pro Ser Gly Val Leu Gly Thr Glu Leu Tyr Lys Ala 210 215 220

Thr Asp Ala Gly Ile Leu Ala Asn Ile Tyr Thr Ser Pro Val Thr Tyr Gln Ile Pro Gly Pro Ala Ile Ile Ser Gly Ala Ser Ala Val Gln Gln Thr Thr Ser Ala Ile Thr Ala Ser Ala Ser Ala Ile Thr Gly Ser Ala 265 Thr Ala Ala Pro Thr Ala Ala Thr Thr Thr Ala Ala Ala Ala Thr Thr Thr Thr Ala Gly Ser Gly Ala Thr Ala Thr Pro Ser Thr Gly Gly Ser Pro Ser Ser Ala Gln Pro Ala Pro Thr Thr Ala Ala Ala Thr Ser Ser Pro Ala Arg Pro Thr Arg Cys Ala Gly Leu Lys Lys Arg Arg Arg His Ala Arg Asp Val Lys Val Ala Leu 340 <210> SEQ ID NO 39 <211> LENGTH: 326 <212> TYPE: PRT <213 > ORGANISM: Myceliophthora thermophila <400> SEOUENCE: 39 Ala His Gly His Val Thr Asn Ile Val Ile Asn Gly Val Ser Tyr Gln Asn Phe Asp Pro Phe Thr His Pro Tyr Met Gln Asn Pro Pro Thr Val 25 Val Gly Trp Thr Ala Ser Asn Thr Asp Asn Gly Phe Val Gly Pro Glu Ser Phe Ser Ser Pro Asp Ile Ile Cys His Lys Ser Ala Thr Asn Ala Gly Gly His Ala Val Val Ala Ala Gly Asp Lys Val Phe Ile Gln Trp 65 70 75 80 Asp Thr Trp Pro Glu Ser His His Gly Pro Val Ile Asp Tyr Leu Ala Asp Cys Gly Asp Ala Gly Cys Glu Lys Val Asp Lys Thr Thr Leu Lys Phe Phe Lys Ile Ser Glu Ser Gly Leu Leu Asp Gly Thr Asn Ala Pro Gly Lys Trp Ala Ser Asp Thr Leu Ile Ala Asn Asn Asn Ser Trp Leu Val Gln Ile Pro Pro Asn Ile Ala Pro Gly Asn Tyr Val Leu Arg His Glu Ile Ile Ala Leu His Ser Ala Gly Gln Gln Asn Gly Ala Gln Asn Tyr Pro Gln Cys Phe Asn Leu Gln Val Thr Gly Ser Gly Thr Gln Lys 185 Pro Ser Gly Val Leu Gly Thr Glu Leu Tyr Lys Ala Thr Asp Ala Gly Ile Leu Ala Asn Ile Tyr Thr Ser Pro Val Thr Tyr Gln Ile Pro Gly Pro Ala Ile Ile Ser Gly Ala Ser Ala Val Gln Gln Thr Thr Ser Ala

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Ile Thr Ala Ser Ala Ser Ala Ile Thr Gly Ser Ala Thr Ala Ala Pro 245 250 255	
Thr Ala Ala Thr Thr Thr Ala Ala Ala Ala Ala Thr Thr Thr Thr Thr 260 265 270	
Ala Gly Ser Gly Ala Thr Ala Thr Pro Ser Thr Gly Gly Ser Pro Ser 275 280 285	
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cagttctace egggetgeca ecagetecag gteaceggeg geggetecae egtgecetet	600
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Val Ser Arg Ser Ile Pro Gly Asn Gly Pro Val Glu Asp Val Asn Ser 50 55 60	
Ile Asp Leu Gln Cys His Ala Gly Ala Glu Pro Ala Lys Leu His Ala 65 70 75 80	

Pro Ala Ala Ala Gly Ser Thr Val Thr Leu Tyr Trp Thr Leu Trp Pro

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Thr	Gly	Суз 115	Gln	Asp	Trp	Ser	Pro 120	Gly	Thr	Lys	Pro	Val 125	Trp	Phe	Lys
Ile	Lys 130	Glu	Gly	Gly	Arg	Glu 135	Gly	Thr	Ser	Asn	Thr 140	Pro	Leu	Met	Thr
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Gln 225	Pro	Tyr	Thr	Ile	Pro 230	Gly	Pro	Ala	Val	Phe 235	Thr	CAa			
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Arg	Ser	Ile 35	Pro	Gly	Asn	Gly	Pro 40	Val	Glu	Asp	Val	Asn 45	Ser	Ile	Asp
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Gly	Ala	Gln	Phe	Tyr 165	Pro	Gly	Cys	His	Gln 170	Leu	Gln	Val	Thr	Gly 175	Gly
Gly	Ser	Thr	Val 180	Pro	Ser	Thr	Asn	Leu 185	Val	Ser	Phe	Pro	Gly 190	Ala	Tyr
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Val Ser Arg Ser Ile Pro Gly Asn Gly Pro Val Glu Asp Val Asn Ser
Ile Asp Leu Gln Cys His Ala Gly Ala Glu Pro Ala Lys Leu His Ala
Pro Ala Ala Ala Gly Ser Thr Val Thr Leu Tyr Trp Thr Leu Trp Pro
Asp Ser His Val Gly Pro Val Ile Thr Tyr Met Ala Arg Cys Pro Asp
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Thr Gly Cys Gln Asp Trp Ser Pro Gly Thr Lys Pro Val Trp Phe Lys
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Ile Lys Glu Gly Gly Arg Glu Gly Thr Ser Asn Val Trp Ala Ala Thr
Pro Leu Met Thr Ala Pro Ser Ala Tyr Thr Tyr Thr Ile Pro Ser Cys
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Leu Lys Ser Gly Tyr Tyr Leu Val Arg His Glu Ile Ile Ala Leu His
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<210> SEQ 1 <211> LENG <212> TYPE	ΓH: 225					

<213 > ORGANISM: Myceliophthora thermophila

<400> SEQUENCE: 47

Met Arg Tyr Phe Leu Gln Leu Ala Ala Ala Ala Ala Phe Ala Val Asn 1 $$ 5 $$ 10 $$ 15

Ser Ala Ala Gly His Tyr Ile Phe Gln Gln Phe Ala Thr Gly Gly Ser \$20\$

Lys Tyr Pro Pro Trp Lys Tyr Ile Arg Arg Asn Thr Asn Pro Asp Trp $_{\rm 35}$ $_{\rm 40}$ $_{\rm 45}$

Leu Gln Asn Gly Pro Val Thr Asp Leu Ser Ser Thr Asp Leu Arg Cys $50 \hspace{1.5cm} 55 \hspace{1.5cm} 60 \hspace{1.5cm}$

Asn Val Gly Gly Gln Val Ser Asn Gly Thr Glu Thr Ile Thr Leu Asn 65 70 75 80

Ala Gly Asp Glu Phe Ser Phe Ile Leu Asp Thr Pro Val Tyr His Ala 85 90 95

Gly Pro Thr Ser Leu Tyr Met Ser Lys Ala Pro Gly Ala Val Ala Asp 100 105 110

Gly Thr Ser Trp Thr Leu Ser Gly Thr Tyr Thr Gln Arg Ile Pro Lys 130 135 140

His Asn Pro Gly Ala Ala Pro Gln Phe Tyr Ile Ser Cys Ala Gln Val \$165\$

Lys Val Val Asp Gly Gly Ser Thr Asn Pro Thr Pro Thr Ala Gln Ile \$180\$

Pro Gly Ala Phe His Ser Asn Asp Pro Gly Leu Thr Val Asn Ile Tyr 195 $\,$ 200 $\,$ 205

Trp 225

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<211> LENGTH: 205
<212> TYPE: PRT
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Trp Lys Tyr Ile Arg Arg Asn Thr Asn Pro Asp Trp Leu Gln Asn Gly
Pro Val Thr Asp Leu Ser Ser Thr Asp Leu Arg Cys Asn Val Gly Gly
Gln Val Ser Asn Gly Thr Glu Thr Ile Thr Leu Asn Ala Gly Asp Glu
Phe Ser Phe Ile Leu Asp Thr Pro Val Tyr His Ala Gly Pro Thr Ser
Leu Tyr Met Ser Lys Ala Pro Gly Ala Val Ala Asp Tyr Asp Gly Gly
Gly Ala Trp Phe Lys Ile Tyr Asp Trp Gly Pro Ser Gly Thr Ser Trp
Thr Leu Ser Gly Thr Tyr Thr Gln Arg Ile Pro Lys Cys Ile Pro Asp
                          120
Gly Glu Tyr Leu Leu Arg Ile Gln Gln Ile Gly Leu His Asn Pro Gly
Ala Ala Pro Gln Phe Tyr Ile Ser Cys Ala Gln Val Lys Val Val Asp
                   150
Gly Gly Ser Thr Asn Pro Thr Pro Thr Ala Gln Ile Pro Gly Ala Phe
His Ser Asn Asp Pro Gly Leu Thr Val Asn Ile Tyr Asn Asp Pro Leu
                    185
Thr Asn Tyr Val Val Pro Gly Pro Arg Val Ser His Trp
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<211> LENGTH: 1332
<212> TYPE: DNA
<213> ORGANISM: Myceliophthora thermophila
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attequatqq eqaaqaaqqq caatqteqee acceateete teqeaqqeqq teteqaetee
gaagacatgg cctgtggtcg ggatggtcaa gaacccgtgg catttacgtg tccggcccca
                                                                    240
getggtgeca agttgaetet egagtttege atgtgggeeg atgettegea gteeggateg
                                                                    300
atogatocat cocaccitgg cgicatggcc atctaccica agaaggittc cgacatgaaa
                                                                     360
tetgaegegg cegetggeee gggetggtte aagatttggg accaaggeta egaettggeg
                                                                    420
gccaagaagt gggccaccga gaagctcatc gacaacaacg gcctcctgag cgtcaacctt
                                                                     480
ccaaccggct taccaaccgg ctactacctc gcccgccagg agatcatcac gctccaaaac
gttaccaatg acaggccaga gccccagttc tacgtcggct gcgcacagct ctacgtcgag
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ggcacctegg acteacecat ececteggae aagaeggtet ecatteeegg ceacateage
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gaccoggoog accoggoot gacottcaac gtotacacgg gogacgoatc cacctacaag	720
ccgcccggcc ccgaggttta cttccccacc accaccacca ccacctcctc ctcctcc	780
ggaagcagcg acaacaaggg agccaggcgc cagcaaaccc ccgacgacaa gcaggccgac	840
ggcctcgttc cagccgactg cctcgtcaag aacgcgaact ggtgcgccgc tgccctgccg	900
ccgtacaccg acgaggccgg ctgctgggcc gccgccgagg actgcaacaa gcagctggac	960
gcgtgctaca ccagcgcacc cccctcgggc agcaaggggt gcaaggtctg ggaggagcag	1020
gtgtgcaccg tcgtctcgca gaagtgcgag gccggggatt tcaaggggcc cccgcagctc	1080
gggaaggage teggegaggg gategatgag cetatteegg ggggaaaget geeceeggeg	1140
gtcaacgcgg gagagaacgg gaatcatggc ggaggtggtg gtgatgatgg tgatgatgat	1200
aatgatgagg ccggggctgg ggcagcgtcg actccgactt ttgctgctcc tggtgcggcc	1260
aagactcccc aaccaaactc cgagagggcc cggcgccgtg aggcgcattg gcggcgactg	1320
gaatctgctg ag	1332
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Pro Leu Ser Ser Ala His Thr Thr Phe Thr Thr Leu Phe Val Asn Asp 20 25 30	
Val Asn Gln Gly Asp Gly Thr Cys Ile Arg Met Ala Lys Lys Gly Asn 35 40 45	
Val Ala Thr His Pro Leu Ala Gly Gly Leu Asp Ser Glu Asp Met Ala 50 55 60	
Cys Gly Arg Asp Gly Gln Glu Pro Val Ala Phe Thr Cys Pro Ala Pro 65 70 75 80	
Ala Gly Ala Lys Leu Thr Leu Glu Phe Arg Met Trp Ala Asp Ala Ser 85 90 95	
Gln Ser Gly Ser Ile Asp Pro Ser His Leu Gly Val Met Ala Ile Tyr 100 105 110	
Leu Lys Lys Val Ser Asp Met Lys Ser Asp Ala Ala Ala Gly Pro Gly 115 120 125	
Trp Phe Lys Ile Trp Asp Gln Gly Tyr Asp Leu Ala Ala Lys Lys Trp 130 135 140	
Ala Thr Glu Lys Leu Ile Asp Asn Asn Gly Leu Leu Ser Val Asn Leu 145 150 155 160	
Pro Thr Gly Leu Pro Thr Gly Tyr Tyr Leu Ala Arg Gln Glu Ile Ile 165 170 175	
Thr Leu Gln Asn Val Thr Asn Asp Arg Pro Glu Pro Gln Phe Tyr Val 180 185 190	
Gly Cys Ala Gln Leu Tyr Val Glu Gly Thr Ser Asp Ser Pro Ile Pro 195 200 205	
Ser Asp Lys Thr Val Ser Ile Pro Gly His Ile Ser Asp Pro Ala Asp 210 215 220	

Pro Gly Leu Thr Phe Asn Val Tyr Thr Gly Asp Ala Ser Thr Tyr Lys 225 230 230 235

Pro Pro Gly Pro	Glu Val 245	Tyr l	Phe Pro	Thr 250	Thr	Thr	Thr	Thr	Thr 255	Ser
Ser Ser Ser Ser 260	Gly Ser	Ser A	Asp Asr 265		Gly	Ala	Arg	Arg 270	Gln	Gln
Thr Pro Asp Asp 275	Lys Gln		Asp Gly 280	Leu	Val	Pro	Ala 285	Asp	Cya	Leu
Val Lys Asn Ala 290	Asn Trp	Cys 1 295	Ala Ala	Ala	Leu	Pro 300	Pro	Tyr	Thr	Asp
Glu Ala Gly Cys 305	Trp Ala 310	Ala A	Ala Glu	Asp	Суs 315	Asn	Lys	Gln	Leu	Asp 320
Ala Cys Tyr Thr	Ser Ala 325	Pro I	Pro Ser	Gly 330	Ser	ГÀа	Gly	СЛа	335	Val
Trp Glu Glu Gln 340	Val Cys	Thr V	Val Val 345		Gln	Lys	Cys	Glu 350	Ala	Gly
Asp Phe Lys Gly 355	Pro Pro		Leu Gly 360	ГÀа	Glu	Leu	Gly 365	Glu	Gly	Ile
Asp Glu Pro Ile 370	-	375	-			380				-
Glu Asn Gly Asn 385	390				395					400
Asn Asp Glu Ala	Gly Ala 405	Gly A	Ala Ala	Ser 410	Thr	Pro	Thr	Phe	Ala 415	Ala
Pro Gly Ala Ala 420	Lys Thr	Pro (Gln Pro 425		Ser	Glu	Arg	Ala 430	Arg	Arg
Arg Glu Ala His 435	Trp Arg		Leu Glu 440	Ser	Ala	Glu				
<210> SEQ ID NO <211> LENGTH: 42 <212> TYPE: PRT	3			morah i	ila					
<210> SEQ ID NO <211> LENGTH: 42	3 Myceliop			mophi	ila					
<210> SEQ ID NO <211> LENGTH: 42 <212> TYPE: PRT <213> ORGANISM:	3 Mycelior 51	ohthoi	ra ther	_		Val	Asn	Gln	Gly 15	Asp
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<210> SEQ ID NO <211> LENGTH: 42 <212> TYPE: PRT <213> ORGANISM: <400> SEQUENCE: His Thr Thr Phe 1 Gly Thr Cys Ile	3 Myceliop 51 Thr Thr 5 Arg Met	Leu I Ala I Ser (ra ther Phe Val Lys Lys 25	Asn 10 Gly	Asp Asn	Val	Ala	Thr 30	15 His	Pro
<pre><210> SEQ ID NO <211> LENGTH: 42 <212> TYPE: PRT <213> ORGANISM: <400> SEQUENCE: His Thr Thr Phe 1 Gly Thr Cys Ile</pre>	Mycelion 51 Thr Thr 5 Arg Met Leu Asp	Leu l Ala l Ser (ra ther Phe Val Lys Lys 25 Glu Asp 40	Asn 10 Gly Met	Asp Asn Ala	Val Cys	Ala Gly 45	Thr 30 Arg	15 His Asp	Pro Gly
<pre><210> SEQ ID NO <211> LENGTH: 42 <212> TYPE: PRT <213> ORGANISM: <400> SEQUENCE: His Thr Thr Phe 1 Gly Thr Cys Ile</pre>	3 Myceliop 51 Thr Thr 5 Arg Met Leu Asp Ala Phe	Leu I Ala I Ser (Thr (55	ra ther Phe Val Lys Lys 25 Glu Asp 40 Cys Pro	Asn 10 Gly Met	Asp Asn Ala Pro	Val Cys Ala 60	Ala Gly 45 Gly	Thr 30 Arg Ala	15 His Asp Lys	Pro Gly Leu
<pre><210> SEQ ID NO <211> LENGTH: 42 <212> TYPE: PRT <213> ORGANISM: <400> SEQUENCE: His Thr Thr Phe 1 Gly Thr Cys Ile</pre>	Mycelion 51 Thr Thr 5 Arg Met Leu Asp Ala Phe Arg Met 70	Leu I Ala I Ser (Thr (55	ra ther Phe Val Lys Lys 25 Glu Asp 40 Cys Pro	Asn 10 Gly Met Ala	Asp Asn Ala Pro Ser 75	Val Cys Ala 60 Gln	Ala Gly 45 Gly Ser	Thr 30 Arg Ala Gly	His Asp Lys Ser	Pro Gly Leu Ile
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145 150 155 160	
Thr Asn Asp Arg Pro Glu Pro Gln Phe Tyr Val Gly Cys Ala Gln Leu 165 170 175	
Tyr Val Glu Gly Thr Ser Asp Ser Pro Ile Pro Ser Asp Lys Thr Val	
Ser Ile Pro Gly His Ile Ser Asp Pro Ala Asp Pro Gly Leu Thr Phe 195 200 205	
Asn Val Tyr Thr Gly Asp Ala Ser Thr Tyr Lys Pro Pro Gly Pro Glu 210 215 220	
Val Tyr Phe Pro Thr Thr Thr Thr Thr Ser Ser Ser Ser Gly 225 230 235 240	
Ser Ser Asp Asn Lys Gly Ala Arg Arg Gln Gln Thr Pro Asp Asp Lys 245 250 255	
Gln Ala Asp Gly Leu Val Pro Ala Asp Cys Leu Val Lys Asn Ala Asn 260 265 270	
Trp Cys Ala Ala Ala Leu Pro Pro Tyr Thr Asp Glu Ala Gly Cys Trp 275 280 285	
Ala Ala Ala Glu Asp Cys Asn Lys Gln Leu Asp Ala Cys Tyr Thr Ser 290 295 300	
Ala Pro Pro Ser Gly Ser Lys Gly Cys Lys Val Trp Glu Glu Gln Val 305 310 315 320	
Cys Thr Val Val Ser Gln Lys Cys Glu Ala Gly Asp Phe Lys Gly Pro 325 330 335	
Pro Gln Leu Gly Lys Glu Leu Gly Glu Gly Ile Asp Glu Pro Ile Pro 340 345 350	
Gly Gly Lys Leu Pro Pro Ala Val Asn Ala Gly Glu Asn Gly Asn His 355 360 365	
Gly Gly Gly Gly Asp Asp Gly Asp Asp Asp Asp Asp Glu Ala Gly 370 375 380	
Ala Gly Ala Ala Ser Thr Pro Thr Phe Ala Ala Pro Gly Ala Ala Lys 385 390 395 400	
Thr Pro Gln Pro Asn Ser Glu Arg Ala Arg Arg Arg Glu Ala His Trp 405 410 415	
Arg Arg Leu Glu Ser Ala Glu 420	
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	80
	40
	00
aaggtcattc actcgattca aggtggctgc cccgccaggg ccgagacgat cccggattgc 3	60

420

480

agegeacaaa atateaaege etgeaatata aageeegata atgeeeagat ggacaeeeeg

gataagtatg agttcacgat cccggaggat ctccccagtg gcaaggccac cctcgcctgg

acatggatca acactatcgg caaccgcgag ttttatatgg catgcgcccc ggttgagatc atcccgtcca tcggaggaac ctgcgcgacc gaggagggga agtactacga atatcccaac cccggtaagt cggtcgaaac catcccgggc tggaccgatt tggttcccct gcaaggcgaa tgcggtgctg cctccggtgt ctcgggctcc ggcggaaacg ccagcagtgc tacccctgcc gcaggggccg ccccgactcc tgctgtccgc ggccgccgtc ccacctggaa cgcc <210> SEQ ID NO 53 <211> LENGTH: 278 <212> TYPE: PRT <213 > ORGANISM: Myceliophthora thermophila <400> SEQUENCE: 53 Met Phe Ser Leu Lys Phe Phe Ile Leu Ala Gly Gly Leu Ala Val Leu 1 5 10 15 Thr Glu Ala His Ile Arg Leu Val Ser Pro Ala Pro Phe Thr Asn Pro Asp Gln Gly Pro Ser Pro Leu Leu Glu Ala Gly Ser Asp Tyr Pro Cys 40 His Asn Gly Asn Gly Gly Gly Tyr Gln Gly Thr Pro Thr Gln Met Ala 55 Lys Gly Ser Lys Gln Gln Leu Ala Phe Gln Gly Ser Ala Val His Gly Gly Gly Ser Cys Gln Val Ser Ile Thr Tyr Asp Glu Asn Pro Thr Ala 90 Gln Ser Ser Phe Lys Val Ile His Ser Ile Gln Gly Gly Cys Pro Ala 105 Arg Ala Glu Thr Ile Pro Asp Cys Ser Ala Gln Asn Ile Asn Ala Cys Asn Ile Lys Pro Asp Asn Ala Gln Met Asp Thr Pro Asp Lys Tyr Glu Phe Thr Ile Pro Glu Asp Leu Pro Ser Gly Lys Ala Thr Leu Ala Trp Thr Trp Ile Asn Thr Ile Gly Asn Arg Glu Phe Tyr Met Ala Cys Ala Pro Val Glu Ile Thr Gly Asp Gly Gly Ser Glu Ser Ala Leu Ala Ala Leu Pro Asp Met Val Ile Ala Asn Ile Pro Ser Ile Gly Gly Thr Cys Ala Thr Glu Glu Gly Lys Tyr Tyr Glu Tyr Pro Asn Pro Gly Lys Ser 210 215 220 Val Glu Thr Ile Pro Gly Trp Thr Asp Leu Val Pro Leu Gln Gly Glu 230 Cys Gly Ala Ala Ser Gly Val Ser Gly Ser Gly Gly Asn Ala Ser Ser 250 Ala Thr Pro Ala Ala Gly Ala Ala Pro Thr Pro Ala Val Arg Gly Arg

Arg Pro Thr Trp Asn Ala 275

_																	_
<211	L> LE	EQ II ENGTH	H: 25														
		PE:		Мус	eliop	hth	ora t	herr	nophi	lla							
< 400)> SI	EQUE	ICE :	54													
His 1	Ile	Arg	Leu	Val 5	Ser	Pro	Ala	Pro	Phe 10	Thr	Asn	Pro	Asp	Gln 15	Gly		
Pro	Ser	Pro	Leu 20	Leu	Glu	Ala	Gly	Ser 25	Asp	Tyr	Pro	СЛа	His 30	Asn	Gly		
Asn	Gly	Gly 35	Gly	Tyr	Gln	Gly	Thr 40	Pro	Thr	Gln	Met	Ala 45	ГЛа	Gly	Ser		
Lys	Gln 50	Gln	Leu	Ala	Phe	Gln 55	Gly	Ser	Ala	Val	His 60	Gly	Gly	Gly	Ser		
Cya 65	Gln	Val	Ser	Ile	Thr 70	Tyr	Asp	Glu	Asn	Pro 75	Thr	Ala	Gln	Ser	Ser 80		
Phe	ГЛа	Val	Ile	His 85	Ser	Ile	Gln	Gly	Gly 90	Cys	Pro	Ala	Arg	Ala 95	Glu		
Thr	Ile	Pro	Asp 100	CÀa	Ser	Ala	Gln	Asn 105	Ile	Asn	Ala	CÀa	Asn 110	Ile	ГЛа		
Pro	Asp	Asn 115	Ala	Gln	Met	Asp	Thr 120	Pro	Asp	Lys	Tyr	Glu 125	Phe	Thr	Ile		
Pro	Glu 130	Asp	Leu	Pro	Ser	Gly 135	Lys	Ala	Thr	Leu	Ala 140	Trp	Thr	Trp	Ile		
Asn 145	Thr	Ile	Gly	Asn	Arg 150	Glu	Phe	Tyr	Met	Ala 155	CÀa	Ala	Pro	Val	Glu 160		
Ile	Thr	Gly	Asp	Gly 165	Gly	Ser	Glu	Ser	Ala 170	Leu	Ala	Ala	Leu	Pro 175	Asp		
Met	Val	Ile	Ala 180	Asn	Ile	Pro	Ser	Ile 185	Gly	Gly	Thr	CAa	Ala 190	Thr	Glu		
Glu	Gly	Lys 195	Tyr	Tyr	Glu	Tyr	Pro 200	Asn	Pro	Gly	Lys	Ser 205	Val	Glu	Thr		
Ile	Pro 210	Gly	Trp	Thr	Asp	Leu 215	Val	Pro	Leu	Gln	Gly 220	Glu	Cys	Gly	Ala		
Ala 225	Ser	Gly	Val	Ser	Gly 230	Ser	Gly	Gly	Asn	Ala 235	Ser	Ser	Ala	Thr	Pro 240		
Ala	Ala	Gly	Ala	Ala 245	Pro	Thr	Pro	Ala	Val 250	Arg	Gly	Arg	Arg	Pro 255	Thr		
Trp	Asn	Ala															
-210)	EQ II	OM C	55													
		ENGTI															
		(PE : RGANI		Мус	eliop	hth	ora t	herr	nophi	lla							
< 400)> SI	EQUE	ICE :	55													
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gggt	ccaç	gaa a	agtti	ggc	gt gt	acga	agcad	c att	cgca	aga	acac	gaad	cta d	caact	cgccc	12	20
gtta	accga	acc t	gtc	ggaca	ac ca	aacct	gege	c tgo	caacç	gtcg	gcgg	9999	etc (gggca	accagc	18	В0
acca	accgt	gc t	cgad	egte	aa gg	gccg	gagad	c teg	gttca	ecct	tctt	cago	cga o	gttg	gccgtc	24	40
taco	cacca	agg g	ggcc	catci	cc go	ctgt	gcgtg	g gad	ccgga	ecca	gtg	cagaç	gag (catgo	gatgga	3(00
cggg	gaaco	egg a	acato	geget	eg ed	cgaa	ctgg	c tca	acaaq	gctg	gcta	acct	ggc (ggtga	actgac	36	60

20

25

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tacgacgggt ccggtgactg tttcaagatc tatgactggg gaccgacgtt caacgggggc
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<211> LENGTH: 223
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<213 > ORGANISM: Myceliophthora thermophila
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Gln Leu Ser Val Gly Ser Arg Lys Phe Gly Val Tyr Glu His Ile Arg
Lys Asn Thr Asn Tyr Asn Ser Pro Val Thr Asp Leu Ser Asp Thr Asn
                         40
Leu Arg Cys Asn Val Gly Gly Ser Gly Thr Ser Thr Thr Val Leu
                      55
Asp Val Lys Ala Gly Asp Ser Phe Thr Phe Phe Ser Asp Val Ala Val
Tyr His Gln Gly Pro Ile Ser Leu Cys Val Asp Arg Thr Ser Ala Glu
                                   90
Ser Met Asp Gly Arg Glu Pro Asp Met Arg Cys Arg Thr Gly Ser Gln
                           105
Ala Gly Tyr Leu Ala Val Thr Asp Tyr Asp Gly Ser Gly Asp Cys Phe
Lys Ile Tyr Asp Trp Gly Pro Thr Phe Asn Gly Gly Gln Ala Ser Trp
Pro Thr Arg Asn Ser Tyr Glu Tyr Ser Ile Leu Lys Cys Ile Arg Asp
                             155
Gly Glu Tyr Leu Leu Arg Ile Gln Ser Leu Ala Ile His Asn Pro Gly
Ala Leu Pro Gln Phe Tyr Ile Ser Cys Ala Gln Val Asn Val Thr Gly
Gly Gly Thr Val Thr Pro Arg Ser Arg Arg Pro Ile Leu Ile Tyr Phe
Asn Phe His Ser Tyr Ile Val Pro Gly Pro Ala Val Phe Lys Cys
<210> SEQ ID NO 57
<211> LENGTH: 208
<212> TYPE: PRT
<213 > ORGANISM: Myceliophthora thermophila
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Arg Lys Asn Thr Asn Tyr Asn Ser Pro Val Thr Asp Leu Ser Asp Thr
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Asn Leu Arg Cys Asn Val Gly Gly Gly Ser Gly Thr Ser Thr Thr Val Leu Asp Val Lys Ala Gly Asp Ser Phe Thr Phe Phe Ser Asp Val Ala Val Tyr His Gln Gly Pro Ile Ser Leu Cys Val Asp Arg Thr Ser Ala Glu Ser Met Asp Gly Arg Glu Pro Asp Met Arg Cys Arg Thr Gly Ser Gln Ala Gly Tyr Leu Ala Val Thr Asp Tyr Asp Gly Ser Gly Asp Cys Phe Lys Ile Tyr Asp Trp Gly Pro Thr Phe Asn Gly Gly Gln Ala Ser Trp Pro Thr Arg Asn Ser Tyr Glu Tyr Ser Ile Leu Lys Cys Ile Arg Asp Gly Glu Tyr Leu Leu Arg Ile Gln Ser Leu Ala Ile His Asn Pro 150 155 Gly Ala Leu Pro Gln Phe Tyr Ile Ser Cys Ala Gln Val Asn Val Thr Gly Gly Gly Thr Val Thr Pro Arg Ser Arg Arg Pro Ile Leu Ile Tyr 185 Phe Asn Phe His Ser Tyr Ile Val Pro Gly Pro Ala Val Phe Lys Cys 200 <210> SEQ ID NO 58 <211> LENGTH: 642 <212> TYPE: DNA <213> ORGANISM: Myceliophthora thermophila <400> SEQUENCE: 58 atgaageteg ceaegeteet egeegeeete acceteggge teagegtegg gteeagaaag 60 tttggcgtgt acgagcacat tcgcaagaac acgaactaca actcgcccgt taccgacctg teggacacca acetgegetg caacgtegge gggggetegg geaceageae caeegtgete gacgtcaagg ccggagactc gttcaccttc ttcagcgacg ttgccgtcta ccaccagggg cccatctcgc tgtgcgtgga ccggaccagt gcagagagca tggatggacg ggaaccggac atgcgctgcc gaactggctc acaagctggc tacctggcgg tgactgtgat gactgtgact gactacgacg ggtccggtga ctgtttcaag atctatgact ggggaccgac gttcaacggg ggccaggcgt cgtggccgac gaggaattcg tacgagtaca gcatcctcaa gtgcatcagg qacqqcqaat acctactqcq qattcaqtcc ctqqccatcc ataacccaqq tqcccttccq cagttctaca tcagctgcgc ccaggtgaat gtgacgggcg gaggcaccat ctatttcaac 600 642 ttccactcgt atatcgtccc tgggccggca gtgttcaagt gc <210> SEQ ID NO 59 <211> LENGTH: 214 <212> TYPE: PRT <213 > ORGANISM: Myceliophthora thermophila <400> SEOUENCE: 59 Met Lys Leu Ala Thr Leu Leu Ala Ala Leu Thr Leu Gly Leu Ser Val 5

Gly Ser Arg Lys Phe Gly Val Tyr Glu His Ile Arg Lys Asn Thr Asn

20

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			20					25					30		
Tyr	Asn	Ser 35	Pro	Val	Thr	Asp	Leu 40	Ser	Asp	Thr	Asn	Leu 45	Arg	CÀa	Asn
Val	Gly 50	Gly	Gly	Ser	Gly	Thr 55	Ser	Thr	Thr	Val	Leu 60	Asp	Val	Lys	Ala
Gly 65	Asp	Ser	Phe	Thr	Phe 70	Phe	Ser	Asp	Val	Ala 75	Val	Tyr	His	Gln	Gly 80
Pro	Ile	Ser	Leu	Сув 85	Val	Asp	Arg	Thr	Ser 90	Ala	Glu	Ser	Met	Asp 95	Gly
Arg	Glu	Pro	Asp 100	Met	Arg	Cys	Arg	Thr 105	Gly	Ser	Gln	Ala	Gly 110	Tyr	Leu
Ala	Val	Thr 115	Val	Met	Thr	Val	Thr 120	Asp	Tyr	Asp	Gly	Ser 125	Gly	Asp	Cys
Phe	Lys 130	Ile	Tyr	Asp	Trp	Gly 135	Pro	Thr	Phe	Asn	Gly 140	Gly	Gln	Ala	Ser
Trp 145	Pro	Thr	Arg	Asn	Ser 150	Tyr	Glu	Tyr	Ser	Ile 155	Leu	Lys	CÀa	Ile	Arg 160
Asp	Gly	Glu	Tyr	Leu 165	Leu	Arg	Ile	Gln	Ser 170	Leu	Ala	Ile	His	Asn 175	Pro
Gly	Ala	Leu	Pro 180	Gln	Phe	Tyr	Ile	Ser 185	Cys	Ala	Gln	Val	Asn 190	Val	Thr
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100 105 110		
Asp Ser Ile Thr Trp Pro Ser Ala Gly Lys Thr Glu Val Ser Val Thr 115 120 125		
Ile Pro Ser Cys Ile Glu Asp Gly Glu Tyr Leu Leu Arg Val Glu His 130 135 140		
Ile Ala Leu His Ser Ala Ser Ser Val Gly Gly Ala Gln Phe Tyr Ile 145 150 155 160		
Ala Cys Ala Gln Leu Ser Val Thr Gly Gly Ser Gly Thr Leu Asn Thr		
Gly Ser Leu Val Ser Leu Pro Gly Ala Tyr Lys Ala Thr Asp Pro Gly 180 185 190		
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ccgcggccgg agcaggcgaa ctcgccgttg cgcgtgggct ggtcgacggg ggcaatcgac	180	30
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ggctggccgc tcggacacgt ggggccggtg ctgtcgtacc tggcgccctg cggcgggctg	360	0
gaggggtccg agagcgggtg cgccggggtg gacaagcggc agctgcggtg gaccaaggtg	420	20
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Gly Glu Val Tyr Gln Gly Phe Asp Pro Arg Pro Glu Gln Ala Asn Ser 35 40 45		
Pro Leu Arg Val Gly Trp Ser Thr Gly Ala Ile Asp Asp Gly Phe Val		
Ala Pro Ala Asn Tyr Ser Ser Pro Asp Ile Ile Cys His Ile Glu Gly 65 70 75 80		
Ala Ser Pro Pro Ala His Ala Pro Val Arg Ala Gly Asp Arg Val His 85 90 95		
Val Gln Trp Asn Gly Trp Pro Leu Gly His Val Gly Pro Val Leu Ser 100 105 110		

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aagaggetga cagegacete aagaagggaa ctagttgate ategtacecg gtacetetec 1080 gtagetgtet gegeagatt eggegeteat aaggeagag aaaccaacea egaagetttg 1140 agaggegga ataagcaacea tggeggtgtt teagag 1176 2210 SEQ ID NO 79 2211 LENGTH: 392 2212 TYPE: PRT 2213 ORGANISH: Myceliophthora thermophila 2400 SEQUENCE: 79 Met Pro Pro Pro Arg Leu Ser Thr Leu Leu Pro Leu Leu Ala Leu Ile 1 Fs 10 15 Ala Pro Thr Ala Leu Gly His Ser His Leu Gly Tyr Ile Ile Ile Asn 20 25 Gly Glu Val Tyr Gln Gly Phe Asp Pro Arg Pro Glu Gln Ala Asn Ser 35 40 25 Pro Leu Arg Val Gly Trp Ser Thr Gly Ala Ile Asp Asp Gly Phe Val 50 75 80 Ala Pro Ala Asn Tyr Ser Ser Pro Asp Ile Ile Cys His Ile Glu Gly Gly 65 75 80 Ala Ser Pro Pro Ala His Ala Pro Val Arg Ala Gly Asp Arg Val His 85 95 Val Gln Trp Lye Arg Leu Ala Ala Arg Thr Arg Gly Ala Gly Ala Val 100 100 105 Val Pro Gly Ala Leu Arg Arg Ala Gly Gly Val Arg Glu Arg Val Asp 115 120 Asp Ser Leu Pro Ala Met Glu Leu Val Gly Ala Ala Gly Gly Ala Gly 136 140 Gly Glu Asp Asp Gly Ser Gly Ser Asp Gly Ser Gly Ser Gly Gly Ser 145 150 160 Gly Arg Val Gly Val Pro Gly Gln Arg Trp Ala Thr Asp Val Leu Ile 175 160 Gly Arg Val Gly Val Pro Gly Gln Arg Trp Ala Thr Asp Val Leu Ile 175 175 Ala Ala Asn Asn Ser Trp Gln Val Glu Ile Val Ala Leu His Tyr Ala Ala 2195 220 Glu Pro Tyr Val Leu Arg His Glu Ile Val Ala Leu His Tyr Ala Ala 2195 220 Glu Gly Gly Asp Gly Ser Met Glu Leu Asp His Phe Asp Ala Thr Gln 225 220 Glu Gly Gly Asp Gly Ser Met Glu Leu Asp His Phe Asp Ala Thr Gln 225 220 Phe Tyr Arg Pro Asp Asp Pro Gly Ile Leu Leu Asn Val Thr Ala Gly 245 250 Pro Val Pro Tyr Ala Gln Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly	cccttcaccg (eggeacee	ac gccag	ccgag a	cggcagaag	ccaaagg	ggg gag	gtatgat	960
gtagetgtet gegeagatte eggegeteat aaggeageag aaaceaacea egaagetttg 1140 agaaggegegea ataagcaacea tggeggtgtt teagag 1176 <pre> <210 > SEQ ID NO 79 <211 > LENGTHER: 392 <212 > TYPE: PRT <213 > ORGANISM: Myceliophthora thermophila <400 > SEQUENCE: 79 Met Pro Pro Pro Arg Leu Ser Thr Leu Leu Pro Leu Leu Ala Leu IIe 15 Ala Pro Thr Ala Leu Gly His Ser His Leu Gly Tyr IIe IIe IIe Asm 20 Gly Glu Val Tyr Gln Gly Phe Asp Pro Arg Pro Glu Gln Ala Asn Ser 45 Pro Leu Arg Val Gly Trp Ser Thr Gly Ala IIe Asp Asp Gly Phe Val 50 Ala Pro Ala Asn Tyr Ser Pro Asp IIe IIe Cys His IIe Glu Gly 77 Ala Ser Pro Pro Ala His Ala Pro Val Arg Ala Gly Asp Arg Val His 85 85 Val Gln Trp Lys Arg Leu Ala Ala Arg Thr Arg Gly Ala Gly Ala Val 100 Val Pro Gly Ala Leu Arg Arg Ala Gly Gly Val Arg Glu Arg Val Asp 115 Asp Ser Leu Pro Ala Met Glu Leu Val Gly Ala Ala Gly Gly Pla Ala Gly Gly Ser 145 Gly Gly Asp Asp Gly Ser Gly Ser Asp Gly Ser Gly Ser Gly Gly Ser 145 Gly Ala Ola Asp Asp Gly Ser Gly Ser Asp Gly Ser Gly Ser Gly Gly Ser 145 Gly Gly Asp Asp Ser Iteu Pro Ala Met Glu Leu Val Gly Ala Thr Asp Val Leu IIe 175 Ala Ala Asn Asn Asn Ser Trp Gln Val Glu IIe Pro Arg Gly Leu Arg Asp 180 Gly Pro Tyr Val Leu Arg His Glu IIe Val Ala Leu His Tyr Ala Ala 2195 Glu Pro Gly Gly Ala Gln Asn Tyr Pro Leu Cys Val Asn Leu Trp Val 210 Glu Gly Gly Asp Gly Ser Met Glu Leu Asp His Phe Asp Ala Thr Gln 225 Glu Gly Gly Asp Asp Pro Gly IIe Leu Leu Asn Val Thr Ala Gly 245 Dee Tyr Arg Pro Asp Asp Pro Gly IIe Leu Leu Asn Val Thr Ala Gly 255 Leu Arg Ser Tyr Ala Val Pro Gly Pro Thr Leu Ala Ala Ala Gly Ala Thr 260 Pro Val Pro Tyr Ala Gln Gln Asn IIe Ser Ser Ala Arg Ala Asp Gly </pre>	gaccaaaccc g	gaactaaa	ga cctaa	atgaa c	gcttcttt	atagtago	ccg gcc	agaacag	1020
### ### ### ### ### ### ### ### ### ##	aagaggctga d	cagegace	tc aagaa	gggaa c	tagttgatc	atcgtaco	ccg gta	cctctcc	1080
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35	Ala Pro Thr		Gly His		s Leu Gly	Tyr Ile		e Asn	
50 55 60 Ala Pro Ala Asn Tyr Ser Ser Pro Asp 11e I1e Cys His I1e Glu Gly 75 80 Ala Ser Pro Pro Ala His Ala Pro Val Arg Ala Gly Asp Arg Val His 95 95 Val Gln Trp Lys Arg Leu Ala Ala Arg Thr Arg Gly Ala Gly Ala Val 100 100 Val Pro Gly Ala Leu Arg Arg Ala Gly Gly Val Arg Glu Arg Val Asp 115 110 Asp Ser Leu Pro Ala Met Glu Leu Val Gly Ala Ala Gly Gly Ala Gly 140 140 Gly Glu Asp Asp Gly Ser Gly Ser Asp Gly Ser Gly Ser Gly Ser Gly Gly Ser 145 150 Gly Arg Val Gly Val Pro Gly Gln Arg Trp Ala Thr Asp Val Leu Ile 175 165 Ala Ala Asn Asn Ser Trp Gln Val Glu Ile Pro Arg Gly Leu Arg Asp 180 185 Gly Pro Tyr Val Leu Arg His Glu Ile Val Ala Leu His Tyr Ala Ala 205 190 Glu Pro Gly Gly Ala Gln Asn Tyr Pro Leu Cys Val Asn Leu Trp Val 215 220 Glu Gly Gly Asp Gly Ser Met Glu Leu Asp His Phe Asp Ala Thr Gln 235 240 Phe Tyr Arg Pro Asp Asp Asp Pro Gly Ile Leu Leu Asn Val Thr Ala Gly 255 255 Leu Arg Ser Tyr Ala Val Pro Gly Pro Thr Leu Ala Ala Gly Ala Thr 270 Pro Val Pro Tyr Ala Gln Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly		Tyr Gln	Gly Phe		o Arg Pro		Ala As	n Ser	
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S5 90 95 95 96 95 96 95 96 95 96 96		Asn Tyr		Pro As	_	Cys His	Ile Gl	_	
100 105 110 110 105 110	Ala Ser Pro		His Ala	Pro Va	_	Gly Asp	-		
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130	_	Ala Leu	Arg Arg		y Gly Val	-	Arg Va	l Asp	
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Ala Ala Asn Asn Ser Trp Gln Val Glu Ile Pro Arg Gly Leu Arg Asp 180		Asp Gly	_	Ser As		_	Gly Gl	-	
180 185 190 Gly Pro Tyr Val Leu Arg His Glu Ile Val Ala Leu His Tyr Ala Ala 205 Glu Pro Gly Gly Ala Gln Asn Tyr Pro Leu Cys Val Asn Leu Trp Val 210 Glu Gly Gly Asp Gly Ser Met Glu Leu Asp His Phe Asp Ala Thr Gln 225 Phe Tyr Arg Pro Asp Asp Pro Gly Ile Leu Leu Asn Val Thr Ala Gly 245 Leu Arg Ser Tyr Ala Val Pro Gly Pro Thr Leu Ala Ala Gly Ala Thr 270 Pro Val Pro Tyr Ala Gln Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly	Gly Arg Val	-	Pro Gly	Gln Ar		Thr Asp			
Glu Pro Gly Gly Ala Gln Asn Tyr Pro Leu Cys Val Asn Leu Trp Val 210 Glu Gly Gly Asp Gly Ser Met Glu Leu Asp His Phe Asp Ala Thr Gln 225 Phe Tyr Arg Pro Asp Asp Pro Gly Ile Leu Leu Asn Val Thr Ala Gly 255 Leu Arg Ser Tyr Ala Val Pro Gly Pro Thr Leu Ala Ala Gly Ala Thr 270 Pro Val Pro Tyr Ala Gln Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly	Ala Ala Asn		Trp Gln		_	Arg Gly		g Asp	
Glu Gly Gly Asp Gly Ser Met Glu Leu Asp His Phe Asp Ala Thr Gln 225 Phe Tyr Arg Pro Asp Asp Pro Gly Ile Leu Leu Asn Val Thr Ala Gly 245 Leu Arg Ser Tyr Ala Val Pro Gly Pro Thr Leu Ala Ala Gly Ala Thr 260 Pro Val Pro Tyr Ala Gln Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly		Val Leu	Arg His		e Val Ala		Tyr Al	a Ala	
Phe Tyr Arg Pro Asp Asp Pro Gly Ile Leu Leu Asn Val Thr Ala Gly 245 Leu Arg Ser Tyr Ala Val Pro Gly Pro Thr Leu Ala Ala Gly Ala Thr 260 Pro Val Pro Tyr Ala Gln Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly		Gly Ala		-	o Leu Cys		Leu Tr	p Val	
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260 265 270 Pro Val Pro Tyr Ala Gln Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly	Phe Tyr Arg		Asp Pro	Gly Il		Asn Val			
	Leu Arg Ser	_	Val Pro	_		Ala Ala	_	a Thr	
		Tyr Ala	Gln Gln		e Ser Ser	_	Ala As	p Gly	

Thr Pro Val Ile Val Thr Arg Ser Thr Glu Thr Val Pro Phe Thr Ala

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310 316 316 317 328 328 329		290					295					300				
Arg Pro Glu Gln Lys Arg Leu Thr Ala Thr Ser Arg Arg Glu Leu Val 345 Asp His Arg Thr Arg Tyr Leu Ser Val Ala Val Cys Ala Asp Phe Gly 355 Ala His Lys Ala Ala Glu Thr Asn His Glu Ala Leu Arg Gly Gly Asn 370 Ala His Lys Ala Ala Glu Thr Asn His Glu Ala Leu Arg Gly Gly Asn 370 Ala His Lys Ala Ala Glu Thr Asn His Glu Ala Leu Arg Gly Gly Asn 370 Ala His Lys Ala Ala Glu Thr Asn His Glu Ala Leu Arg Gly Gly Asn 370 Ala His Lys Ala Ala Glu Thr Asn His Glu Ala Leu Arg Gly Gly Asn 370 Ala Sec III No 80 All Leu Arg Gly Gly Val Ser Glu Ala Sec III No 80 Ala Sec III Sec III No 80 Ala Sec III Sec III Sec III Sec III III III III III III III III III I		Pro	Thr	Pro	Ala		Thr	Ala	Glu	Ala		Gly	Gly	Arg	Tyr	
Agg His Arg Thr Arg Tyr Leu Ser Val Ala Val Cys Ala Asg Phe Gly Ala Ala Sin Ala	Asp	Gln	Thr	Arg		Lys	Asp	Leu	Asn		Arg	Phe	Phe	Tyr		Ser
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10						Tur	Tla	Tla	Tla	Δan	Glv	Glu	Val	Тул	Gln	Glv
Ser Thr Gly Ala Ile Asp Asp Gly Phe Val Ala Pro Ala Asn Tyr Ser Asp So		DCI	1115	пси	5	171	110	110	110		Cly	Olu	vai	171		Gly
Ser Pro So Asp Ile Ile Cys Sis His Sis Ile Glu Gly Ala Ser Go Pro Pro Pro Pro Ala His Sign Sign Arg Ala Ser Go Pro Pro Pro Ala His Sign Arg Ala Gly Ala Ser Arg Sign Arg Ala Ser Tro Go Pro Pro Ala His Sign Arg Ala Ser Tro Go Pro Sign Arg Sign Arg Ala Sign Arg Sign Ar	Phe	Asp	Pro	_	Pro	Glu	Gln	Ala		Ser	Pro	Leu	Arg		Gly	Trp
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Arg Ala Gly Val Arg Glu Arg Glu Arg Val Arg A		Pro	Val	Arg	Ala		Asp	Arg	Val	His		Gln	Trp	Lys	Arg	
Ser 100 105 110	Ala	Ala	Arg	Thr		Gly	Ala	Gly	Ala		Val	Pro	Gly	Ala		Arg
115	Arg	Ala	Gly		Val	Arg	Glu	Arg		Asp	Asp	Ser	Leu		Ala	Met
130	Glu	Leu		Gly	Ala	Ala	Gly		Ala	Gly	Gly	Glu		Asp	Gly	Ser
145 150 155 160 Gln Val Glu Ile Pro 165 Arg Gly Leu Arg 170 Asp Gly Pro Tyr Val Leu Arg 175 Arg 175 His Glu Ile Val Ala Leu His Tyr Ala Ala Ala Glu Pro Gly Gly Ala Gln 180 Fro Gly 190 Asp Gly Pro Tyr Arg Gly Ser 205 Asn Tyr Pro 195 Leu Cys Val Asn Leu Trp Val Glu Gly Gly Asp Gly Ser 205 Asp Gly Ser 205 Met Glu Leu Asp His Pro 215 Ala Thr Gln Pro Tyr Arg Pro Asp Asp 220 Pro Gly Ile Leu Leu Asn Val 230 Thr Ala Gly Leu Arg Ser Tyr Ala Val 240 Pro Gly Pro Thr Leu Ala Ala Gly Ala Thr Pro Val Pro Tyr Ala Gln	Gly		Asp	Gly	Ser	Gly		Gly	Gly	Ser	Gly		Val	Gly	Val	Pro
His Glu Ile Val Ala Leu His Tyr Ala Ala Glu Pro Gly Gly Ala Gln 180		Gln	Arg	Trp	Ala		Asp	Val	Leu	Ile		Ala	Asn	Asn	Ser	
Asn Tyr Pro Leu Cys Val Asn Leu Trp Val Glu Gly Gly Asp Gly Ser 200 Trp Val Glu Gly Gly Asp Gly Ser 200 Trp Val Glu Gly Gly Asp Gly Ser 200 Trp Val Glu Gly Gly Asp Gly Ser 200 Trp Gly Ile Leu Leu Asn Val Thr Ala Gly Leu Arg Ser Tyr Ala Val 225 Trp Gly Pro Thr Leu Ala Ala Gly Ala Thr Pro Val Pro Tyr Ala Gln	Gln	Val	Glu	Ile		Arg	Gly	Leu	Arg		Gly	Pro	Tyr	Val		Arg
Met Glu Leu Asp His Phe Asp Ala Thr Gln Phe Tyr Arg Pro Asp Asp Asp 210	His	Glu	Ile		Ala	Leu	His	Tyr		Ala	Glu	Pro	Gly	_	Ala	Gln
210 215 220 210 215 220 210 210 210 210 210 210 210 210 210	Asn	Tyr		Leu	CÀa	Val	Asn		Trp	Val	Glu	Gly	_	Asp	Gly	Ser
225 230 235 240 Pro Gly Pro Thr Leu Ala Ala Gly Ala Thr Pro Val Pro Tyr Ala Gln	Met		Leu	Asp	His	Phe		Ala	Thr	Gln	Phe		Arg	Pro	Asp	Asp
		Gly	Ile	Leu	Leu		Val	Thr	Ala	Gly		Arg	Ser	Tyr	Ala	
	Pro	Gly	Pro	Thr		Ala	Ala	Gly	Ala		Pro	Val	Pro	Tyr		Gln

Gln Asn Ile Ser Ser Ala Arg Ala Asp Gly Thr Pro Val Ile Val Thr 260 265 270	
Arg Ser Thr Glu Thr Val Pro Phe Thr Ala Ala Pro Thr Pro Ala Glu 275 280 285	
Thr Ala Glu Ala Lys Gly Gly Arg Tyr Asp Asp Gln Thr Arg Thr Lys 290 295 300	
Asp Leu Asn Glu Arg Phe Phe Tyr Ser Ser Arg Pro Glu Gln Lys Arg 305 310 315 320	
Leu Thr Ala Thr Ser Arg Arg Glu Leu Val Asp His Arg Thr Arg Tyr 325 330 335	
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ggcaagtgcc ccgtgaaggc tggcggcacc gtcaccatcg agatgcacca gcaacccggc 24	10
gaccgcagct gcaacaacga agccatcgga ggggcgcatt ggggccccgt ccaggtgtac 30	00
ctgaccaagg ttcaggacgc cgcgacggcc gacggctcga cgggctggtt caagatcttc 36	50
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Asp Phe Val Cys Asn Ala Gly Thr Arg Pro Val Ser Gly Lys Cys Pro 50 60	
Val Lys Ala Gly Gly Thr Val Thr Ile Glu Met His Gln Gln Pro Gly 65 70 75 80	
Asp Arg Ser Cys Asn Asn Glu Ala Ile Gly Gly Ala His Trp Gly Pro 85 90 95	
Val Gln Val Tyr Leu Thr Lys Val Gln Asp Ala Ala Thr Ala Asp Gly 100 105 110	

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780 837

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Val Arg Leu Pro Ala Ser Asn Ser Pro Val Thr Asn Val Gly Ser Arg 35 40 45
Asp Phe Val Cys Asn Ala Gly Thr Arg Pro Val Ser Gly Lys Cys Pro 50 60
Val Lys Ala Gly Gly Thr Val Thr Ile Glu Met His Gln Gln Pro Gly 70 75 80
Asp Arg Ser Cys Asn Asn Glu Ala Ile Gly Gly Ala His Trp Gly Pro 85 90 95
Val Gln Val Tyr Leu Thr Lys Val Gln Asp Ala Ala Thr Ala Asp Gly 100 105 110
Ser Thr Gly Trp Phe Lys Ile Phe Ser Asp Ser Trp Ser Lys Lys Pro 115 120 125
Gly Gly Asn Ser Gly Asp Asp Asp Asn Trp Gly Thr Arg Asp Leu Asn 130 135 140
Ala Cys Cys Gly Lys Met Asp Val Ala Ile Pro Ala Asp Ile Ala Ser 145 150 155 160
Gly Asp Tyr Leu Leu Arg Ala Glu Ala Leu Ala Leu His Thr Ala Gly 165 170 175
Gln Ala Gly Gly Ala Gln Phe Tyr Met Ser Cys Tyr Gln Met Thr Val 180 185 190
Glu Gly Gly Ser Gly Thr Ala Asn Pro Pro Thr Val Lys Phe Pro Gly 195 200 205
Ala Tyr Ser Ala Asn Asp Pro Gly Ile Leu Val Asn Ile His Ala Pro 210 215 220
Leu Ser Ser Tyr Thr Ala Pro Gly Pro Ala Val Tyr Ala Gly Gly Thr 225 230 235 240
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Val Gly Ser Ser Pro Ser Ala Val Ala Pro Gly Ser Gly Ala Gly Asn 260 265 270
Gly Gly Phe Gln Pro Arg 275
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Cys Asn Ala Gly Thr Arg Pro Val Ser Gly Lys Cys Pro Val Lys Ala 35 40 45
Gly Gly Thr Val Thr Ile Glu Met His Gln Gln Pro Gly Asp Arg Ser 50 55 60
Cys Asn Asn Glu Ala Ile Gly Gly Ala His Trp Gly Pro Val Gln Val 65 70 75 80
Tyr Leu Thr Lys Val Gln Asp Ala Ala Thr Ala Asp Gly Ser Thr Gly 85 90 95
Trp Phe Lys Ile Phe Ser Asp Ser Trp Ser Lys Lys Pro Gly Gly Asn 100 105 110
Ser Gly Asp Asp Asp Asn Trp Gly Thr Arg Asp Leu Asn Ala Cys Cys 115 120 125
Gly Lys Met Asp Val Ala Ile Pro Ala Asp Ile Ala Ser Gly Asp Tyr 130 135 140
Leu Leu Arg Ala Glu Ala Leu Ala Leu His Thr Ala Gly Gln Ala Gly 145 150 155 160
Gly Ala Gln Phe Tyr Met Ser Cys Tyr Gln Met Thr Val Glu Gly Gly 165 170 175
Ser Gly Thr Ala Asn Pro Pro Thr Val Lys Phe Pro Gly Ala Tyr Ser 180 185 190
Ala Asn Asp Pro Gly Ile Leu Val Asn Ile His Ala Pro Leu Ser Ser 195 200 205
Tyr Thr Ala Pro Gly Pro Ala Val Tyr Ala Gly Gly Thr Ile Arg Glu 210 215 220
Ala Gly Ser Ala Cys Thr Gly Cys Ala Gln Thr Cys Lys Val Gly Ser 225 230 235 240
Ser Pro Ser Ala Val Ala Pro Gly Ser Gly Ala Gly Asn Gly Gly Gly 245 250 255
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atcegetege eggegaceaa egacecegtg egegacetet egagegeege categtgtge 180
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ggactccact ttaagatcta catcggtcag gacagccagt atgtggcccc ggggccgcgg	720
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Gln Gly Asp Gly Leu Asn Lys Tyr Ile Arg Ser Pro Ala Thr Asn Asp 35 40 45	
Pro Val Arg Asp Leu Ser Ser Ala Ala Ile Val Cys Asn Thr Gln Gly 50 60	
Ser Lys Ala Ala Pro Asp Phe Val Arg Ala Ala Ala Gly Asp Lys Leu 65 70 75 80	
Thr Phe Leu Trp Ala His Asp Asn Pro Asp Asp Pro Val Asp Tyr Val 85 90 95	
Leu Asp Pro Ser His Lys Gly Ala Ile Leu Thr Tyr Val Ala Ala Tyr 100 105 110	
Pro Ser Gly Asp Pro Thr Gly Pro Ile Trp Ser Lys Leu Ala Glu Glu 115 120 125	
Gly Phe Thr Gly Gly Gln Trp Ala Thr Ile Lys Met Ile Asp Asn Gly 130 135 140	
Gly Lys Val Asp Val Thr Leu Pro Glu Ala Leu Ala Pro Gly Lys Tyr 145 150 155 160	
Leu Ile Arg Gln Glu Leu Leu Ala Leu His Arg Ala Asp Phe Ala Cys 165 170 175	
Asp Asp Pro Ala His Pro Asn Arg Gly Ala Glu Ser Tyr Pro Asn Cys 180 185 190	
Val Gln Val Glu Val Ser Gly Ser Gly Asp Lys Lys Pro Asp Gln Asn 195 200 205	
Phe Asp Phe Asn Lys Gly Tyr Thr Cys Asp Asn Lys Gly Leu His Phe 210 215 220	
Lys Ile Tyr Ile Gly Gln Asp Ser Gln Tyr Val Ala Pro Gly Pro Arg 225 230 235 240	
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Asp	Leu	Ser 35	Ser	Ala	Ala	Ile	Val 40	Cas	Asn	Thr	Gln	Gly 45	Ser	Lys	Ala	
	D-		D.						a.		.		mı	D1		
Ala	Pro 50	Asp	Рne	val	Arg	Ala 55	ALA	ALA	GTÀ	Asp	FÀS	ьeu	Thr	rne	ьеи	
Trp	Ala	His	Asp	Asn	Pro	Asp	Asp	Pro	Val	Asp	Tyr	Val	Leu	Asp	Pro	
65			-		70	-	-			75	-			•	80	
Ser	His	Lys	Gly		Ile	Leu	Thr	Tyr		Ala	Ala	Tyr	Pro		Gly	
				85					90					95		
Asp	Pro	Thr	Gly 100	Pro	Ile	Trp	Ser	Lys 105	Leu	Ala	Glu	Glu	Gly 110	Phe	Thr	
a.	G7	a.		7.7	m'	T 7	Ŧ.		T.7		7.	a.			**- 1	
GIY	GIY	115	Trp	Ala	Thr	iie	ьуs 120	Met	IIe	Aap	Asn	125	GIY	гуз	vai	
Asp	Val	Thr	Leu	Pro	Glu	Ala	Leu	Ala	Pro	Glv	Lvs	Tvr	Leu	Ile	Ara	
~ P	130					135				1	140	- 1 -			3	
	Glu	Leu	Leu	Ala	Leu	His	Arg	Ala	Asp		Ala	Cys	Asp	Asp		
145					150					155					160	
Ala	His	Pro	Asn	_	Gly	Ala	Glu	Ser	-	Pro	Asn	CAa	Val		Val	
				165					170					175		
Glu	Val	Ser	Gly 180	Ser	Gly	Asp	Lys	Lys 185	Pro	Asp	Gln	Asn	Phe 190	Asp	Phe	
Aan	Larc	G1++		Thr	Cys	Δar	Δαν		Gl v	Len	шіс	Dhe		T1e	Тугт	
ASII	пув	195	ıyr	1111	cyn	vsb	200	пув	GTÅ	ьeu	чта	205	пув	тте	түт	
Ile	Gly	Gln	Asp	Ser	Gln	Tyr	Val	Ala	Pro	Gly	Pro	Arg	Pro	Trp	Asn	
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gtca	tgaa	act a	atct:	egee.	ca t	gca	ccaat	ga (cgact	tgca	agt	cttt	caa q	gggcg	gacagc	120
ggca	acgt	ct q	gggt	caag	at c	gagc	agct	g gc	gtac	aacc	cgt	cagc	caa o	cccc	cctgg	180
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gtco	caago	geg a	aata	tata	ct g	egge	acga	g at	cctg	gggt	tgc.	acgt	ege a	aggaa	accgtg	300
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cago	etge	ect o	caaa.	tatt	gc g	ctcc	cagg	get	ttac	ggcc	cac	aaga	cga q	gggta	atcttg	420
gtcg	gactt	gt	ggag	ggtt	aa c	cagg	gcca	g gt	caact	taca	cgg	egeet	tgg a	aggad	ccgtt	480
tgga	gcga	aag o	cgtg	ggac	ac c	gagti	ttgg	gg:	gtcc	aaca	cga	ccga	gtg (egeca	accatg	540
ctcc	aca:	add t	tact:	agaa.	ta di	at.aa	aaa.	g as	caac	gadt	aus.	t.daa.	eta 1	gadd	gcctag	600
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Cys Lys Ser Phe Lys Gly Asp Ser Gly Asn Val Trp Val Lys Ile Glu 35 40 45	
Gln Leu Ala Tyr Asn Pro Ser Ala Asn Pro Pro Trp Ala Ser Asp Leu 50 55 60	
Leu Arg Glu His Gly Ala Lys Trp Lys Val Thr Ile Pro Pro Ser Leu 65 70 75 80	
Val Pro Gly Glu Tyr Leu Leu Arg His Glu Ile Leu Gly Leu His Val 85 90 95	
Ala Gly Thr Val Met Gly Ala Gln Phe Tyr Pro Gly Cys Thr Gln Ile 100 105 110	
Arg Val Thr Glu Gly Gly Ser Thr Gln Leu Pro Ser Gly Ile Ala Leu 115 120 125	
Pro Gly Ala Tyr Gly Pro Gln Asp Glu Gly Ile Leu Val Asp Leu Trp 130 135 140	
Arg Val Asn Gln Gly Gln Val Asn Tyr Thr Ala Pro Gly Gly Pro Val 145 150 155 160	
Trp Ser Glu Ala Trp Asp Thr Glu Phe Gly Gly Ser Asn Thr Thr Glu 165 170 175	
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cccggcgaat atctgctgcg gcacgagatc ctggggttgc acgtcgcagg aaccgtgatg	240
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ctgccctcgg gtattgcgct cccaggcgct tacggcccac aagacgaggg tatcttggtc	360
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Pro	Ser	Ala 35	Asn	Pro	Pro	Trp	Ala 40	Ser	Asp	Leu	Leu	Arg 45	Glu	His	Gly	
Ala	Lys 50	Trp	ГÀа	Val	Thr	Ile 55	Pro	Pro	Ser	Leu	Val 60	Pro	Gly	Glu	Tyr	
Leu 65	Leu	Arg	His	Glu	Ile 70	Leu	Gly	Leu	His	Val 75	Ala	Gly	Thr	Val	Met 80	
Gly	Ala	Gln	Phe	Tyr 85	Pro	Gly	Cys	Thr	Gln 90	Ile	Arg	Val	Thr	Glu 95	Gly	
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Pro	Gln	Asp 115	Glu	Gly	Ile	Leu	Val 120	Asp	Leu	Trp	Arg	Val 125	Asn	Gln	Gly	
Gln	Val 130	Asn	Tyr	Thr	Ala	Pro 135	Gly	Gly	Pro	Val	Trp 140	Ser	Glu	Ala	Trp	
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Asp	Asp	Leu	Leu	Asp 165	Tyr	Met	Ala	Ala	Asn 170	Asp	Asp	Pro	Сла	Cys 175	Thr	
Asp	Gln	Asn	Gln 180	Phe	Gly	Ser	Leu	Glu 185	Pro	Gly	Ser	Lys	Ala 190	Ala	Gly	
Gly	Ser	Pro 195	Ser	Leu	Tyr	Asp	Thr 200	Val	Leu	Val	Pro	Val 205	Leu	Gln	ГЛа	
Lys	Val 210	Pro	Thr	ГЛа	Leu	Gln 215	Trp	Ser	Gly	Pro	Ala 220	Ser	Val	Asn	Gly	
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aag	tagta	cca t	cctco	ccac	cc gg	ggaco	ccato	g gco	cttct	caca	ttg	ccaa	ggt 1	caaq	gccggc	300
cag	tagga	ccg (ccaco	ctgg	ga co	gtaa	aggg	gc gc	egtet	ggt	ccaa	agat	cca (ccago	gagatg	360
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atc	aaaa	get (gtate	gcag	ga co	gega	agtat	ctg	gctg	gtg	caga	agca	cat 1	gaa	ctccac	480
agc	gccgg	gca q	gccc	egge	gg cg	gecea	agtto	c tac	catt	ctt	gtg	ccca	gct (ctcaç	gtcacc	540
ggc	ggcag	geg (ggaco	ctgga	aa co	ccaç	ggaad	c aag	ggtgt	cgt	tcc	ccgg	ege (ctaca	aaggcc	600

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Val	Arg	Ile 35	Thr	Thr	Asn	Phe	Gln 40	Ser	Asn	Gly	Pro	Val 45	Thr	Asp	Val	
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ГÀа	Ser	Ser	Ile	Ser 85	His	Pro	Gly	Pro	Met 90	Ala	Phe	Tyr	Ile	Ala 95	Lys	
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Trp	Ser	Lys 115	Ile	His	Gln	Glu	Met 120	Pro	His	Phe	Gly	Thr 125	Ser	Leu	Thr	
Trp	Asp 130	Ser	Asn	Gly	Arg	Thr 135	Ser	Met	Pro	Val	Thr 140	Ile	Pro	Arg	Cys	
Leu 145	Gln	Asp	Gly	Glu	Tyr 150	Leu	Leu	Arg	Ala	Glu 155	His	Ile	Ala	Leu	His 160	
Ser	Ala	Gly	Ser	Pro 165	Gly	Gly	Ala	Gln	Phe 170	Tyr	Ile	Ser	Сув	Ala 175	Gln	
Leu	Ser	Val	Thr 180	Gly	Gly	Ser	Gly	Thr 185	Trp	Asn	Pro	Arg	Asn 190	Lys	Val	
Ser	Phe	Pro 195	Gly	Ala	Tyr	Lys	Ala 200	Thr	Asp	Pro	Gly	Ile 205	Leu	Ile	Asn	
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Asp 225	Thr	Сув														
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Ser	Asp	Gln 35	Ile	Arg	Cys	Tyr	Glu 40	Arg	Asn	Pro	Gly	Thr 45	Gly	Ala	Pro	
Gly	Ile	Tyr	Asn	Val	Thr	Ala	Gly	Thr	Thr	Ile	Asn	Tyr	Asn	Ala	Lys	

50 55 60	
Ser Ser Ile Ser His Pro Gly Pro Met Ala Phe Tyr Ile Ala Lys Val 65 70 75 80	
Pro Ala Gly Gln Ser Ala Ala Thr Trp Asp Gly Lys Gly Ala Val Trp 85 90 95	
Ser Lys Ile His Gln Glu Met Pro His Phe Gly Thr Ser Leu Thr Trp	
Asp Ser Asn Gly Arg Thr Ser Met Pro Val Thr Ile Pro Arg Cys Leu 115 120 125	
Gln Asp Gly Glu Tyr Leu Leu Arg Ala Glu His Ile Ala Leu His Ser 130 135 140	
Ala Gly Ser Pro Gly Gly Ala Gln Phe Tyr Ile Ser Cys Ala Gln Leu	
Ser Val Thr Gly Gly Ser Gly Thr Trp Asn Pro Arg Asn Lys Val Ser	
Phe Pro Gly Ala Tyr Lys Ala Thr Asp Pro Gly Ile Leu Ile Asn Ile	
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Ala Pro Gly Asn Tyr Leu Val Arg His Glu Leu Ile Ala Leu His Gln 145 150 150 160	
Ala Asn Asn Pro Gln Phe Tyr Pro Glu Cys Ala Gln Ile Lys Val Thr 165 170 175	
Gly Ser Gly Thr Ala Glu Pro Ala Ala Ser Tyr Lys Ala Ala Ile Pro 180 185 190	
Gly Tyr Cys Gln Gln Ser Asp Pro Asn Ile Ser Phe Asn Ile Asn Asp 195 200 205	
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cccagtatet accaeeeegg ecceatgeag ttetaeetgg eccgegttee ggaeggaeag	300
gacgtcaagt cgtggaccgg cgagggtgcc gtgtggttca aggtgtacga ggagcagcct	360
caatttggcg cccagctgac ctggcctagc aacggcaaga gctcgttcga ggttcctatc	420
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Asn Ser Glu Gln Ile Arg Cys Phe Gln Ala Thr Pro Ala Gly Ala Gln 50 55 60	

Asp Val Tyr Thr Val Gln Ala Gly Ser Thr Val Thr Tyr His Ala Asn

65					70					75					80
	_		_			~ 7	_		~ 7		_			_	
Pro	ser	IIe	Tyr	н1s 85	Pro	GIY	Pro	Met	90	Pne	Tyr	Leu	Ala	Arg 95	Val
Pro .	Asp	Gly	Gln 100	Asp	Val	ГÀЗ	Ser	Trp 105	Thr	Gly	Glu	Gly	Ala 110	Val	Trp
Phe	Lys	Val 115	Tyr	Glu	Glu	Gln	Pro 120	Gln	Phe	Gly	Ala	Gln 125	Leu	Thr	Trp
Pro	Ser 130	Asn	Gly	ГЛа	Ser	Ser 135	Phe	Glu	Val	Pro	Ile 140	Pro	Ser	Cys	Ile
Arg	Ala	Gly	Asn	Tyr	Leu 150	Leu	Arg	Ala	Glu	His 155	Ile	Ala	Leu	His	Val 160
Ala	Gln	Ser	Gln	Gly 165	Gly	Ala	Gln	Phe	Tyr 170	Ile	Ser	CAa	Ala	Gln 175	Leu
Gln	Val	Thr	Gly 180	Gly	Gly	Ser	Thr	Glu 185	Pro	Ser	Gln	Lys	Val 190	Ser	Phe
Pro	Gly	Ala 195	Tyr	Lys	Ser	Thr	Asp 200	Pro	Gly	Ile	Leu	Ile 205	Asn	Ile	Asn
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Ser	Glu	Gln 35	Ile	Arg	CAa	Phe	Gln 40	Ala	Thr	Pro	Ala	Gly 45	Ala	Gln	Asp
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Asp	Gly	Gln	Asp	Val 85	Lys	Ser	Trp	Thr	Gly 90	Glu	Gly	Ala	Val	Trp 95	Phe
Lys	Val	Tyr	Glu 100	Glu	Gln	Pro	Gln	Phe 105	Gly	Ala	Gln	Leu	Thr 110	Trp	Pro
Ser .	Asn	Gly 115	Lys	Ser	Ser	Phe	Glu 120	Val	Pro	Ile	Pro	Ser 125	Cha	Ile	Arg
Ala	Gly 130	Asn	Tyr	Leu	Leu	Arg 135	Ala	Glu	His	Ile	Ala 140	Leu	His	Val	Ala
Gln 145	Ser	Gln	Gly	Gly	Ala 150	Gln	Phe	Tyr	Ile	Ser 155	Сув	Ala	Gln	Leu	Gln 160
Val	Thr	Gly	Gly	Gly 165	Ser	Thr	Glu	Pro	Ser 170	Gln	Lys	Val	Ser	Phe 175	Pro
Gly	Ala	Tyr	Lys 180	Ser	Thr	Asp	Pro	Gly 185	Ile	Leu	Ile	Asn	Ile 190	Asn	Tyr

Pro	Val	Pro 195	Thr	Ser	Tyr	Gln	Asn 200	Pro	Gly	Pro	Ala	Val 205	Phe	Arg	CÀa	
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cggt	acat	tgc t	gag	cacca	ac co	ggat	egge	2 220	caaco	gaca	tcat	ggad	cag (cagco	cacaag	300
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Gln	Gly	Val 35	Arg	Val	Pro	Ser	Tyr 40	Asn	Gly	Pro	Ile	Glu 45	Asp	Val	Thr	
Ser	Asn 50	Ser	Ile	Ala	CAa	Asn 55	Gly	Pro	Pro	Asn	Pro 60	Thr	Thr	Pro	Thr	
Asn 65	Lys	Val	Ile	Thr	Val 70	Arg	Ala	Gly	Glu	Thr 75	Val	Thr	Ala	Val	Trp 80	
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Ala	Thr	Thr 115	Asp	Ser	Gly	Val	Gly 120	Gly	Gly	Trp	Phe	Lys 125	Ile	Gln	Glu	
Asp	Gly 130	Leu	Thr	Asn	Gly	Val 135	Trp	Gly	Thr	Glu	Arg 140	Val	Ile	Asn	Gly	
Gln 145	Gly	Arg	His	Asn	Ile 150	Lys	Ile	Pro	Glu	Сув 155	Ile	Ala	Pro	Gly	Gln 160	
Tyr	Leu	Leu	Arg	Ala 165	Glu	Met	Leu	Ala	Leu 170	His	Gly	Ala	Ser	Asn 175	Tyr	

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Trp Ala Glu Gly Glu Ala Gln Phe Tyr Pro Phe Pro Leu Phe Pro Phe 35 40 45	
Phe Pro Ser Leu Leu Ser Gly Asn Tyr Thr Ile Pro Gly Pro Ala 50 55 60	
Ile Trp Lys Cys Pro Glu Ala Gln Gln Asn Glu 65 70 75	
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5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	-

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gaco	gacgo	gca d	ccaco	ctcgg	gg ca	aagct	cgcc	c cgo	catco	gagc	gcgt	cta	gt	ccago	gacggc	900
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Thr 65	Glu	Asp	Gly	Ser	Lys 70	Ser	Сув	Ile	Thr	Ser 75	Ser	Gly	Val	Asp	Arg 80	
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Ser	Pro 130	Arg	Val	Tyr	Leu	Leu 135	Gly	Gly	Asp	Gly	Asn 140	Tyr	Val	Val	Leu	
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Pro	Cys	Gly	Glu	Asn 165	Ala	Ala	Leu	Tyr	Leu 170	Ser	Glu	Met	Asp	Ala 175	Thr	
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Tyr	Cys	Asp 195	Ala	Gln	CAa	Pro	Val 200	Gln	Asn	Trp	Asn	Asn 205	Gly	Thr	Leu	
Asn	Thr 210	Gly	Arg	Val	Gly	Ser 215	Cys	Cys	Asn	Glu	Met 220	Asp	Ile	Leu	Glu	

Ala Asn Ser Lys Ala Glu Ala Phe Thr Pro His Pro Cys Ile Gly Asn

225					230					235					240
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Thr	Ala	Thr	Thr 420	Thr	Ser	Ser	Gly	Pro 425	Ala	Glu	Pro	Thr	Gln 430	Thr	His
Tyr	Gly	Gln 435	CAa	Gly	Gly	Lys	Gly 440	Trp	Thr	Gly	Pro	Thr 445	Arg	Сла	Glu
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Lys	Gly	Ser	Asp	Gly	Ala	Ile	Asn	Ser 105	Val	Ser	Pro	Arg	Val 110	Tyr	Leu
Leu	Gly	Gly 115	Asp	Gly	Asn	Tyr	Val 120	Val	Leu	Lys	Leu	Leu 125	Gly	Gln	Glu

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Pro Val Gln Asn Trp Asn Asn Gly Thr Leu Asn Thr Gly Arg Val Gly 180 185 190
Ser Cys Cys Asn Glu Met Asp Ile Leu Glu Ala Asn Ser Lys Ala Glu 195 200 205
Ala Phe Thr Pro His Pro Cys Ile Gly Asn Ser Cys Asp Lys Ser Gly 210 215 220
Cys Gly Phe Asn Ala Tyr Ala Arg Gly Tyr His Asn Tyr Trp Ala Pro 225 230 235 240
Gly Gly Thr Leu Asp Thr Ser Arg Pro Phe Thr Met Ile Thr Arg Phe 245 250 255
Val Thr Asp Asp Gly Thr Thr Ser Gly Lys Leu Ala Arg Ile Glu Arg 260 265 270
Val Tyr Val Gln Asp Gly Lys Lys Val Pro Ser Ala Ala Pro Gly Gly 275 280 285
Asp Val Ile Thr Ala Asp Gly Cys Thr Ser Ala Gln Pro Tyr Gly Gly 290 295 300
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Ser Asn Gly Pro Cys Ser Asp Thr Glu Gly Asn Pro Ser Asn Ile Leu 340 345 350
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Thr Asp Cys Val Ser Gly Tyr His Cys Val Tyr Gln Asn Asp Trp Tyr 35 40 45

Ser Gln Cys Val Pro Gly Ala Ala Ser Thr Thr Leu Gln Thr Ser Thr 50 55 60

Thr Ser Arg Pro Thr Ala Thr Ser Thr Ala Pro Pro Ser Ser Thr Thr 65 70 75 80

Ser Pro Ser Lys Gly Lys Leu Lys Trp Leu Gly Ser Asn Glu Ser Gly 85 90 95

Ala Glu Phe Gly Glu Gly Asn Tyr Pro Gly Leu Trp Gly Lys His Phe $100 \,$ $105 \,$ $110 \,$

Ile Phe Pro Ser Thr Ser Ala Ile Gln Thr Leu Ile Asn Asp Gly Tyr 115 120 125

Asn Ile Phe Arg Ile Asp Phe Ser Met Glu Arg Leu Val Pro Asn Gln

Leu Thr Ser Ser Phe Asp Glu Gly Tyr Leu Arg Asn Leu Thr Glu Val 145 $$ 150 $$ 155 $$ 160

Val Asn Phe Val Thr Asn Ala Gly Lys Tyr Ala Val Leu Asp Pro His 165 170 175

Asn Tyr Gly Arg Tyr Tyr Gly Asn Val Ile Thr Asp Thr Asn Ala Phe \$180\$

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Val	Ile 210	Phe	Asp	Thr	Asn	Asn 215	Glu	Tyr	Asn	Thr	Met 220	Asp	Gln	Thr	Leu
Val 225	Leu	Asn	Leu	Asn	Gln 230	Ala	Ala	Ile	Asp	Gly 235	Ile	Arg	Ala	Ala	Gly 240
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Gln	Asn	Lys 275	Ile	Val	Tyr	Glu	Met 280	His	Gln	Tyr	Leu	Asp 285	Ser	Asp	Ser
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Thr	Gly	Leu	Leu 340	Asp	His	Leu	Gln	Asp 345	Asn	Ser	Glu	Val	Trp 350	Leu	Gly
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Thr Gly Leu Leu Asp His Leu Gln Asp Asn Ser Glu Val Trp Leu Gly 325 330 335	
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Arg	Glu	Ile	Ala	Ala 405	Lys	Gly	Thr	Val	Leu 410	Leu	Lys	Asn	Thr	Gly 415	Ser
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Trp Ala Glu Ala Tyr Ala Gln Ala Lys Ser Phe Val Ser Gln Met Thr 50 55 60

Leu Leu Glu Lys Val Asn Leu Thr Thr Gly Val Gly Trp Gly Ala Glu 65 $$ 70 $$ 75 $$ 80

Gln Cys Val Gly Gln Val Gly Ala Ile Pro Arg Leu Gly Leu Arg Ser \$90\$

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Pro	Glu	Ala 195	Gln	Gly	Tyr	Gly	Tyr 200	Asn	Ile	Ser	Glu	Thr 205	Leu	Ser	Ser
Asn	Ile 210	Asp	Asp	rys	Thr	Met 215	His	Glu	Leu	Tyr	Leu 220	Trp	Pro	Phe	Ala
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Gly	Asn	Asn	Ser	Tyr 245	Ala	Cys	Gln	Asn	Ser 250	Lys	Leu	Leu	Asn	Asp 255	Leu
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Ala	Gln	His 275	Thr	Gly	Ala	Ala	Ser 280	Ala	Val	Ala	Gly	Leu 285	Asp	Met	Ser
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Tyr 465	Glu	Ser	Val	Leu	Ser 470	Asn	Tyr	Ala	Glu	Glu 475	Asn	Thr	Lys	Ala	Leu 480
Val	Ser	Gln	Ala	Asn 485	Ala	Thr	Ala	Ile	Val 490	Phe	Val	Asn	Ala	Asp 495	Ser
Gly	Glu	Gly	Tyr 500	Ile	Asn	Val	Asp	Gly 505	Asn	Glu	Gly	Asp	Arg 510	Lys	Asn
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Trp	Cys 530	Ser	Asn	Thr	Ile	Val 535	Val	Ile	His	Ser	Val 540	Gly	Pro	Val	Leu
Leu 545	Thr	Asp	Trp	Tyr	Asp 550	Asn	Pro	Asn	Ile	Thr 555	Ala	Ile	Leu	Trp	Ala 560
Gly	Leu	Pro	Gly	Gln 565	Glu	Ser	Gly	Asn	Ser 570	Ile	Thr	Asp	Val	Leu 575	Tyr
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Arg	Glu	Ser 595	Tyr	Gly	Ala	Asp	Val 600	Leu	Tyr	Lys	Pro	Asn 605	Asn	Gly	Asn
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Ser	Asn	Val	Ser 660	Glu	Tyr	Arg	Pro	Thr 665	Thr	Gly	Thr	Thr	Ile 670	Gln	Ala
Pro	Thr	Phe 675	Gly	Asn	Phe	Ser	Thr 680	Asp	Leu	Glu	Asp	Tyr 685	Leu	Phe	Pro
ГÀа	Asp 690	Glu	Phe	Pro	Tyr	Ile 695	Pro	Gln	Tyr	Ile	Tyr 700	Pro	Tyr	Leu	Asn
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Ala	Glu	Glu	Phe	Leu 725	Pro	Pro	His	Ala	Thr 730	Asp	Asp	Asp	Pro	Gln 735	Pro
Leu	Leu	Arg	Ser 740	Ser	Gly	Gly	Asn	Ser 745	Pro	Gly	Gly	Asn	Arg 750	Gln	Leu
Tyr	Asp	Ile 755	Val	Tyr	Thr	Ile	Thr 760	Ala	Asp	Ile	Thr	Asn 765	Thr	Gly	Ser
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Pro	Gly	Glu	Thr	Arg 805	Gln	Phe	Thr	Gly	Arg 810	Leu	Thr	Arg	Arg	Asp 815	Leu
Ser	Asn	Trp	Asp 820	Val	Thr	Val	Gln	Asp 825	Trp	Val	Ile	Ser	Arg 830	Tyr	Pro
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Ala Val Val Leu Asp Ala Asn Trp Arg Trp Val His Asp Val Asn Gly

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Phe	Val	Thr 115	Gly	Ser	Asn	Val	Gly 120	Ser	Arg	Leu	Tyr	Leu 125	Leu	Gln	Asp
Asp	Ser 130	Thr	Tyr	Gln	Ile	Phe 135	Lys	Leu	Leu	Asn	Arg 140	Glu	Phe	Ser	Phe
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Lys	Ala	Gly	Ala 180	Lys	Tyr	Gly	Thr	Gly 185	Tyr	Cys	Asp	Ser	Gln 190	CÀa	Pro
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Pro	Ser 210	Ser	Asn	Asn	Ala	Asn 215	Thr	Gly	Ile	Gly	Asp 220	His	Gly	Ser	Cya
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Ser	Asp	Ile	Ser 340	Gly	Val	Thr	Gly	Asn 345	Ser	Ile	Thr	Thr	Glu 350	Phe	Cys
Thr	Ala	Gln 355	ГЛа	Gln	Ala	Phe	Gly 360	Asp	Thr	Asp	Asp	Phe 365	Ser	Gln	His
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Val 385	Met	Ser	Leu	Trp	390 Asp	Asp	Tyr	Ala	Ala	Gln 395	Met	Leu	Trp	Leu	Asp 400
Ser	Asp	Tyr	Pro	Thr 405	Asp	Ala	Asp	Pro	Thr 410	Thr	Pro	Gly	Ile	Ala 415	Arg
Gly	Thr	СЛа	Pro 420	Thr	Asp	Ser	Gly	Val 425	Pro	Ser	Asp	Val	Glu 430	Ser	Gln
Ser	Pro	Asn 435	Ser	Tyr	Val	Thr	Tyr 440	Ser	Asn	Ile	Lys	Phe 445	Gly	Pro	Ile
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Val	Leu	Asp 35	Ala	Asn	Trp	Arg	Trp 40	Val	His	Asp	Val	Asn 45	Gly	Tyr	Thr
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His 225	Pro	Сув	Asp	Thr	Pro 230	Gly	Gln	Thr	Met	Сув 235	Ser	Gly	Asp	Asp	Cys 240
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Pro	Gly	Lys 275	Ile	Ile	Asp	Thr	Thr 280	Lys	Pro	Phe	Thr	Val 285	Val	Thr	Gln
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Gln	Lys	Gln	Ala 340	Phe	Gly	Asp	Thr	Asp 345	Asp	Phe	Ser	Gln	His 350	Gly	Gly
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Tyr Pro Thr Asp Ala Asp Pro Thr Thr Pro Gly Ile Ala Arg Gly Thr 385 390 395 400	
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Thr 305	Val	Val	Thr	Gln	Phe 310	Leu	Lys	Asn	Ser	Ala 315	Gly	Glu	Leu	Ser	Glu 320
Ile	Lys	Arg	Phe	Tyr 325	Val	Gln	Asn	Gly	1330	Val	Ile	Pro	Asn	Ser 335	Glu
Ser	Thr	Ile	Pro 340	Gly	Val	Glu	Gly	Asn 345	Ser	Ile	Thr	Gln	Asp 350	Trp	Cys

Gly Gly Met Val Gln Met Gly Lys Ala Leu Ala Gly Pro Met Val Leu Val Met Ser Ile Trp Asp Asp His Ala Val Asn Met Leu Trp Leu Asp Ser Thr Trp Pro Ile Asp Gly Ala Gly Lys Pro Gly Ala Glu Arg Gly Ala Cys Pro Thr Thr Ser Gly Val Pro Ala Glu Val Glu Ala Glu Ala Pro Asn Ser Asn Val Ile Phe Ser Asn Ile Arg Phe Gly Pro Ile Gly Ser Thr Val Ser Gly Leu Pro Asp Gly Gly Ser Gly Asn Pro Asn Pro 455 Pro Val Ser Ser Ser Thr Pro Val Pro Ser Ser Ser Thr Thr Ser Ser 470 Gly Ser Ser Gly Pro Thr Gly Gly Thr Gly Val Ala Lys His Tyr Glu Gln Cys Gly Gly Ile Gly Phe Thr Gly Pro Thr Gln Cys Glu Ser Pro 505 Tyr Thr Cys Thr Lys Leu Asn Asp Trp Tyr Ser Gln Cys Leu <210> SEQ ID NO 129 <211> LENGTH: 509 <212> TYPE: PRT <213> ORGANISM: Myceliophthora thermophila <400> SEQUENCE: 129 Gln Asn Ala Cys Thr Leu Thr Ala Glu Asn His Pro Ser Leu Thr Tyr Ser Lys Cys Thr Ser Gly Gly Ser Cys Thr Ser Val Gln Gly Ser Ile Thr Ile Asp Ala Asn Trp Arg Trp Thr His Arg Thr Asp Ser Ala Thr Asn Cys Tyr Glu Gly Asn Lys Trp Asp Thr Ser Trp Cys Ser Asp Gly Pro Ser Cys Ala Ser Lys Cys Cys Ile Asp Gly Ala Asp Tyr Ser Ser 65 70 75 80 Thr Tyr Gly Ile Thr Thr Ser Gly Asn Ser Leu Asn Leu Lys Phe Val Thr Lys Gly Gln Tyr Ser Thr Asn Ile Gly Ser Arg Thr Tyr Leu Met Glu Ser Asp Thr Lys Tyr Gln Met Phe Gln Leu Leu Gly Asn Glu Phe 120 Thr Phe Asp Val Asp Val Ser Asn Leu Gly Cys Gly Leu Asn Gly Ala 135 Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Met Ser Lys Tyr Ser Gly Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Glu Ala Asn Val Glu Asn

Asp Arg Gln Lys Ala Ala Phe Gly Asp Val Thr Asp Phe Gln Asp Lys

60

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Ser Cys C	Cys Ser	Glu	Met	Asp 215	Val	Trp	Glu	Ala	Asn 220	Asn	Met	Ala	Ala
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Val Val 1 290	Thr Gln	Phe	Leu	Lуs 295	Asn	Ser	Ala	Gly	Glu 300	Leu	Ser	Glu	Ile
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Thr Trp E 385	Pro Ile		Gly 390	Ala	Gly	Lys	Pro	Gly 395	Ala	Glu	Arg	Gly	Ala 400
Cys Pro 1	Thr Thr	Ser 405	Gly	Val	Pro	Ala	Glu 410	Val	Glu	Ala	Glu	Ala 415	Pro
Asn Ser A	Asn Val 420		Phe	Ser	Asn	Ile 425	Arg	Phe	Gly	Pro	Ile 430	Gly	Ser
Thr Val S	Ser Gly 135	Leu	Pro	Asp	Gly 440	Gly	Ser	Gly	Asn	Pro 445	Asn	Pro	Pro
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Ser Ser 0 465	Gly Pro		Gly 470		Thr	Gly		Ala 475		His	Tyr	Glu	Gln 480
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Gly Pro Ser Cys Ala Ser Lys Cys Cys Ile Asp Gly Ala Asp Tyr Ser

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Val 385	Met	Ser	Ile	Trp	390	Asp	His	Ala	Val	Asn 395	Met	Leu	Trp	Leu	Asp 400
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Pro 465	Val	Ser	Ser	Ser	Thr 470	Pro	Val	Pro	Ser	Ser 475	Ser	Thr	Thr	Ser	Ser 480
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Gln	Cys	Gly	Gly 500	Ile	Gly	Phe	Thr	Gly 505	Pro	Thr	Gln	Cys	Glu 510	Ser	Pro
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CAa	Pro	Arg	Asp 180	Leu	Lys	Phe	Ile	Asn 185	Gly	Glu	Ala	Asn	Val 190	Glu	Asn
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Ser	Cys 210	Cys	Ser	Glu	Met	Asp 215	Val	Trp	Glu	Ala	Asn 220	Asn	Met	Ala	Ala
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Ile Thr Ile Asp Ala Asn Trp Arg Trp Thr His Arg Thr Asp Ser Ala 50 55 60	
Thr Asn Cys Tyr Glu Gly Asn Lys Trp Asp Thr Ser Tyr Cys Ser Asp 65 70 75 80	
Gly Pro Ser Cys Ala Ser Lys Cys Cys Ile Asp Gly Ala Asp Tyr Ser 85 90 95	
Ser Thr Tyr Gly Ile Thr Thr Ser Gly Asn Ser Leu Asn Leu Lys Phe 100 105 110	
Val Thr Lys Gly Gln Tyr Ser Thr Asn Ile Gly Ser Arg Thr Tyr Leu 115 120 125	
Met Glu Ser Asp Thr Lys Tyr Gln Met Phe Gln Leu Leu Gly Asn Glu 130 135 140	
Phe Thr Phe Asp Val Asp Val Ser Asn Leu Gly Cys Gly Leu Asn Gly 145 150 155 160	
Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Met Ser Lys Tyr 165 170 175	
Ser Gly Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser 180 185 190	
Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Glu Ala Asn Val Glu 195 200 205	

Asn Trp Gln Ser Ser Thr Asn Asp Ala Asn Ala Gly Thr Gly Lys Tyr

210 215 220

Gly 225	Ser	Cys	CÀa	Ser	Glu 230	Met	Asp	Val	Trp	Glu 235	Ala	Asn	Asn	Met	Ala 240
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Glu	Gly	Asp	Ser 260	Cys	Gly	Gly	Thr	Tyr 265	Ser	Thr	Asp	Arg	Tyr 270	Ala	Gly
Ile	Cys	Asp 275	Pro	Asp	Gly	CAa	Asp 280	Phe	Asn	Ser	Tyr	Arg 285	Gln	Gly	Asn
Lys	Thr 290	Phe	Tyr	Gly	Lys	Gly 295	Met	Thr	Val	Asp	Thr 300	Thr	Lys	Lys	Ile
Thr 305	Val	Val	Thr	Gln	Phe 310	Leu	Lys	Asn	Ser	Ala 315	Gly	Glu	Leu	Ser	Glu 320
Ile	Lys	Arg	Phe	Tyr 325	Val	Gln	Asn	Gly	330 Lys	Val	Ile	Pro	Asn	Ser 335	Glu
Ser	Thr	Ile	Pro 340	Gly	Val	Glu	Gly	Asn 345	Ser	Ile	Thr	Gln	Glu 350	Tyr	Cys
Asp	Arg	Gln 355	Lys	Ala	Ala	Phe	Gly 360	Asp	Val	Thr	Asp	Phe 365	Gln	Asp	Lys
Gly	Gly 370	Met	Val	Gln	Met	Gly 375	Lys	Ala	Leu	Ala	Gly 380	Pro	Met	Val	Leu
Val 385	Met	Ser	Ile	Trp	390	Asp	His	Ala	Asp	Asn 395	Met	Leu	Trp	Leu	Asp 400
Ser	Thr	Trp	Pro	Ile 405	Asp	Gly	Ala	Gly	Lys 410	Pro	Gly	Ala	Glu	Arg 415	Gly
Ala	Cys	Pro	Thr 420	Thr	Ser	Gly	Val	Pro 425	Ala	Glu	Val	Glu	Ala 430	Glu	Ala
Pro	Asn	Ser 435	Asn	Val	Ile	Phe	Ser 440	Asn	Ile	Arg	Phe	Gly 445	Pro	Ile	Gly
Ser	Thr 450	Val	Ser	Gly	Leu	Pro 455	Asp	Gly	Gly	Ser	Gly 460	Asn	Pro	Asn	Pro
Pro 465	Val	Ser	Ser	Ser	Thr 470	Pro	Val	Pro	Ser	Ser 475	Ser	Thr	Thr	Ser	Ser 480
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Gln	Cys	Gly	Gly 500	Ile	Gly	Phe	Thr	Gly 505	Pro	Thr	Gln	Cys	Glu 510	Ser	Pro
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)> SI					4		_		-	·				
					T	7	7.7 -	G7	7	TT# -	D	C	T e	ml	Was
Gln 1	Asn	Ala	cys	Thr 5	ьeu	Asn	Ala	GIU	Asn 10	HIS	Pro	ser	ьeu	Thr 15	Trp
Ser	ГЛа	СЛа	Thr 20	Ser	Gly	Gly	Ser	Сув 25	Thr	Ser	Val	Gln	Gly 30	Ser	Ile
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Thr	Tyr	Gly	Ile	Thr 85	Thr	Ser	Gly	Asn	Ser 90	Leu	Asn	Leu	Lys	Phe 95	Val
Thr	Lys	Gly	Gln 100	Tyr	Ser	Thr	Asn	Ile 105	Gly	Ser	Arg	Thr	Tyr 110	Leu	Met
Glu	Ser	Asp 115	Thr	Lys	Tyr	Gln	Met 120	Phe	Gln	Leu	Leu	Gly 125	Asn	Glu	Phe
Thr	Phe 130	Asp	Val	Asp	Val	Ser 135	Asn	Leu	Gly	Càa	Gly 140	Leu	Asn	Gly	Ala
Leu 145	Tyr	Phe	Val	Ser	Met 150	Asp	Ala	Asp	Gly	Gly 155	Met	Ser	TÀa	Tyr	Ser 160
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CÀa	Pro	Arg	Asp 180	Leu	Lys	Phe	Ile	Asn 185	Gly	Glu	Ala	Asn	Val 190	Glu	Asn
Trp	Gln	Ser 195	Ser	Thr	Asn	Asp	Ala 200	Asn	Ala	Gly	Thr	Gly 205	Lys	Tyr	Gly
Ser	Cys 210	Cys	Ser	Glu	Met	Asp 215	Val	Trp	Glu	Ala	Asn 220	Asn	Met	Ala	Ala
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Cys	Asp	Pro	Asp 260	Gly	Cys	Asp	Phe	Asn 265	Ser	Tyr	Arg	Gln	Gly 270	Asn	Lys
Thr	Phe	Tyr 275	Gly	Lys	Gly	Met	Thr 280	Val	Asp	Thr	Thr	Lys 285	Lys	Ile	Thr
Val	Val 290	Thr	Gln	Phe	Leu	Lys 295	Asn	Ser	Ala	Gly	Glu 300	Leu	Ser	Glu	Ile
305 Tàs	Arg	Phe	Tyr	Val	Gln 310	Asn	Gly	ГЛа	Val	Ile 315	Pro	Asn	Ser	Glu	Ser 320
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Arg	Gln	ГЛа	Ala 340	Ala	Phe	Gly	Asp	Val 345	Thr	Asp	Phe	Gln	Asp 350	Lys	Gly
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Cys	Pro	Thr	Thr	Ser 405	Gly	Val	Pro	Ala	Glu 410	Val	Glu	Ala	Glu	Ala 415	Pro
Asn	Ser	Asn	Val 420	Ile	Phe	Ser	Asn	Ile 425	Arg	Phe	Gly	Pro	Ile 430	Gly	Ser
Thr	Val	Ser 435	Gly	Leu	Pro	Asp	Gly 440	Gly	Ser	Gly	Asn	Pro 445	Asn	Pro	Pro

Val Ser Ser Ser Thr Pro Val Pro Ser Ser Ser Thr Thr Ser Ser Gly 450 455 460	
Ser Ser Gly Pro Thr Gly Gly Thr Gly Val Ala Lys His Tyr Glu Gln 465 470 475 480	
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gcccaacteg tegtetaega ceteecegae egtgaetgtg eegeegetge gtccaaegge	660
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gccaacatgg tgaccaacat gaacgtggcc aagtgcagca acgccgcgtc gacgtaccac	840
gagttgaccg tgtacgcgct caagcagctg aacctgccca acgtcgccat gtatctcgac	900
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geoggeatet acaatgatge eggeaageog getgeegtee geggeetgge cactaaegte	1020
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Gln	Cys	Gly 35	Gly	Asn	Gly	Trp	Gln 40	Gly	Pro	Thr	CÀa	Суз 45	Ala	Ser	Gly
Ser	Thr 50	Cys	Val	Ala	Gln	Asn 55	Glu	Trp	Tyr	Ser	Gln 60	CÀa	Leu	Pro	Asn
Ser 65	Gln	Val	Thr	Ser	Ser 70	Thr	Thr	Pro	Ser	Ser 75	Thr	Ser	Thr	Ser	Gln 80
Arg	Ser	Thr	Ser	Thr 85	Ser	Ser	Ser	Thr	Thr 90	Arg	Ser	Gly	Ser	Ser 95	Ser
Ser	Ser	Ser	Thr 100	Thr	Pro	Pro	Pro	Val 105	Ser	Ser	Pro	Val	Thr 110	Ser	Ile
Pro	Gly	Gly 115	Ala	Thr	Ser	Thr	Ala 120	Ser	Tyr	Ser	Gly	Asn 125	Pro	Phe	Ser
Gly	Val 130	Arg	Leu	Phe	Ala	Asn 135	Asp	Tyr	Tyr	Arg	Ser 140	Glu	Val	His	Asn
Leu 145	Ala	Ile	Pro	Ser	Met 150	Thr	Gly	Thr	Leu	Ala 155	Ala	ГÀв	Ala	Ser	Ala 160
Val	Ala	Glu	Val	Pro 165	Ser	Phe	Gln	Trp	Leu 170	Asp	Arg	Asn	Val	Thr 175	Ile
Asp	Thr	Leu	Met 180	Val	Gln	Thr	Leu	Ser 185	Gln	Val	Arg	Ala	Leu 190	Asn	Lys
Ala	Gly	Ala 195	Asn	Pro	Pro	Tyr	Ala 200	Ala	Gln	Leu	Val	Val 205	Tyr	Asp	Leu
Pro	Asp 210	Arg	Asp	Сла	Ala	Ala 215	Ala	Ala	Ser	Asn	Gly 220	Glu	Phe	Ser	Ile
Ala 225	Asn	Gly	Gly	Ala	Ala 230	Asn	Tyr	Arg	Ser	Tyr 235	Ile	Asp	Ala	Ile	Arg 240
ГÀа	His	Ile	Ile	Glu 245	Tyr	Ser	Asp	Ile	Arg 250	Ile	Ile	Leu	Val	Ile 255	Glu
Pro	Asp	Ser	Met 260	Ala	Asn	Met	Val	Thr 265	Asn	Met	Asn	Val	Ala 270	Lys	Cys
Ser	Asn	Ala 275	Ala	Ser	Thr	Tyr	His 280	Glu	Leu	Thr	Val	Tyr 285	Ala	Leu	ГÀа
Gln	Leu 290	Asn	Leu	Pro	Asn	Val 295	Ala	Met	Tyr	Leu	300	Ala	Gly	His	Ala
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Ala	Gly	Ile	Tyr	Asn 325	Asp	Ala	Gly	Lys	Pro 330	Ala	Ala	Val	Arg	Gly 335	Leu
Ala	Thr	Asn	Val 340	Ala	Asn	Tyr	Asn	Ala 345	Trp	Ser	Ile	Ala	Ser 350	Ala	Pro
Ser	Tyr	Thr 355	Ser	Pro	Asn	Pro	Asn 360	Tyr	Asp	Glu	Lys	His 365	Tyr	Ile	Glu
Ala	Phe 370	Ser	Pro	Leu	Leu	Asn 375	Ser	Ala	Gly	Phe	Pro 380	Ala	Arg	Phe	Ile
Val 385	Asp	Thr	Gly	Arg	Asn 390	Gly	Lys	Gln	Pro	Thr 395	Gly	Gln	Gln	Gln	Trp 400

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Gly	Gly	Glu 435	Ser	Asp	Gly	Thr	Ser 440	Asp	Thr	Ser	Ala	Ala 445	Arg	Tyr	Asp
Tyr	His 450	Cys	Gly	Leu	Ser	Asp 455	Ala	Leu	Gln	Pro	Ala 460	Pro	Glu	Ala	Gly
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Thr	Cys	Val 35	Ala	Gln	Asn	Glu	Trp 40	Tyr	Ser	Gln	Cys	Leu 45	Pro	Asn	Ser
Gln	Val 50	Thr	Ser	Ser	Thr	Thr 55	Pro	Ser	Ser	Thr	Ser 60	Thr	Ser	Gln	Arg
Ser 65	Thr	Ser	Thr	Ser	Ser 70	Ser	Thr	Thr	Arg	Ser 75	Gly	Ser	Ser	Ser	Ser 80
Ser	Ser	Thr	Thr	Pro 85	Pro	Pro	Val	Ser	Ser 90	Pro	Val	Thr	Ser	Ile 95	Pro
Gly	Gly	Ala	Thr 100	Ser	Thr	Ala	Ser	Tyr 105	Ser	Gly	Asn	Pro	Phe 110	Ser	Gly
Val	Arg	Leu 115	Phe	Ala	Asn	Asp	Tyr 120	Tyr	Arg	Ser	Glu	Val 125	His	Asn	Leu
Ala	Ile 130	Pro	Ser	Met	Thr	Gly 135	Thr	Leu	Ala	Ala	Lys 140	Ala	Ser	Ala	Val
145				Ser	150		_		_	155					160
Thr	Leu	Met	Val	Gln 165	Thr	Leu	Ser	Gln	Val 170	Arg	Ala	Leu	Asn	Lys 175	Ala
Gly	Ala	Asn	Pro 180	Pro	Tyr	Ala	Ala	Gln 185	Leu	Val	Val	Tyr	Asp 190	Leu	Pro
Asp	Arg	Asp 195	Cya	Ala	Ala	Ala	Ala 200	Ser	Asn	Gly	Glu	Phe 205	Ser	Ile	Ala
Asn	Gly 210	Gly	Ala	Ala	Asn	Tyr 215	Arg	Ser	Tyr	Ile	Asp 220	Ala	Ile	Arg	Lys
His 225	Ile	Ile	Glu	Tyr	Ser 230	Asp	Ile	Arg	Ile	Ile 235	Leu	Val	Ile	Glu	Pro 240
Asp	Ser	Met	Ala	Asn 245	Met	Val	Thr	Asn	Met 250	Asn	Val	Ala	Lys	Сув 255	Ser
Asn	Ala	Ala	Ser 260	Thr	Tyr	His	Glu	Leu 265	Thr	Val	Tyr	Ala	Leu 270	Lys	Gln

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Thr	Asn	Val	Ala	Asn 325	Tyr	Asn	Ala	Trp	Ser 330	Ile	Ala	Ser	Ala	Pro 335	Ser	
Tyr	Thr	Ser	Pro 340	Asn	Pro	Asn	Tyr	Asp 345	Glu	Lys	His	Tyr	Ile 350	Glu	Ala	
Phe	Ser	Pro 355	Leu	Leu	Asn	Ser	Ala 360	Gly	Phe	Pro	Ala	Arg 365	Phe	Ile	Val	
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Asp 385	Trp	Cys	Asn	Val	390 Lys	Gly	Thr	Gly	Phe	Gly 395	Val	Arg	Pro	Thr	Ala 400	
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Gly	Glu	Ser	Asp 420	Gly	Thr	Ser	Asp	Thr 425	Ser	Ala	Ala	Arg	Tyr 430	Asp	Tyr	
His	Cys	Gly 435	Leu	Ser	Asp	Ala	Leu 440	Gln	Pro	Ala	Pro	Glu 445	Ala	Gly	Gln	
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Ser Thr Cys Val Ala Gln Asn Glu Trp Tyr Ser Gln Cys Leu Pro Asn 50 55 60	
Ser Gln Val Thr Ser Ser Thr Thr Pro Ser Ser Thr Ser Thr Ser Gln	
Arg Ser Thr Ser Thr Ser Ser Ser Thr Thr Arg Ser Gly Ser Ser Ser	
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Ser Ser Ser Thr Thr Pro Thr Pro Val Ser Ser Pro Val Thr Ser Ile 100 105 110	
Pro Gly Gly Ala Thr Ser Thr Ala Ser Tyr Ser Gly Asn Pro Phe Ser 115 120 125	
Gly Val Arg Leu Phe Ala Asn Asp Tyr Tyr Arg Ser Glu Val His Asn 130 135 140	
Leu Ala Ile Pro Ser Met Thr Gly Thr Leu Ala Ala Lys Ala Ser Ala 145 150 155 160	
Val Ala Glu Val Pro Ser Phe Gln Trp Leu Asp Arg Asn Val Thr Ile 165 170 175	
Asp Thr Leu Met Val Pro Thr Leu Ser Arg Val Arg Ala Leu Asn Lys 180 185 190	
Ala Gly Ala Asn Pro Pro Tyr Ala Ala Gln Leu Val Val Tyr Asp Leu 195 200 205	

Lys	His	Ile	Ile	Glu 245	Tyr	Ser	Asp	Ile	Arg 250	Ile	Ile	Leu	Val	Ile 255	Glu
Pro	Asp	Ser	Met 260	Ala	Asn	Met	Val	Thr 265	Asn	Met	Asn	Val	Ala 270	Lys	Сув
Ser	Asn	Ala 275	Ala	Ser	Thr	Tyr	His 280	Glu	Leu	Thr	Val	Tyr 285	Ala	Leu	Lys
Gln	Leu 290	Asn	Leu	Pro	Asn	Val 295	Ala	Met	Tyr	Leu	300	Ala	Gly	His	Ala
Gly 305	Trp	Leu	Gly	Trp	Pro 310	Ala	Asn	Ile	Gln	Pro 315	Ala	Ala	Glu	Leu	Phe 320
Ala	Gly	Ile	Tyr	Asn 325	Asp	Ala	Gly	Lys	Pro 330	Ala	Ala	Val	Arg	Gly 335	Leu
Ala	Thr	Asn	Val 340	Ala	Asn	Tyr	Asn	Ala 345	Trp	Ser	Ile	Ala	Ser 350	Ala	Pro
Ser	Tyr	Thr 355	Ser	Pro	Asn	Pro	Asn 360	Tyr	Asp	Glu	Lys	His 365	Tyr	Ile	Glu
Ala	Phe 370	Ser	Pro	Leu	Leu	Asn 375	Ser	Ala	Gly	Phe	Pro 380	Ala	Arg	Phe	Ile
Val 385	Asp	Thr	Gly	Arg	Asn 390	Gly	ГÀв	Gln	Pro	Thr 395	Gly	Gln	Gln	Gln	Trp 400
Gly	Asp	Trp	Сув	Asn 405	Val	Lys	Gly	Thr	Gly 410	Phe	Gly	Val	Arg	Pro 415	Thr
Ala	Asn	Thr	Gly 420	His	Glu	Leu	Val	Asp 425	Ala	Phe	Val	Trp	Val 430	Lys	Pro
Gly	Gly	Glu 435	Ser	Asp	Gly	Thr	Ser 440	Asp	Thr	Ser	Ala	Ala 445	Arg	Tyr	Asp
Tyr	His 450	Сув	Gly	Leu	Ser	Asp 455	Ala	Leu	Gln	Pro	Ala 460	Pro	Glu	Ala	Gly
Gln 465	Trp	Phe	Gln	Ala	Tyr 470	Phe	Glu	Gln	Leu	Leu 475	Thr	Asn	Ala	Asn	Pro 480
Pro	Phe														
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Thr	Сув	Val 35	Ala	Gln	Asn	Glu	Trp 40	Tyr	Ser	Gln	CAa	Leu 45	Pro	Asn	Ser
Gln	Val 50	Thr	Ser	Ser	Thr	Thr 55	Pro	Ser	Ser	Thr	Ser 60	Thr	Ser	Gln	Arg
Ser 65	Thr	Ser	Thr	Ser	Ser 70	Ser	Thr	Thr	Arg	Ser 75	Gly	Ser	Ser	Ser	Ser 80
Ser	Ser	Thr	Thr	Pro	Thr	Pro	Val	Ser	Ser	Pro	Val	Thr	Ser	Ile	Pro

Ala Asn Gly Gly Ala Ala Asn Tyr Arg Ser Tyr Ile Asp Ala Ile Arg 225 230 235 240

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Val	Arg	Leu 115	Phe	Ala	Asn	Asp	Tyr 120	Tyr	Arg	Ser	Glu	Val 125	His	Asn	Leu
Ala	Ile 130	Pro	Ser	Met	Thr	Gly 135	Thr	Leu	Ala	Ala	Lys 140	Ala	Ser	Ala	Val
Ala 145	Glu	Val	Pro	Ser	Phe 150	Gln	Trp	Leu	Asp	Arg 155	Asn	Val	Thr	Ile	Asp 160
Thr	Leu	Met	Val	Pro 165	Thr	Leu	Ser	Arg	Val 170	Arg	Ala	Leu	Asn	Lys 175	Ala
Gly	Ala	Asn	Pro 180	Pro	Tyr	Ala	Ala	Gln 185	Leu	Val	Val	Tyr	Asp 190	Leu	Pro
Asp	Arg	Asp 195	CÀa	Ala	Ala	Ala	Ala 200	Ser	Asn	Gly	Glu	Phe 205	Ser	Ile	Ala
Asn	Gly 210	Gly	Ala	Ala	Asn	Tyr 215	Arg	Ser	Tyr	Ile	Asp 220	Ala	Ile	Arg	ГÀа
His 225	Ile	Ile	Glu	Tyr	Ser 230	Asp	Ile	Arg	Ile	Ile 235	Leu	Val	Ile	Glu	Pro 240
Asp	Ser	Met	Ala	Asn 245	Met	Val	Thr	Asn	Met 250	Asn	Val	Ala	Lys	Сув 255	Ser
Asn	Ala	Ala	Ser 260	Thr	Tyr	His	Glu	Leu 265	Thr	Val	Tyr	Ala	Leu 270	Lys	Gln
Leu	Asn	Leu 275	Pro	Asn	Val	Ala	Met 280	Tyr	Leu	Asp	Ala	Gly 285	His	Ala	Gly
Trp	Leu 290	Gly	Trp	Pro	Ala	Asn 295	Ile	Gln	Pro	Ala	Ala 300	Glu	Leu	Phe	Ala
Gly 305	Ile	Tyr	Asn	Asp	Ala 310	Gly	Lys	Pro	Ala	Ala 315	Val	Arg	Gly	Leu	Ala 320
Thr	Asn	Val	Ala	Asn 325	Tyr	Asn	Ala	Trp	Ser 330	Ile	Ala	Ser	Ala	Pro 335	Ser
Tyr	Thr	Ser	Pro 340	Asn	Pro	Asn	Tyr	Asp 345	Glu	Lys	His	Tyr	Ile 350	Glu	Ala
Phe	Ser	Pro 355	Leu	Leu	Asn	Ser	Ala 360	Gly	Phe	Pro	Ala	Arg 365	Phe	Ile	Val
Asp	Thr 370	Gly	Arg	Asn	Gly	Lys 375	Gln	Pro	Thr	Gly	Gln 380	Gln	Gln	Trp	Gly
385	Trp	Cys	Asn	Val	390	Gly	Thr	Gly	Phe	Gly 395	Val	Arg	Pro	Thr	Ala 400
Asn	Thr	Gly	His	Glu 405	Leu	Val	Asp	Ala	Phe 410	Val	Trp	Val	Lys	Pro 415	Gly
Gly	Glu	Ser	Asp 420	Gly	Thr	Ser	Asp	Thr 425	Ser	Ala	Ala	Arg	Tyr 430	Asp	Tyr
His	Сув	Gly 435	Leu	Ser	Asp	Ala	Leu 440	Gln	Pro	Ala	Pro	Glu 445	Ala	Gly	Gln
Trp	Phe 450	Gln	Ala	Tyr	Phe	Glu 455	Gln	Leu	Leu	Thr	Asn 460	Ala	Asn	Pro	Pro
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465

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<223> OTHER INFORMATION: Synthetic polynucleotide.
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tgcctgccca acagccaggt gacgagttcc accactccgt cgtcgacttc cacctcgcag
cqcaqcacca qcacctccaq caqcaccacc aqqaqcqqca qctcctcctc ctcctccacc
acgececege cegtetecag eccegtgace ageatteceg geggtgegac etceaeggeg
                                                                     360
agetactetg geaacceett etegggegte eggetetteg ceaacgaeta etacaggtee
                                                                     420
                                                                     480
gaggtccaca atctcgccat tcctagcatg actggtactc tggcggccaa ggcttccgcc
qtcqccqaaq tccctaqctt ccaqtqqctc qaccqqaacq tcaccatcqa caccctqatq
                                                                     540
qtcccqactc tqtcccqcqt ccqqqctctc aataaqqccq qtqccaatcc tccctatqct
                                                                     600
geceaacteg tegtetaega ceteceegae egtgaetgtg cegeegetge gtecaaegge
                                                                     660
                                                                     720
qaqttttcqa ttqcaaacqq cqqcqccqcc aactacaqqa qctacatcqa cqctatccqc
aagcacatca aggagtactc ggacatccgg atcatcctgg ttatcgagcc cgactcgatg
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gccaacatgg tgaccaacat gaacgtggcc aagtgcagca acgccgcgtc gacgtaccac
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gagttgaccg tgtacgcgct caagcagctg aacctgccca acgtcgccat gtatctcgac
                                                                     900
geeggeeaeg eeggetgget eggetggeee geeaaeatee ageeegeege egagetgttt
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geeggeatet acaatgatge eggeaageeg getgeegtee geggeetgge cactaaegte
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gccaactaca acgcctggag catcgcttcg gccccgtcgt acacgtcgcc taaccctaac
                                                                    1080
tacgacgaga agcactacat cgaggcette agecegetet tgaacgacge eggetteece
                                                                    1140
gcacgettea ttgtegaeae tggeegeaae ggeaaaeaae etaceggeea acaaeagtgg
                                                                    1200
ggtgactggt gcaatgtcaa gggcaccggc tttggcgtgc gcccgacggc caacacgggc
                                                                    1260
cacgagetgg tegatgeett tgtetgggte aageeeggeg gegagteega eggeacaage
gacaccageg cegecegeta egactaceae tgeggeetgt cegatgeeet geageetgee
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<223> OTHER INFORMATION: Synthetic polypeptides.
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40

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Arg	Ser	Thr	Ser	Thr 85	Ser	Ser	Ser	Thr	Thr 90	Arg	Ser	Gly	Ser	Ser 95	Ser
Ser	Ser	Ser	Thr 100	Thr	Pro	Pro	Pro	Val 105	Ser	Ser	Pro	Val	Thr 110	Ser	Ile
Pro	Gly	Gly 115	Ala	Thr	Ser	Thr	Ala 120	Ser	Tyr	Ser	Gly	Asn 125	Pro	Phe	Ser
Gly	Val 130	Arg	Leu	Phe	Ala	Asn 135	Asp	Tyr	Tyr	Arg	Ser 140	Glu	Val	His	Asn
Leu 145	Ala	Ile	Pro	Ser	Met 150	Thr	Gly	Thr	Leu	Ala 155	Ala	Lys	Ala	Ser	Ala 160
Val	Ala	Glu	Val	Pro 165	Ser	Phe	Gln	Trp	Leu 170	Asp	Arg	Asn	Val	Thr 175	Ile
Asp	Thr	Leu	Met 180	Val	Pro	Thr	Leu	Ser 185	Arg	Val	Arg	Ala	Leu 190	Asn	ГЛа
Ala	Gly	Ala 195	Asn	Pro	Pro	Tyr	Ala 200	Ala	Gln	Leu	Val	Val 205	Tyr	Asp	Leu
Pro	Asp 210	Arg	Asp	Cys	Ala	Ala 215	Ala	Ala	Ser	Asn	Gly 220	Glu	Phe	Ser	Ile
Ala 225	Asn	Gly	Gly	Ala	Ala 230	Asn	Tyr	Arg	Ser	Tyr 235	Ile	Asp	Ala	Ile	Arg 240
ГÀа	His	Ile	Lys	Glu 245	Tyr	Ser	Asp	Ile	Arg 250	Ile	Ile	Leu	Val	Ile 255	Glu
Pro	Asp	Ser	Met 260	Ala	Asn	Met	Val	Thr 265	Asn	Met	Asn	Val	Ala 270	Lys	CÀa
Ser	Asn	Ala 275	Ala	Ser	Thr	Tyr	His 280	Glu	Leu	Thr	Val	Tyr 285	Ala	Leu	Lys
Gln	Leu 290	Asn	Leu	Pro	Asn	Val 295	Ala	Met	Tyr	Leu	Asp	Ala	Gly	His	Ala
Gly 305	Trp	Leu	Gly	Trp	Pro 310	Ala	Asn	Ile	Gln	Pro 315	Ala	Ala	Glu	Leu	Phe 320
Ala	Gly	Ile	Tyr	Asn 325	Asp	Ala	Gly	Lys	Pro 330	Ala	Ala	Val	Arg	Gly 335	Leu
Ala	Thr	Asn	Val 340	Ala	Asn	Tyr	Asn	Ala 345	Trp	Ser	Ile	Ala	Ser 350	Ala	Pro
Ser	Tyr	Thr 355	Ser	Pro	Asn	Pro	Asn 360	Tyr	Asp	Glu	Lys	His 365	Tyr	Ile	Glu
Ala	Phe 370	Ser	Pro	Leu	Leu	Asn 375	Asp	Ala	Gly	Phe	Pro 380	Ala	Arg	Phe	Ile
Val 385	Asp	Thr	Gly	Arg	Asn 390	Gly	Lys	Gln	Pro	Thr 395	Gly	Gln	Gln	Gln	Trp 400
Gly	Asp	Trp	Cys	Asn 405	Val	Lys	Gly	Thr	Gly 410	Phe	Gly	Val	Arg	Pro 415	Thr
Ala	Asn	Thr	Gly 420	His	Glu	Leu	Val	Asp 425	Ala	Phe	Val	Trp	Val 430	Lys	Pro
Gly	Gly	Glu 435	Ser	Asp	Gly	Thr	Ser 440	Asp	Thr	Ser	Ala	Ala 445	Arg	Tyr	Asp

Tyr	His 450	Cys	Gly	Leu	Ser	Asp 455	Ala	Leu	Gln	Pro	Ala 460	Pro	Glu	Ala	Gly
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Pro	Phe														
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Thr	Cys	Val 35	Ala	Gln	Asn	Glu	Trp 40	Tyr	Ser	Gln	CÀa	Leu 45	Pro	Asn	Ser
Gln	Val 50	Thr	Ser	Ser	Thr	Thr 55	Pro	Ser	Ser	Thr	Ser 60	Thr	Ser	Gln	Arg
Ser 65	Thr	Ser	Thr	Ser	Ser 70	Ser	Thr	Thr	Arg	Ser 75	Gly	Ser	Ser	Ser	Ser 80
Ser	Ser	Thr	Thr	Pro 85	Pro	Pro	Val	Ser	Ser 90	Pro	Val	Thr	Ser	Ile 95	Pro
Gly	Gly	Ala	Thr 100	Ser	Thr	Ala	Ser	Tyr 105	Ser	Gly	Asn	Pro	Phe 110	Ser	Gly
Val	Arg	Leu 115	Phe	Ala	Asn	Asp	Tyr 120	Tyr	Arg	Ser	Glu	Val 125	His	Asn	Leu
Ala	Ile 130	Pro	Ser	Met	Thr	Gly 135	Thr	Leu	Ala	Ala	Lys 140	Ala	Ser	Ala	Val
Ala 145	Glu	Val	Pro	Ser	Phe 150	Gln	Trp	Leu	Asp	Arg 155	Asn	Val	Thr	Ile	Asp 160
Thr	Leu	Met	Val	Pro 165	Thr	Leu	Ser	Arg	Val 170	Arg	Ala	Leu	Asn	Lys 175	Ala
Gly	Ala	Asn	Pro 180	Pro	Tyr	Ala	Ala	Gln 185	Leu	Val	Val	Tyr	Asp 190	Leu	Pro
Asp	_	Asp 195	_							_				Ile	Ala
Asn	Gly 210	Gly	Ala	Ala	Asn	Tyr 215	Arg	Ser	Tyr	Ile	Asp 220	Ala	Ile	Arg	Lys
His 225	Ile	Lys	Glu	Tyr	Ser 230	Asp	Ile	Arg	Ile	Ile 235	Leu	Val	Ile	Glu	Pro 240
Asp	Ser	Met	Ala	Asn 245	Met	Val	Thr	Asn	Met 250	Asn	Val	Ala	Lys	Сув 255	Ser
Asn	Ala	Ala	Ser 260	Thr	Tyr	His	Glu	Leu 265	Thr	Val	Tyr	Ala	Leu 270	Lys	Gln
Leu	Asn	Leu 275	Pro	Asn	Val	Ala	Met 280	Tyr	Leu	Asp	Ala	Gly 285	His	Ala	Gly
Trp	Leu 290	Gly	Trp	Pro	Ala	Asn 295	Ile	Gln	Pro	Ala	Ala 300	Glu	Leu	Phe	Ala
Gly	Ile	Tyr	Asn	Aap	Ala	Gly	Lys	Pro	Ala	Ala	Val	Arg	Gly	Leu	Ala

Concinuca	
305 310 315 32	20
Thr Asn Val Ala Asn Tyr Asn Ala Trp Ser Ile Ala Ser Ala Pro Se 325 330 335	er
Tyr Thr Ser Pro Asn Pro Asn Tyr Asp Glu Lys His Tyr Ile Glu Al	La
Phe Ser Pro Leu Leu Asn Asp Ala Gly Phe Pro Ala Arg Phe Ile Va 355 360 365	al
Asp Thr Gly Arg Asn Gly Lys Gln Pro Thr Gly Gln Gln Gln Trp Gl 370 375 380	Ly
Asp Trp Cys Asn Val Lys Gly Thr Gly Phe Gly Val Arg Pro Thr Al 385 390 395 40	
Asn Thr Gly His Glu Leu Val Asp Ala Phe Val Trp Val Lys Pro Gl	ц
Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser Ala Ala Arg Tyr Asp Ty 420 425 430	yr
His Cys Gly Leu Ser Asp Ala Leu Gln Pro Ala Pro Glu Ala Gly Gl 435 440 445	Ln
Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu Thr Asn Ala Asn Pro Pr 450 455 460	co
Phe 465	
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ggtcccacat gctgcgcctc gggctcgacc tgcgttgcgc agaacgagtg gtactct	cag 180
tgcctgccca acagccaggt gacgagttcc accactccgt cgtcgacttc cacctcg	gcag 240
egeageacea geacetecag cageaceace aggageggea getectecte etecte	cacc 300
acgcccaccc ccgtctccag ccccgtgacc agcattcccg gcggtgcgac ctccacg	ggcg 360
agetactetg geaacceett etegggegte eggetetteg ecaaegaeta etaeage	gtee 420
gaggtcatga atctcgccat tcctagcatg actggtactc tggcggccaa ggcttcc	ggc 480
gtcgccgaag tccctagctt ccagtggctc gaccggaacg tcaccatcga caccctg	gatg 540
gtcaccactc tgtcccaggt ccgggctctc aataaggccg gtgccaatcc tccctat	get 600
gcccaactcg togtctacga cotccccgac cgtgactgtg ccgccgctgc gtccaac	cggc 660
gagttttcga ttgcaaacgg cggcagcgcc aactacagga gctacatcga cgctatc	ccgc 720
aagcacatca ttgagtactc ggacatccgg atcatcctgg ttatcgagcc cgactcg	gatg 780
gccaacatgg tgaccaacat gaacgtggcc aagtgcagca acgccgcgtc gacgtac	ccac 840
gagttgaccg tgtacgcgct caagcagctg aacctgccca acgtcgccat gtatctc	cgac 900
geoggecaeg ceggetgget eggetggeee gecaacatee ageoegeege egagetg	gttt 960

gccggcatct acaatgatgc cggcaagccg gctgccgtcc gcggcctggc cactaacgtc 1020

gccaactaca acgcctggag catcgcttcg gccccgtcgt acacgcagcc taaccctaac	1080
tacgacgaga agcactacat cgaggcette agcccgetet tgaactegge eggetteece	1140
gcacgettca ttgtcgacac tggccgcaac ggcaaacaac ctaccggcca acaacagtgg	1200
ggtgactggt gcaatgtcaa gggcaccggc tttggcgtgc gcccgacggc caacacgggc	1260
cacgagetgg tegatgeett tgtetgggte aageeeggeg gegagteega eggeacaage	1320
gacaccageg eegecegeta egactaceae tgeggeetgt eegatgeeet geageetgee	1380
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Gln Cys Gly Gly Asn Gly Trp Gln Gly Pro Thr Cys Cys Ala Ser Gly 35 40 45	
Ser Thr Cys Val Ala Gln Asn Glu Trp Tyr Ser Gln Cys Leu Pro Asn 50 55 60	
Ser Gln Val Thr Ser Ser Thr Thr Pro Ser Ser Thr Ser Thr Ser Gln 65 70 80	
Arg Ser Thr Ser Thr Ser Ser Ser Thr Thr Arg Ser Gly Ser Ser Ser 85 90 95	
Ser Ser Ser Thr Thr Pro Thr Pro Val Ser Ser Pro Val Thr Ser Ile 100 105 110	
Pro Gly Gly Ala Thr Ser Thr Ala Ser Tyr Ser Gly Asn Pro Phe Ser 115 120 125	
Gly Val Arg Leu Phe Ala Asn Asp Tyr Tyr Arg Ser Glu Val Met Asn 130 135 140	
Leu Ala Ile Pro Ser Met Thr Gly Thr Leu Ala Ala Lys Ala Ser Ala 145 150 160	
Val Ala Glu Val Pro Ser Phe Gln Trp Leu Asp Arg Asn Val Thr Ile 165 170 175	
Asp Thr Leu Met Val Thr Thr Leu Ser Gln Val Arg Ala Leu Asn Lys 180 185 190	
Ala Gly Ala Asn Pro Pro Tyr Ala Ala Gln Leu Val Val Tyr Asp Leu 195 200 205	
Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly Glu Phe Ser Ile 210 215 220	
Ala Asn Gly Gly Ser Ala Asn Tyr Arg Ser Tyr Ile Asp Ala Ile Arg 225 230 240	
Lys His Ile Ile Glu Tyr Ser Asp Ile Arg Ile Ile Leu Val Ile Glu 245 250 255	

Pro Asp Ser Met Ala Asn Met Val Thr Asn Met Asn Val Ala Lys Cys 260 270

280 Gln Leu Asn Leu Pro Asn Val Ala Met Tyr Leu Asp Ala Gly His Ala Gly Trp Leu Gly Trp Pro Ala Asn Ile Gln Pro Ala Ala Glu Leu Phe Ala Gly Ile Tyr Asn Asp Ala Gly Lys Pro Ala Ala Val Arg Gly Leu Ala Thr Asn Val Ala Asn Tyr Asn Ala Trp Ser Ile Ala Ser Ala Pro Ser Tyr Thr Gln Pro Asn Pro Asn Tyr Asp Glu Lys His Tyr Ile Glu Ala Phe Ser Pro Leu Leu Asn Ser Ala Gly Phe Pro Ala Arg Phe Ile 375 Val Asp Thr Gly Arg Asn Gly Lys Gln Pro Thr Gly Gln Gln Gln Trp 390 395 Gly Asp Trp Cys Asn Val Lys Gly Thr Gly Phe Gly Val Arg Pro Thr Ala Asn Thr Gly His Glu Leu Val Asp Ala Phe Val Trp Val Lys Pro 425 Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser Ala Ala Arg Tyr Asp 440 Tyr His Cys Gly Leu Ser Asp Ala Leu Gln Pro Ala Pro Glu Ala Gly 455 Gln Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu Thr Asn Ala Asn Pro 470 Pro Phe <210> SEQ ID NO 147 <211> LENGTH: 465 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Synthetic polypeptides. <400> SEQUENCE: 147 Ala Pro Val Ile Glu Glu Arg Gln Asn Cys Gly Ala Val Trp Thr Gln Cys Gly Gly Asn Gly Trp Gln Gly Pro Thr Cys Cys Ala Ser Gly Ser Thr Cys Val Ala Gln Asn Glu Trp Tyr Ser Gln Cys Leu Pro Asn Ser Gln Val Thr Ser Ser Thr Thr Pro Ser Ser Thr Ser Thr Ser Gln Arg 55 Ser Thr Ser Thr Ser Ser Ser Thr Thr Arg Ser Gly Ser Ser Ser Ser Ser Thr Thr Pro Thr Pro Val Ser Ser Pro Val Thr Ser Ile Pro 90 Gly Gly Ala Thr Ser Thr Ala Ser Tyr Ser Gly Asn Pro Phe Ser Gly 105 Val Arg Leu Phe Ala Asn Asp Tyr Tyr Arg Ser Glu Val Met Asn Leu 120

Ser Asn Ala Ala Ser Thr Tyr His Glu Leu Thr Val Tyr Ala Leu Lys

Ala		Pro	Ser	Met	Thr		Thr	Leu	Ala	Ala		Ala	Ser	Ala	Val
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Ala 145	Glu	Val	Pro	Ser	Phe 150	Gln	Trp	Leu	Asp	Arg 155	Asn	Val	Thr	Ile	Asp 160
Thr	Leu	Met	Val	Thr 165	Thr	Leu	Ser	Gln	Val 170	Arg	Ala	Leu	Asn	Lys 175	Ala
Gly	Ala	Asn	Pro 180	Pro	Tyr	Ala	Ala	Gln 185	Leu	Val	Val	Tyr	Asp 190	Leu	Pro
Asp	Arg	Asp 195	Суз	Ala	Ala	Ala	Ala 200	Ser	Asn	Gly	Glu	Phe 205	Ser	Ile	Ala
Asn	Gly 210	Gly	Ser	Ala	Asn	Tyr 215	Arg	Ser	Tyr	Ile	Asp 220	Ala	Ile	Arg	Lys
His 225	Ile	Ile	Glu	Tyr	Ser 230	Asp	Ile	Arg	Ile	Ile 235	Leu	Val	Ile	Glu	Pro 240
Asp	Ser	Met	Ala	Asn 245	Met	Val	Thr	Asn	Met 250	Asn	Val	Ala	Lys	Сув 255	Ser
Asn	Ala	Ala	Ser 260	Thr	Tyr	His	Glu	Leu 265	Thr	Val	Tyr	Ala	Leu 270	Lys	Gln
Leu	Asn	Leu 275	Pro	Asn	Val	Ala	Met 280	Tyr	Leu	Asp	Ala	Gly 285	His	Ala	Gly
Trp	Leu 290	Gly	Trp	Pro	Ala	Asn 295	Ile	Gln	Pro	Ala	Ala 300	Glu	Leu	Phe	Ala
Gly 305	Ile	Tyr	Asn	Asp	Ala 310	Gly	Lys	Pro	Ala	Ala 315	Val	Arg	Gly	Leu	Ala 320
Thr	Asn	Val	Ala	Asn 325	Tyr	Asn	Ala	Trp	Ser 330	Ile	Ala	Ser	Ala	Pro 335	Ser
Tyr	Thr	Gln	Pro 340	Asn	Pro	Asn	Tyr	Asp 345	Glu	Lys	His	Tyr	Ile 350	Glu	Ala
Phe	Ser	Pro 355	Leu	Leu	Asn	Ser	Ala 360	Gly	Phe	Pro	Ala	Arg 365	Phe	Ile	Val
Asp	Thr 370	Gly	Arg	Asn	Gly	Lys 375	Gln	Pro	Thr	Gly	Gln 380	Gln	Gln	Trp	Gly
Asp 385	Trp	Сув	Asn	Val	Lys 390	Gly	Thr	Gly	Phe	Gly 395	Val	Arg	Pro	Thr	Ala 400
Asn	Thr	Gly	His	Glu 405	Leu	Val	Asp	Ala	Phe 410	Val	Trp	Val	Lys	Pro 415	Gly
Gly	Glu	Ser	Asp 420	Gly	Thr	Ser	Asp	Thr 425	Ser	Ala	Ala	Arg	Tyr 430	Asp	Tyr
His	Cys	Gly 435	Leu	Ser	Asp	Ala	Leu 440	Gln	Pro	Ala	Pro	Glu 445	Ala	Gly	Gln
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Phe 465															
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		rgan. Equei		•	≈±±0]	PII L FI	∍⊥a 1	erī	"obu:	тта					
		~													

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Gly Gln Leu Asn Asp Leu Ala Val Arg Ala Gly Leu Lys Tyr Phe Gly $20 \\ 25 \\ 30$

Thr Ala Leu Ser Glu Ser Val Ile Asn Ser Asp Thr Arg Tyr Ala Ala 35 404045

Ile Leu Ser Asp Lys Ser Met Phe Gly Gln Leu Val Pro Glu Asn Gly

Met Lys Trp Asp Ala Thr Glu Pro Ser Arg Gly Gln Phe Asn Tyr Ala 65 70 75 80

Ser Gly Asp Ile Thr Ala Asn Thr Ala Lys Lys Asn Gly Gln Gly Met

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Ser Gly Ser Trp Thr Arg Asp Ser Leu Thr Ser Val Ile Glu Thr His

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Val Ile Asn Glu Ala Ile Asn Asp Asp Gly Asn Ser Trp Arg Asp Asn

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Gly 225	His	Leu	Ile	Val	Gly 230	Ser	Thr	Pro	Thr	Arg 235	Ser	Gln	Leu	Ala	Thr 240
Ala	Leu	Gln	Arg	Phe 245	Thr	Ala	Leu	Gly	Leu 250	Glu	Val	Ala	Tyr	Thr 255	Glu
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Thr	Gln	Gly 275	Asn	Asp	Phe	Ala	Asn 280	Val	Val	Gly	Ser	Сув 285	Leu	Asp	Thr
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Trp 305	Ile	Pro	Asn	Thr	Phe 310	Pro	Gly	Gln	Gly	Asp 315	Ala	Leu	Ile	Tyr	Asp 320
Ser	Asn	Tyr	Asn	Lys 325	Lys	Pro	Ala	Trp	Thr 330	Ser	Ile	Ser	Ser	Val 335	Leu
Ala	Ala	ГЛа	Ala 340	Thr	Gly	Ala	Pro	Pro 345	Ala	Ser	Ser	Ser	Thr 350	Thr	Leu
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Ser	Ser 370	Ala	Thr	Pro	Thr	Ser 375	Val	Pro	Thr	Gln	Thr 380	Arg	Trp	Gly	Gln
Cys 385	Gly	Gly	Ile	Gly	Trp 390	Thr	Gly	Pro	Thr	Gln 395	CÀa	Glu	Ser	Pro	Trp 400
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	Leu				Ala	Val	Arg	Ala	Gly 10	Leu	Lys	Tyr	Phe	Gly 15	Thr
	Leu	Ser			Val	Ile	Asn			Thr	Arg	Tyr			Ile
т	C.s.	7 ~~	20	Con	Mot	Db.	C1	25 Cln	Lon	T7~7	D	G1	30 3an	C1	Mot
	Ser	35	•				40					45		•	
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Ile As		ı Ala	Ile	Asn	Asp 135	Asp	Gly	Asn	Ser	Trp 140	Arg	Asp	Asn	Val	
Phe Le 145	u Arg	J Thr	Phe	Gly 150	Thr	Asp	Tyr	Phe	Ala 155	Leu	Ser	Phe	Asn	Leu 160	
Ala Ly	a Lys	8 Ala	Asp 165	Pro	Asp	Thr	Lys	Leu 170	Tyr	Tyr	Asn	Asp	Tyr 175	Asn	
Leu Gl	u Tyr	180	Gln	Ala	Lys	Thr	Asp 185	Arg	Ala	Val	Glu	Leu 190	Val	Lys	
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Leu Gl 225	n Arç	y Phe	Thr	Ala 230	Leu	Gly	Leu	Glu	Val 235	Ala	Tyr	Thr	Glu	Leu 240	
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Gln Gl	y Asr	1 Asp 260		Ala	Asn	Val	Val 265	Gly	Ser	Cys	Leu	Asp 270	Thr	Ala	
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Ile Pr 29		n Thr	Phe	Pro	Gly 295	Gln	Gly	Asp	Ala	Leu 300	Ile	Tyr	Asp	Ser	
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Thr Il	e Thi	Thr 340	Pro	Pro	Pro	Ala	Ser 345	Thr	Thr	Ala	Ser	Ser 350	Ser	Ser	
Ser Al	a Thi 355		Thr	Ser	Val	Pro 360	Thr	Gln	Thr	Arg	Trp 365	Gly	Gln	СЛа	
Gly Gl 37		e Gly	Trp	Thr	Gly 375	Pro	Thr	Gln	Сув	Glu 380	Ser	Pro	Trp	Thr	
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Arg Leu Gly Thr Ile Glu Asp Asp Gly Gly Val Tyr Asp Ile Tyr Lys 130 135 140								
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Thr Gly Lys His Phe Asp Glu Trp Lys Arg Gln Gly Asn Leu Gln Leu 180 185 190								
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His Thr Leu Leu Trp His Ser Gln Leu Pro Gln Trp Val Gln Asn Ile 165 170 175	
Asn Asp Arg Asn Thr Leu Thr Gln Val Ile Glu Asn His Val Thr Thr 180 185 190	
Leu Val Thr Arg Tyr Lys Gly Lys Ile Leu His Trp Asp Val Val Asn 195 200 205	
Glu Ile Phe Ala Glu Asp Gly Ser Leu Arg Asp Ser Val Phe Ser Arg 210 215 220	
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Ala Asn Tyr Ala Lys Val Thr Arg Gly Met Val Glu Lys Val Asn Lys 260 265 270	
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Leu Ala Gly Pro Gly Gly Trp Asn Thr Ala Ala Gly Val Pro Asp Ala 290 295 300	
Leu Lys Ala Leu Ala Ala Asn Val Lys Glu Ile Ala Ile Thr Glu 305 310 315 320	
Leu Asp Ile Ala Gly Ala Ser Ala Asn Asp Tyr Leu Thr Val Met Asn	

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				325					330					335	
Ala	Сув	Leu	Gln 340	Val	Ser	Lys	Сув	Val 345	Gly	Ile	Thr	Val	Trp 350	Gly	Val
Ser	Asp	Lys 355	Asp	Ser	Trp	Arg	Ser 360	Ser	Ser	Asn	Pro	Leu 365	Leu	Phe	Asp
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Ala	Val 130	Val	Asn	Phe	Ala	Gln 135	Ala	Asn	Gly	Lys	Leu 140	Ile	Arg	Gly	His
Thr 145	Leu	Leu	Trp	His	Ser 150	Gln	Leu	Pro	Gln	Trp 155	Val	Gln	Asn	Ile	Asn 160
Asp	Arg	Asn	Thr	Leu 165	Thr	Gln	Val	Ile	Glu 170	Asn	His	Val	Thr	Thr 175	Leu
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Leu	Gly 210	Glu	Asp	Phe	Val	Gly 215	Ile	Ala	Phe	Arg	Ala 220	Ala	Arg	Ala	Ala
Asp 225	Pro	Asn	Ala	ГÀа	Leu 230	Tyr	Ile	Asn	Asp	Tyr 235	Asn	Leu	Asp	Ile	Ala 240
Asn	Tyr	Ala	Lys	Val 245	Thr	Arg	Gly	Met	Val 250	Glu	Lys	Val	Asn	Lys 255	Trp
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Ala	Gly	Pro 275	Gly	Gly	Trp	Asn	Thr 280	Ala	Ala	Gly	Val	Pro 285	Asp	Ala	Leu
rys	Ala 290	Leu	Ala	Ala	Ala	Asn 295	Val	Lys	Glu	Ile	Ala 300	Ile	Thr	Glu	Leu

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Thr Leu Leu Glu Gly Ser	His Tyr Gln Val Asn 75	Trp Arg Asn Thr Gly							
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Tyr Gly Trp Thr His Asn	Pro Leu Ile Glu Tyr 120	Tyr Val Val Glu Ser 125							

Tyr Gly Thr Tyr Asn Pro Gly Ser Gln Ala Gln Tyr Lys Gly Ser Phe Gln Ser Asp Gly Gly Thr Tyr Asn Ile Tyr Val Ser Thr Arg Tyr Asn Ala Pro Ser Ile Glu Gly Thr Arg Thr Phe Gln Gln Tyr Trp Ser Ile Arg Thr Ser Lys Arg Val Gly Gly Ser Val Thr Met Gln Asn His Phe Asn Ala Trp Ala Gln His Gly Met Pro Leu Gly Ser His Asp Tyr Gln Ile Val Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser Asp Ile Tyr Val Gln Thr His <210> SEQ ID NO 159 <211> LENGTH: 208 <212> TYPE: PRT <213 > ORGANISM: Myceliophthora thermophila <400> SEQUENCE: 159 Arg Pro Phe Asp Phe Asp Asp Gly Asn Ser Thr Glu Ala Leu Ala Lys Arg Gln Val Thr Pro Asn Ala Gln Gly Tyr His Ser Gly Tyr Phe Tyr Ser Trp Trp Ser Asp Gly Gly Gly Gln Ala Thr Phe Thr Leu Leu Glu 40 Gly Ser His Tyr Gln Val Asn Trp Arg Asn Thr Gly Asn Phe Val Gly Gly Lys Gly Trp Asn Pro Gly Thr Gly Arg Thr Ile Asn Tyr Gly Gly Ser Phe Asn Pro Ser Gly Asn Gly Tyr Leu Ala Val Tyr Gly Trp Thr His Asn Pro Leu Ile Glu Tyr Tyr Val Val Glu Ser Tyr Gly Thr Tyr Asn Pro Gly Ser Gln Ala Gln Tyr Lys Gly Ser Phe Gln Ser Asp Gly Gly Thr Tyr Asn Ile Tyr Val Ser Thr Arg Tyr Asn Ala Pro Ser Ile Glu Gly Thr Arg Thr Phe Gln Gln Tyr Trp Ser Ile Arg Thr Ser Lys Arg Val Gly Gly Ser Val Thr Met Gln Asn His Phe Asn Ala Trp Ala 170 Gln His Gly Met Pro Leu Gly Ser His Asp Tyr Gln Ile Val Ala Thr 185 Glu Gly Tyr Gln Ser Ser Gly Ser Ser Asp Ile Tyr Val Gln Thr His 200 <210> SEQ ID NO 160 <211> LENGTH: 681 <212> TYPE: DNA <213 > ORGANISM: Myceliophthora thermophila <400> SEQUENCE: 160

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Asn Gly Tyr Ser Val Ser Phe Ser Asn Ala Gly Asp Phe Val Val Gly 65 70 75 80	
Lys Gly Trp Arg Thr Gly Ala Thr Arg Asn Ile Thr Phe Ser Gly Ser	
85 90 95	
Thr Gln His Thr Ser Gly Thr Val Leu Val Ser Val Tyr Gly Trp Thr 100 105 110	
Arg Asn Pro Leu Ile Glu Tyr Tyr Val Gln Glu Tyr Thr Ser Asn Gly 115 120 125	
Ala Gly Ser Ala Gln Gly Glu Lys Leu Gly Thr Val Glu Ser Asp Gly 130 135 140	
Gly Thr Tyr Glu Ile Trp Arg His Gln Gln Val Asn Gln Pro Ser Ile 145 150 155 160	
Glu Gly Thr Ser Thr Phe Trp Gln Tyr Ile Ser Asn Arg Val Ser Gly 165 170 175	
Gln Arg Pro Asn Gly Gly Thr Val Thr Leu Ala Asn His Phe Ala Ala 180 185 190	
Trp Gln Lys Leu Gly Leu Asn Leu Gly Gln His Asp Tyr Gln Val Leu 195 200 205	

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Ser Phe Ser Asn Ala Gly Asp Phe Val Val Gly Lys Gly Trp Arg Thr
Gly Ala Thr Arg Asn Ile Thr Phe Ser Gly Ser Thr Gln His Thr Ser
Gly Thr Val Leu Val Ser Val Tyr Gly Trp Thr Arg Asn Pro Leu Ile
Glu Tyr Tyr Val Gln Glu Tyr Thr Ser Asn Gly Ala Gly Ser Ala Gln
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Gly Glu Lys Leu Gly Thr Val Glu Ser Asp Gly Gly Thr Tyr Glu Ile
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Trp Arg His Gln Gln Val Asn Gln Pro Ser Ile Glu Gly Thr Ser Thr
Phe Trp Gln Tyr Ile Ser Asn Arg Val Ser Gly Gln Arg Pro Asn Gly
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                          155
Gly Thr Val Thr Leu Ala Asn His Phe Ala Ala Trp Gln Lys Leu Gly
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Ile His Ala Ser Lys Asp Leu Gln Asn Trp Lys Leu Ile Gly His Val 65 70 75 80	
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Thr Ser Gly Ile Trp Ala Pro Thr Leu Arg Phe His Asp Asp Thr Phe 100 105 110	
Trp Leu Val Thr Thr Leu Val Asp Asp Asp Arg Pro Gln Glu Asp Ala	

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Ala	Trp	His	Val 180	Gly	Pro	Tyr	Ile	Gln 185	Gln	Ala	Glu	Val	Asp 190	Leu	Asp
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Ala	Arg	Ser	Arg	Lys 245	Ile	Ser	Ser	Pro	Tyr 250	Glu	Ser	Asn	Pro	Asn 255	Asn
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Arg	Thr	Ala 435	Gly	Glu	Glu	Ala	Gly 440	Val	Thr	Ala	Phe	Leu 445	Thr	Gln	Asn
His	His 450	Leu	Asp	Leu	Gly	Val 455	Val	Leu	Leu	Pro	Arg 460	Gly	Ser	Ala	Thr
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Arg	Thr	Val	Val	Met 565	Glu	Ala	Ser	Asn	Glu 570	Ala	Val	Ser	Trp	Gly 575	Phe
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240

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Gln Lys Gly Phe Ile Val Ile Tyr Pro Glu Ser Pro Tyr Ser Gly Thr 100 105 110	
Cys Trp Asp Val Ser Ser Arg Ala Ala Leu Thr His Asn Gly Gly 115 120 125	
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Tyr Asp Gly Pro Arg Pro Lys Met Gln Ile Tyr His Gly Ser Ala Asp

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Pro	Lys 210	Met	Gln	Ile	Tyr	His 215	Gly	Ser	Ala	Asp	Gly 220	Thr	Leu	Arg	Pro
Ser 225	Asn	Tyr	Asn	Glu	Thr 230	Ile	Lys	Gln	Trp	Cys 235	Gly	Val	Phe	Gly	Phe 240
Asp	Tyr	Thr	Arg	Pro 245	Asp	Thr	Thr	Gln	Ala 250	Asn	Ser	Pro	Gln	Ala 255	Gly
Tyr	Thr	Thr	Tyr 260	Thr	Trp	Gly	Glu	Gln 265	Gln	Leu	Val	Gly	Ile 270	Tyr	Ala

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Gln Leu Pro Asn Asn Tyr Asp Ala Asn Lys Ala His Arg Val Val Ile
Gly Tyr His Trp Arg Asp Gly Ser Met Asn Asp Val Ala Asn Gly Gly
Phe Tyr Asp Leu Arg Ser Arg Ala Gly Asp Ser Thr Ile Phe Val Ala
Pro Asn Gly Leu Asn Ala Gly Trp Ala Asn Val Gly Gly Glu Asp Ile
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Thr Phe Thr Asp Gln Ile Val Asp Met Leu Lys Asn Asp Leu Cys Val
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Asp Glu Thr Gln Phe Phe Ala Thr Gly Trp Ser Tyr Gly Gly Ala Met
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	210					215					220				
Val 225	Pro	Asp	Pro	Thr	Gly 230	Asn	Asn	Gly	Val	Lys 235	Phe	Ala	Pro	Gln	Glu 240
Thr	Trp	Asp	Phe	Phe 245	Asp	Ala	Ala	Val	Gly 250	Ala	Ala	Gly	Ala	Gln 255	Ser
Pro	Met	Thr													

We claim:

- 1. A non-naturally occurring polynucleotide sequence encoding a glycoside hydrolase 61 (GH61) variant protein that is at least about 90% identical to SEQ ID NO:4.
- 2. A non-naturally occurring polynucleotide sequence of claim 1, wherein said non-naturally occurring polynucleotide encodes a GH61 variant protein, wherein said GH61 variant protein is at least 90% identical to SEQ ID NO:5.
- 3. A recombinant nucleic acid construct comprising the non-naturally occurring polynucleotide sequence of claim 1.
- **4.** The recombinant nucleic acid construct of claim **3**, wherein said non-naturally occurring polynucleotide sequence encoding a glycoside hydrolase (GH61) variant protein is operably linked to a promoter.
- 5. The recombinant nucleic acid construct of claim 3, wherein said construct further encodes at least one enzyme in addition to said GH61 variant protein.
- **6**. The nucleic acid construct of claim **5**, wherein said at least one additional enzyme is selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases.

- 7. A host cell comprising the nucleic acid construct of claim 3.
- **8**. The host cell of claim 7, wherein said host cell further produces at least one enzyme selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases.
- 9. The host cell of claim 7, wherein said host cell is a yeast or filamentous fungal cell.
- 10. The host cell of claim 9, wherein said host cell is *Myceliophthora thermophila*.
- 11. A method of producing a GH61 variant protein comprising culturing the host cell set forth in claim 7, under conditions such that said host cell produces said GH61 variant proteins.
- 12. The method of claim 11, wherein said host cell further produces at least one additional enzyme selected from wild-type GH61 enzymes, endoglucanases (EG), beta-glucosidases (BGL), Type 1 cellobiohydrolases (CBH1), Type 2 cellobiohydrolases (CBH2), cellulases, hemicellulases, xylanases, xylosidases, amylases, glucoamylases, proteases, esterases, and lipases.

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