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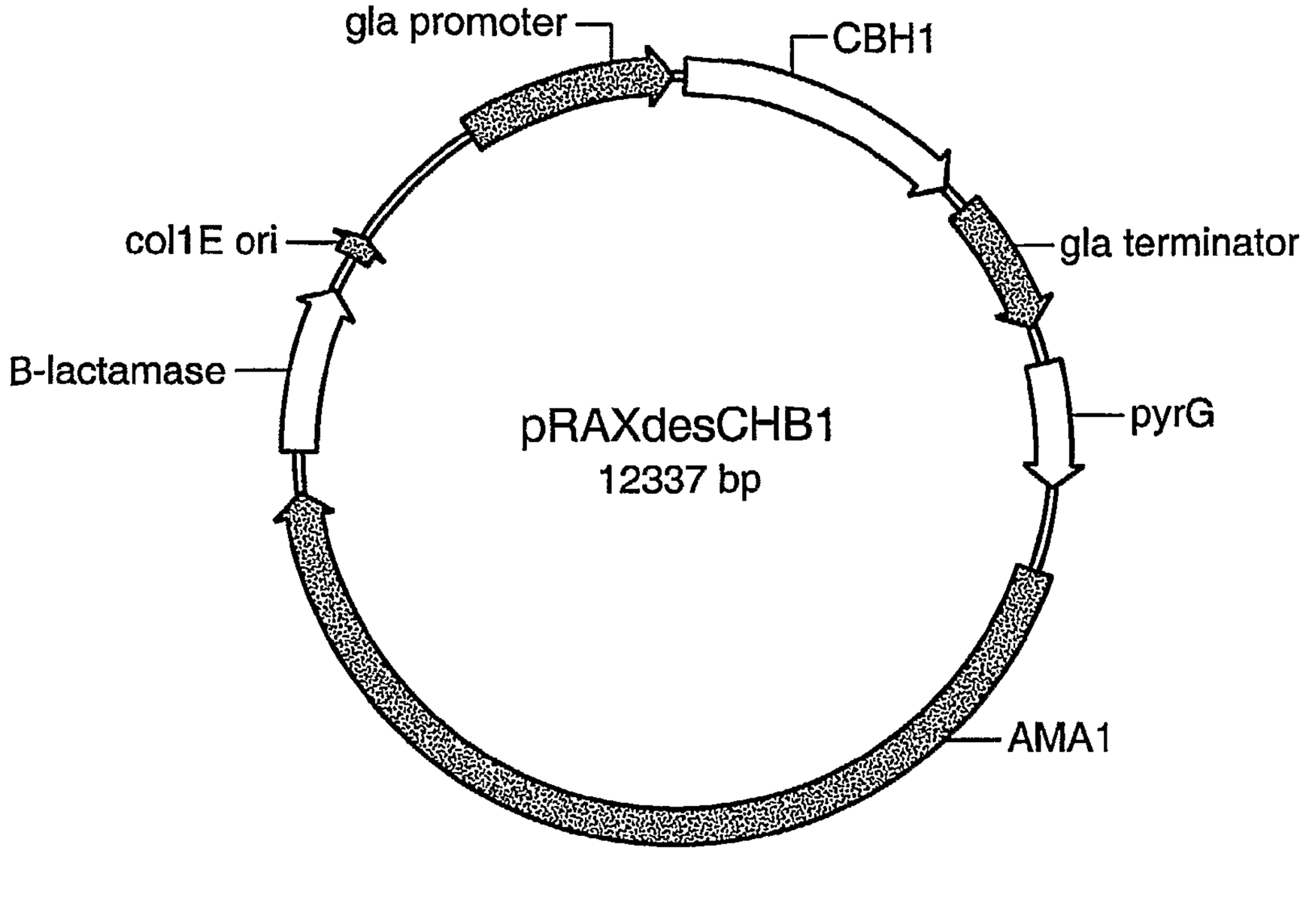
(54) Title: NOVEL VARIANT HYPROCREA JECORINA CBH1 CELLULASES

Replicative Expression pRAXdesCBH1 Vector of CBH1 Genes Under the Control of the Glucoamylase Promoter



(57) Abrégé/Abstract:
Described herein are variants of H. jecorina CBH I, a Cel7 enzyme. The present invention provides novel cellobiohydrolases that have improved thermostability and reversibility.

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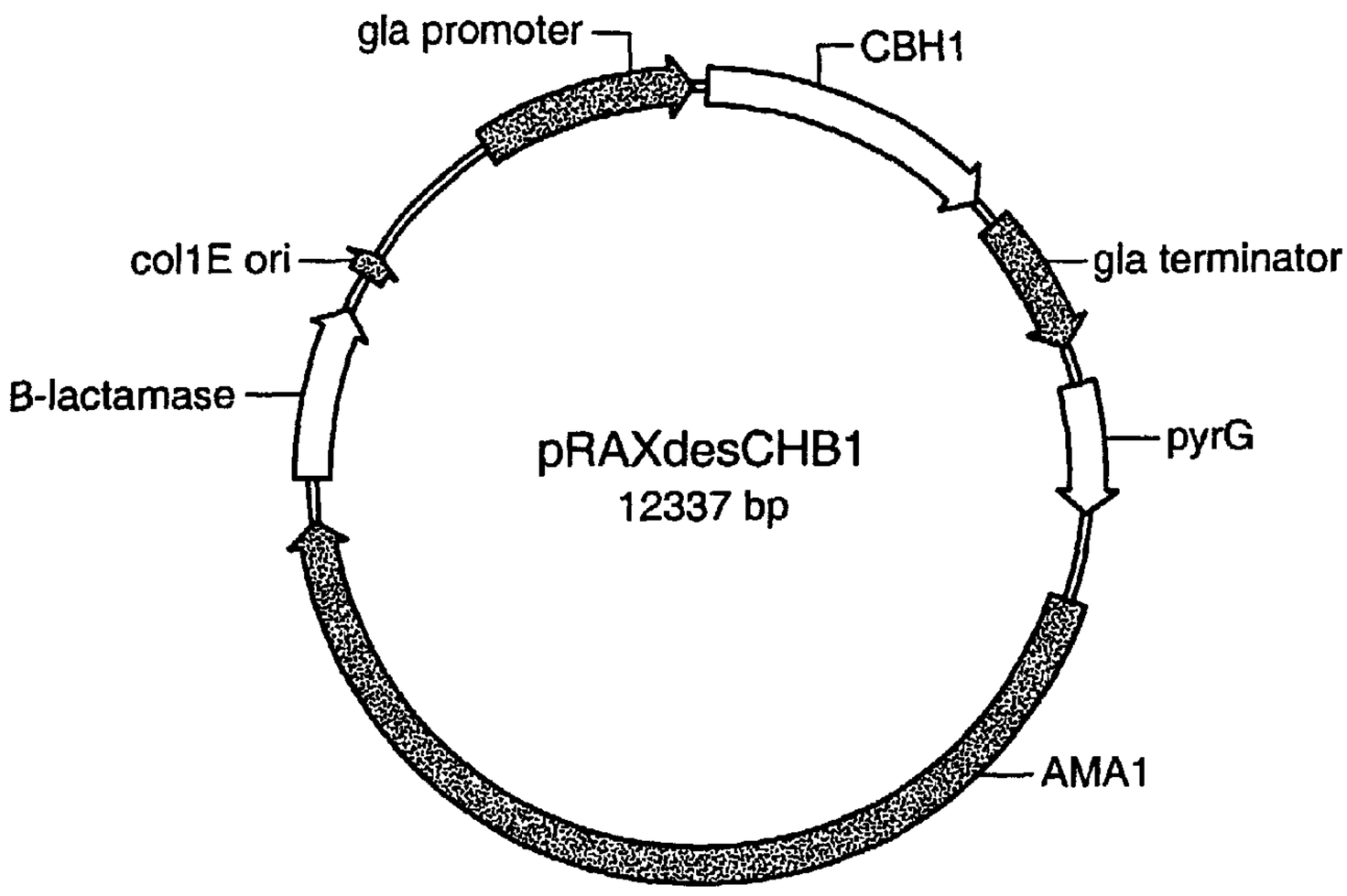
(54) Title: NOVEL VARIANT *HYPROCREA JECORINA* CBH1 CELLULASES

Replicative Expression pRAXdesCBH1 Vector of CBH1 Genes Under the Control of the Glucoamylase Promoter



(57) Abstract: Described herein are variants of *H. jecorina* CBH I, a Cel7 enzyme. The present invention provides novel cellobiohydrolases that have improved thermostability and reversibility.

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NOVEL VARIANT *HYPROCREA JECORINA* CBH1 CELLULASES

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[02] Portions of this work were funded by Subcontract No. ZCO-0-30017-01 with the National Renewable Energy Laboratory under Prime Contract No. DE-AC36-99GO10337 with the U.S. Department of Energy. Accordingly, the United States Government may have certain rights in this invention.

FIELD OF THE INVENTION

[03] The present invention relates to variant cellobiohydrolase enzymes and isolated nucleic acid sequences which encode polypeptides having cellobiohydrolase activity. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing recombinant variant CBH polypeptides.

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PDB reference **1A39**: Davies, G. J., Ducros, V., Lewis, R. J., Borchert, T. V., Schulein, M. *Journal of Biotechnology* 57 *pp.* 91 (1997)
7. PDB reference **6CEL**: Divne, C., Stahlberg, J., Teeri, T. T., Jones, T. A. *Journal of Molecular Biology* 275 *pp.* 309 (1998)

8. PDB reference **1EG1**: Kleywegt, G. J., Zou, J. Y., Divne, C., Davies, G. J., Sinning, I., Stahlberg, J., Reinikainen, T., Srisodsuk, M., Teeri, T. T., Jones, T. A. *Journal of Molecular Biology* 272 *pp.* 383 (1997)

9. PDB reference 1DY4 (8Cel): Stahlberg, J., Henriksson, H., Divne, C., Isaksson, R., Pettersson, G., Johansson, G., Jones, T.A. Journal: (2001) J. Mol. Biol. 305: 79

BACKGROUND OF THE INVENTION

[04] Cellulose and hemicellulose are the most abundant plant materials produced by photosynthesis. They can be degraded and used as an energy source by numerous microorganisms, including bacteria, yeast and fungi, that produce extracellular enzymes capable of hydrolysis of the polymeric substrates to monomeric sugars (Aro *et al.*, J. Biol. Chem., vol. 276, no. 26, pp. 24309-24314, June 29, 2001). As the limits of non-renewable resources approach, the potential of cellulose to become a major renewable energy resource is enormous (Krishna *et al.*, Bioresource Tech. 77:193-196, 2001). The effective utilization of cellulose through biological processes is one approach to overcoming the shortage of foods, feeds, and fuels (Ohmiya *et al.*, Biotechnol. Gen. Engineer. Rev. vol. 14, pp. 365-414, 1997).

[05] Cellulases are enzymes that hydrolyze cellulose (beta-1,4-glucan or beta D-glucosidic linkages) resulting in the formation of glucose, cellobiose, cellooligosaccharides, and the like. Cellulases have been traditionally divided into three major classes: endoglucanases (EC 3.2.1.4) ("EG"), exoglucanases or cellobiohydrolases (EC 3.2.1.91) ("CBH") and beta-glucosidases ([beta] -D-glucoside glucohydrolase; EC 3.2.1.21) ("BG"). (Knowles *et al.*, TIBTECH 5, 255-261, 1987; Shulein, Methods Enzymol., 160, 25, pp. 234-243, 1988). Endoglucanases act mainly on the amorphous parts of the cellulose fibre, whereas cellobiohydrolases are also able to degrade crystalline cellulose (Nevalainen and Penttila, Mycota, 303-319, 1995). Thus, the presence of a cellobiohydrolase in a cellulase system is required for efficient solubilization of crystalline cellulose (Suurnakki, *et al.* Cellulose 7:189-209, 2000). Beta-glucosidase acts to liberate D-glucose units from cellobiose, cello-oligosaccharides, and other glucosides (Freer, J. Biol. Chem. vol. 268, no. 13, pp. 9337-9342, 1993).

[06] Cellulases are known to be produced by a large number of bacteria, yeast and fungi. Certain fungi produce a complete cellulase system capable of degrading crystalline forms of cellulose, such that the cellulases are readily produced in large quantities via fermentation. Filamentous fungi play a special role since many yeast, such as *Saccharomyces cerevisiae*, lack the ability to hydrolyze cellulose. See, *e.g.*, Aro *et al.*,

2001; Aubert *et al.*, 1988; Wood *et al.*, Methods in Enzymology, vol. 160, no. 9, pp. 87-116, 1988, and Coughlan, *et al.*, "Comparative Biochemistry of Fungal and Bacterial Cellulolytic Enzyme Systems" Biochemistry and Genetics of Cellulose Degradation, pp. 11-30 1988..

[07] The fungal cellulase classifications of CBH, EG and BG can be further expanded to include multiple components within each classification. For example, multiple CBHs, EGs and BGs have been isolated from a variety of fungal sources including *Trichoderma* reesei which contains known genes for 2 CBHs, *i.e.*, CBH I and CBH II, at least 8 EGs, *i.e.*, EG I, EG II , EG III, EGIV, EGV, EGVI, EGVII and EGVIII, and at least 5 BGs, i.e., BG1, BG2, BG3, BG4 and BG5.

[08] In order to efficiently convert crystalline cellulose to glucose the complete cellulase system comprising components from each of the CBH, EG and BG classifications is required, with isolated components less effective in hydrolyzing crystalline cellulose (Filho *et al.*, Can. J. Microbiol. 42:1-5, 1996). A synergistic relationship has been observed between cellulase components from different classifications. In particular, the EG-type cellulases and CBH- type cellulases synergistically interact to more efficiently degrade cellulose. See, *e.g.*, Wood, Biochemical Society Transactions, 611th Meeting, Galway, vol. 13, pp. 407-410, 1985.

[09] Cellulases are known in the art to be useful in the treatment of textiles for the purposes of enhancing the cleaning ability of detergent compositions, for use as a softening agent, for improving the feel and appearance of cotton fabrics, and the like (Kumar *et al.*, Textile Chemist and Colorist, 29:37-42, 1997).

[10] Cellulase-containing detergent compositions with improved cleaning performance (US Pat. No. 4,435,307; GB App. Nos. 2,095,275 and 2,094,826) and for use in the treatment of fabric to improve the feel and appearance of the textile (US Pat. Nos. 5,648,263, 5,691,178, and 5,776,757; GB App. No. 1,358,599; The Shizuoka Prefectural Hammamatsu Textile Industrial Research Institute Report, Vol. 24, pp. 54-61, 1986), have been described.

[11] Hence, cellulases produced in fungi and bacteria have received significant attention. In particular, fermentation of *Trichoderma spp.* (*e.g.*, *Trichoderma longibrachiatum* or *Trichoderma reesei*) has been shown to produce a complete cellulase system capable of degrading crystalline forms of cellulose.

[12] Although cellulase compositions have been previously described, there remains a need for new and improved cellulase compositions for use in household detergents,

stonewashing compositions or laundry detergents, etc. Cellulases that exhibit improved performance are of particular interest.

BRIEF SUMMARY OF THE INVENTION

[13] The invention provides an isolated cellulase protein, identified herein as variant CBH I, and nucleic acids which encode a variant CBH I.

[14] In one embodiment the invention is directed to a variant CBH I cellulase, wherein said variant comprises a substitution or deletion at a position corresponding to one or more of residues S8, Q17, G22, T41, N49, S57, N64, A68, A77, N89, S92, N103, A112, S113, E193, S196, M213, L225, T226, P227, T246, D249, R251, Y252, T255, D257, D259, S278, S279, K286, L288, E295, T296, S297, A299, N301, E325, T332, F338, S342, F352, T356, Y371, T380, Y381, V393, R394, S398, V403, S411, G430, G440, T445, T462, T484, Q487, and P491 in CBH I from *Hypocrea jecorina* (SEQ ID NO: 2). In first aspect, the invention encompasses an isolated nucleic acid encoding a polypeptide having cellobiohydrolase activity, which polypeptide is a variant of a glycosyl hydrolase of family 7, and wherein said nucleic acid encodes a substitution at a residue which is sensitive to temperature stress in the polypeptide encoded by said nucleic acid, wherein said variant cellobiohydrolase is derived from *H. jecorina* cellobiohydrolase. In second aspect, the invention encompasses an isolated nucleic acid encoding a polypeptide having cellobiohydrolase activity, which polypeptide is a variant of a glycosyl hydrolase of family 7, and wherein said nucleic acid encodes a substitution at a residue which is effects enzyme processitivity in the polypeptide encoded by said nucleic acid, wherein said variant cellobiohydrolase is derived from *H. jecorina* cellobiohydrolase. In third aspect, the invention encompasses an isolated nucleic acid encoding a polypeptide having cellobiohydrolase activity, which polypeptide is a variant of a glycosyl hydrolase of family 7, and wherein said nucleic acid encodes a substitution at a residue which is effects product inhibition in the polypeptide encoded by said nucleic acid, wherein said variant cellobiohydrolase is derived from *H. jecorina* cellobiohydrolase.

[15] In a second embodiment the invention is directed to a variant CBH I cellulose comprising a substitution at a position corresponding to one or more of residues S8P, Q17L, G22D, T41I, N49S, S57N, N64D, A68T, A77D, N89D, S92T, N103I, A112E, S113(T/N/D), E193V, S196T, M213I, L225F, T226A, P227(L/T/A), T246(C/A), D249K, R251A, Y252(A/Q), T255P, D257E, D259W, S278P, S279N, K286M, L288F, E295K, T296P, S297T, A299E, N301(R/K), E325K, T332(K/Y/H), F338Y, S342Y, F352L, T356L, Y371C, T380G, Y381D, V393G, R394A, S398T, V403D, S411F, G430F, G440R, T462I,

T484S, Q487L and/or P491L in CBH I from *Hypocrea jecorina* (SEQ ID NO: 2). In one aspect of this embodiment the variant CBH I cellulase further comprises a deletion at a position corresponding to T445 in CBH I from *Hypocrea jecorina* (SEQ ID NO: 2). In a second aspect of this embodiment the variant CBH I cellulase further comprises the deletion of residues corresponding to residues 382-393 in CBH I of *Hypocrea jecorina* (SEQ ID NO: 2).

[16] In a third embodiment the invention is directed to a variant CBH I cellulase, wherein said variant comprises a substitution at a position corresponding to a residue selected from the group consisting of S8P, N49S, A68T, A77D, N89D, S92T, S113(N/D), L225F, P227(A/L/T), D249K, T255P, D257E, S279N, L288F, E295K, S297T, A299E, N301K, T332(K/Y/H), F338Y, T356L, V393G, G430F in CBH I from *Hypocrea jecorina* (SEQ ID NO: 2).

[17] In a fourth embodiment the invention is directed to a variant CBH I consists essentially of the mutations selected from the group consisting of

i. A112E/T226A;
ii. S196T/S411F;
iii. E295K/S398T;
iv. T246C/Y371C;
v. T41I plus deletion at T445
vi. A68T/G440R/P491L;
vii. G22D/S278P/T296P;
viii. T246A/R251A/Y252A;
ix. T380G/Y381D/R394A;
x. T380G/Y381D/R394A plus deletion of 382-393, inclusive;
xi. Y252Q/D259W/S342Y;
xii. S113T/T255P/K286M;
xiii. P227L/E325K/Q487L;
xiv. P227T/T484S/F352L;
xv. Q17L/E193V/M213I/F352L;
xvi. S8P/N49S/A68T/S113N;
xvii. S8P/N49S/A68T/S113N/P227L;
xviii. T41I/A112E/P227L/S278P/T296P;
xix. S8P/N49S/A68T/A112E/T226A;
xx. S8P/N49S/A68T/A112E/P227L;
xxi. S8P/T41I/N49S/A68T/A112E/P227L;

xxii. G22D/N49S/A68T/P227L/S278P/T296P;

xxiii. S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/S278P/T296P;

xxiv. G22D/N49S/A68T/N103I/S113N/P227L/S278P/T296P;

xxv. G22D/N49S/A68T/N103I/A112E/P227L/S278P/T296P;

xxvi. G22D/N49S/N64D/A68T/N103I/S113N/S278P/T296P;

xxvii. S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/D249K/S278P/T296P;

xxviii. S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/S278P/T296P/N301R;

xxix. S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/D249K/S278P/T296P/N301R

xxx. S8P/G22D/T41I/N49S/A68T/S113N/P227L/D249K/S278P/T296P/N301R;

xxxi. S8P/T41I/N49S/S57N/A68T/S113N/P227L/D249K/S278P/T296P/N301R;

xxxii. S8P/G22D/T41I/N49S/A68T/S113N/P227L/D249K/S278P/N301R;

xxxiii. S8P/T41I/N49S/A68T/S92T/S113N/P227L/D249K/V403D/T462I;

xxxiv. S8P/G22D/T41I/N49S/A68T/S92T/S113N/P227L/D249K/V403D/T462I;

xxxv. S8P/T41I/N49S/A68T/S92T/S113N/P227L/D249K/S411F;

xxxvi. S8P/G22D/T41I/N49S/A68T/S92T/S113N/P227L/D249K/S411F;

xxxvii. S8P/G22D/T41I/N49S/A68T/S92T/S113N/S196T/P227L/D249K/T255P/ S278P/T296P/N301R/E325K/S411F;

xxxviii. S8P/T41I/N49S/A68T/S92T/S113N/S196T/P227L/D249K/T255P/S278P/T296P/N301R/E325K/V403D/S411F/T462I;

xxxix. S8P/G22D/T41I/N49S/A68T/S92T/S113N/S196T/P227L/D249K/T255P/ S278P/T296P/N301R/E325K/V403D/S411F/T462I;

in CBH I from *Hypocrea jecorina* (SEQ ID NO:2).

[18] In an fifth embodiment the invention is directed to a vector comprising a nucleic acid encoding a variant CBH I. In another aspect there is a construct comprising the nucleic acid of encoding the variant CBH I operably linked to a regulatory sequence.

[19] In a sixth embodiment the invention is directed to a host cell transformed with the vector comprising a nucleic acid encoding a CBH I variant.

[20] In a seventh embodiment the invention is directed to a method of producing a CBH I variant comprising the steps of:

(a) culturing a host cell transformed with the vector comprising a nucleic acid encoding a CBH I variant in a suitable culture medium under suitable conditions to produce CBH I variant;

(b) obtaining said produced CBH I variant.

[21] In an eighth embodiment the invention is directed to a detergent composition comprising a surfactant and a CBH I variant. In one aspect of this embodiment the detergent is a laundry detergent. In a second aspect of this embodiment the detergent is a dish detergent. In third aspect of this invention, the variant CBH I cellulase is used in the treatment of a cellulose containing textile, in particular, in the stonewashing or indigo dyed denim.

[22] In a ninth embodiment the invention is directed to a feed additive comprising a CBH I variant.

[23] In a tenth embodiment the invention is directed to a method of treating wood pulp comprising contacting said wood pulp with a CBH I variant.

[24] In a eleventh embodiment the invention is directed to a method of converting biomass to sugars comprising contacting said biomass with a CBH I variant.

[25] In an embodiment, the cellulase is derived from a fungus, bacteria or Actinomycete. In another aspect, the cellulase is derived from a fungus. In a most preferred embodiment, the fungus is a filamentous fungus. It is preferred the filamentous fungus belong to Euascomycete, in particular, *Aspergillus spp., Gliocladium spp., Fusarium spp., Acremonium spp., Myceliophtora spp., Verticillium spp., Myrothecium spp.,* or *Penicillium spp.* In a further aspect of this embodiment, the cellulase is a cellobiohydrolase.

[26] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the scope and spirit of the invention will become apparent to one skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[27] Figure 1 is the nucleic acid (lower line; SEQ ID NO: 1) and amino acid (upper line; SEQ ID NO: 2) sequence of the wild type Cel7A (CBH I) from *H. jecorina*.

[28] Figure 2 is the 3-D structure of *H. jecorina* CBH I.

[29] Figure 3 shows the amino acid alignment of the Cel7 family members for which there were crystal structures available. The sequences are: 2OVW - Fusarium oxysporum Cel7B (SEQ ID NO:32), 1A39 - Humicola insolens Cel7B (SEQ ID NO:33), 6CEL - Hypocrea jecorina Cel7A (SEQ ID NO:34), 1EG1 - Hypocrea jecorina Cel7B (SEQ ID NO:35), and the consensus (SEQ ID NO:77).

[30] Figure 4 illustrates the crystal structures from the catalytic domains of these four Cel7 homologues aligned and overlayed as described herein.

[31] Figure 5 A-M is the nucleic acid sequence and deduced amino acid sequence for eight single residue mutations and five multiple mutation variants (SEQ ID NO:5-30).

[32] Figure 6 A-D is the nucleic acid sequence for pTrex2 (SEQ ID NO:31).

[33] Figure 7 A & B depicts the construction of the expression plasmid pTEX.

[34] Figure 8 A-J is the amino acid alignment of all 42 members of the Cel7 family (SEQ ID NO:32-74).

[35] Figure 9A is a representation of the thermal profiles of the wild type and eight single residue variants. Figure 9B is a representation of the thermal profiles of the wild type and five variants. Legend for Figure 9B: Cel7A = wild-type H. jecorina CBH I; N301K = N301K variant; 334 = P227L variant; 340 = S8P/N49S/A68T/S113N variant; 350 = S8P/N49S/A68T/S113N/ P227L variant; and 363 = S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/S278P/T296P variant.

[36] Figure 10 is the pRAX1 vector. This vector is based on the plasmid pGAPT2 except a 5259bp HindIII fragment of Aspergillus nidulans genomic DNA fragment AMA1 sequence (Molecular Microbiology 1996 19:565-574) was inserted. Base 1 to 1134 contains Aspergillus niger glucoamylase gene promoter. Base 3098 to 3356 and 4950 to 4971 contains Aspergillus niger glucoamylase terminator. Aspergillus nidulans pyrG gene was inserted from 3357 to 4949 as a marker for fungal transformation. There is a multiple cloning site (MCS) into which genes may be inserted.

[37] Figure 11 is the pRAXdes2 vector backbone. This vector is based on the plasmid vector pRAX1. A Gateway cassette has been inserted into pRAX1 vector (indicated by the arrow on the interior of the circular plasmid). This cassette contains recombination sequence attR1 and attR2 and the selection marker catH and ccdB. The vector has been made according to the manual given in Gateway™ Cloning Technology: version 1 page 34-38 and can only replicate in *E. coli* DB3.1 from Invitrogen; in other *E. coli* hosts the ccdB gene is lethal. First a PCR fragment is made with primers containing attB1/2 recombination sequences. This fragment is recombined with pDONR201 (commercially available from Invitrogen); this vector contains attP1/2 recombination sequences with catH and ccdB in between the recombination sites. The BP clonase enzymes from Invitrogen are used to recombine the PCR fragment in this so-called ENTRY vector, clones with the

PCR fragment inserted can be selected at 50µg/ml kanamycin because clones expressing ccdB do not survive. Now the att sequences are altered and called attL1 and attL2. The second step is to recombine this clone with the pRAXdes2 vector (containing attR1 and attR2 catH and ccdB in between the recombination sites). The LR clonase enzymes from Invitrogen are used to recombine the insert from the ENTRY vector in the destination vector. Only pRAXCBH1 vectors are selected using 100µg/ml ampicillin because ccdB is lethal and the ENTRY vector is sensitive to ampicillin. By this method the expression vector is now prepared and can be used to transform *A. niger*.

[38] Figure 12 provides an illustration of the pRAXdes2cbh1 vector which was used for expression of the nucleic acids encoding the CBH1 variants in Aspergillus. A nucleic acid encoding a CBH1 enzyme homolog or variant was cloned into the vector by homologous recombination of the att sequences.

DETAILED DESCRIPTION

[39] The invention will now be described in detail by way of reference only using the following definitions and examples.

[40] 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. Singleton, *et al.*, DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this invention. 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. Numeric ranges are inclusive of the numbers defining the range. 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. Practitioners are particularly directed to Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL (Second Edition), Cold Spring Harbor Press, Plainview, N.Y., 1989, and Ausubel FM *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1993, for definitions and terms of the art. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary.

[41] The headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

I. DEFINITIONS

[43] The term "polypeptide" as used herein refers to a compound made up of a single chain of amino acid residues linked by peptide bonds. The term "protein" as used herein may be synonymous with the term "polypeptide" or may refer, in addition, to a complex of two or more polypeptides.

[44] "Variant" means a protein which is derived from a precursor protein (*e.g.*, the native protein) by addition of one or more amino acids to either or both the C- and N-terminal end, substitution of one or more amino acids at one or a number of different sites in the amino acid sequence, or deletion of one or more amino acids at either or both ends of the protein or at one or more sites in the amino acid sequence. The preparation of an enzyme variant is preferably achieved by modifying a DNA sequence which encodes for the native protein, transformation of that DNA sequence into a suitable host, and expression of the modified DNA sequence to form the derivative enzyme. The variant CBH I enzyme of the invention includes peptides comprising altered amino acid sequences in comparison with a precursor enzyme amino acid sequence wherein the variant CBH enzyme retains the characteristic cellulolytic nature of the precursor enzyme but which may have altered properties in some specific aspect. For example, a variant CBH enzyme may have an increased pH optimum or increased temperature or oxidative stability but will retain its characteristic cellulolytic activity. It is contemplated that the variants according to the present invention may be derived from a DNA fragment encoding a cellulase variant CBH enzyme wherein the functional activity of the expressed cellulase derivative is retained. For example, a DNA fragment encoding a cellulase may further include a DNA sequence or portion thereof encoding a hinge or linker attached to the cellulase DNA sequence at either the 5' or 3' end wherein the functional activity of the encoded cellulase domain is retained.

[45] "Equivalent residues" may also be defined by determining homology at the level of tertiary structure for a precursor cellulase whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic

coordinates of two or more of the main chain atoms of a particular amino acid residue of a cellulase and *Hypocrea jecorina* CBH (N on N, CA on CA, C on C and O on O) are within 0.13nm and preferably 0.1nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the cellulase in question to the *H. jecorina* CBH I. The best model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available.

$$R\,factor = \frac{\Sigma_h |Fo(h)| - |Fc(h)|}{\Sigma_h |Fo(h)|}$$

[46] Equivalent residues which are functionally analogous to a specific residue of *H. jecorina* CBH I are defined as those amino acids of a cellulase which may adopt a conformation such that they either alter, modify or contribute to protein structure, substrate binding or catalysis in a manner defined and attributed to a specific residue of the *H. jecorina* CBH I. Further, they are those residues of the cellulase (for which a tertiary structure has been obtained by x-ray crystallography) which occupy an analogous position to the extent that, although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two of the side chain atoms of the residue lie with 0.13nm of the corresponding side chain atoms of *H. jecorina* CBH. The crystal structure of *H. jecorina* CBH I is shown in Figure 2.

[47] The term "nucleic acid molecule" includes RNA, DNA and cDNA molecules. It will be understood that, as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding a given protein such as CBH I may be produced. The present invention contemplates every possible variant nucleotide sequence, encoding CBH I, all of which are possible given the degeneracy of the genetic code.

[48] A "heterologous" nucleic acid construct or sequence has a portion of the sequence which is not native to the cell in which it is expressed. Heterologous, with respect to a control sequence refers to a control sequence (*i.e.* promoter or enhancer) that does not function in nature to regulate the same gene the expression of which it is currently regulating. Generally, heterologous nucleic acid sequences are not endogenous to the cell or part of the genome in which they are present, and have been added to the cell, by infection, transfection, transformation, microinjection, electroporation, or the like. A "heterologous" nucleic acid construct may contain a control sequence/DNA coding

sequence combination that is the same as, or different from a control sequence/DNA coding sequence combination found in the native cell.

[49] As used herein, the term "vector" refers to a nucleic acid construct designed for transfer between different host cells. An "expression vector" refers to a vector that has the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

[50] Accordingly, an “expression cassette” or “expression vector” is a nucleic acid construct generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter.

[51] As used herein, the term "plasmid" refers to a circular double-stranded (ds) DNA construct used as a cloning vector, and which forms an extrachromosomal self-replicating genetic element in many bacteria and some eukaryotes.

[52] As used herein, the term "selectable marker-encoding nucleotide sequence" refers to a nucleotide sequence which is capable of expression in cells and where expression of the selectable marker confers to cells containing the expressed gene the ability to grow in the presence of a corresponding selective agent, or under corresponding selective growth conditions.

[53] As used herein, the term "promoter" refers to a nucleic acid sequence that functions to direct transcription of a downstream gene. The promoter will generally be appropriate to the host cell in which the target gene is being expressed. The promoter together with other transcriptional and translational regulatory nucleic acid sequences (also termed "control sequences") are necessary to express a given gene. In general, the transcriptional and translational regulatory sequences include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences.

[54] "Chimeric gene" or “heterologous nucleic acid construct”, as defined herein refers to a non-native gene (*i.e.*, one that has been introduced into a host) that may be composed of parts of different genes, including regulatory elements. A chimeric gene construct for transformation of a host cell is typically composed of a transcriptional regulatory region (promoter) operably linked to a heterologous protein coding sequence,

or, in a selectable marker chimeric gene, to a selectable marker gene encoding a protein conferring antibiotic resistance to transformed cells. A typical chimeric gene of the present invention, for transformation into a host cell, includes a transcriptional regulatory region that is constitutive or inducible, a protein coding sequence, and a terminator sequence. A chimeric gene construct may also include a second DNA sequence encoding a signal peptide if secretion of the target protein is desired.

[55] A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA encoding a secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors, linkers or primers for PCR are used in accordance with conventional practice.

[56] As used herein, the term "gene" means the segment of DNA involved in producing a polypeptide chain, that may or may not include regions preceding and following the coding region, *e.g.* 5' untranslated (5' UTR) or "leader" sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[57] In general, nucleic acid molecules which encode the variant CBH I will hybridize, under moderate to high stringency conditions to the wild type sequence provided herein as SEQ ID NO:1. However, in some cases a CBH I-encoding nucleotide sequence is employed that possesses a substantially different codon usage, while the protein encoded by the CBH I-encoding nucleotide sequence has the same or substantially the same amino acid sequence as the native protein. For example, the coding sequence may be modified to facilitate faster expression of CBH I in a particular prokaryotic or eukaryotic expression system, in accordance with the frequency with which a particular codon is utilized by the host. Te'o, *et al.* (FEMS Microbiology Letters 190:13-19, 2000), for example, describes the optimization of genes for expression in filamentous fungi.

[58] A nucleic acid sequence is considered to be "selectively hybridizable" to a reference nucleic acid sequence if the two sequences specifically hybridize to one another under moderate to high stringency hybridization and wash conditions. Hybridization

conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe. For example, "maximum stringency" typically occurs at about Tm-5°C (5° below the Tm of the probe); "high stringency" at about 5-10° below the Tm; "moderate " or "intermediate stringency" at about 10-20° below the Tm of the probe; and "low stringency" at about 20-25° below the Tm. Functionally, maximum stringency conditions may be used to identify sequences having strict identity or near-strict identity with the hybridization probe; while high stringency conditions are used to identify sequences having about 80% or more sequence identity with the probe.

[59] Moderate and high stringency hybridization conditions are well known in the art (see, for example, Sambrook, *et al,* 1989, Chapters 9 and 11, and in Ausubel, F.M., *et al.*, 1993). An example of high stringency conditions includes hybridization at about 42°C in 50% formamide, 5X SSC, 5X Denhardt's solution, 0.5% SDS and 100 μg/ml denatured carrier DNA followed by washing two times in 2X SSC and 0.5% SDS at room temperature and two additional times in 0.1X SSC and 0.5% SDS at 42°C.

[60] As used herein, "recombinant" includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid sequence or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found in identical form within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all as a result of deliberate human intervention.

[61] As used herein, the terms "transformed", "stably transformed" or "transgenic" with reference to a cell means the cell has a non-native (heterologous) nucleic acid sequence integrated into its genome or as an episomal plasmid that is maintained through multiple generations.

[62] As used herein, the term "expression" refers to the process by which a polypeptide is produced based on the nucleic acid sequence of a gene. The process includes both transcription and translation.

[63] The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means "transfection", or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid sequence into a eukaryotic or prokaryotic cell where the nucleic acid sequence may be incorporated into the genome of the cell (for example, chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (for example, transfected mRNA).

[64] It follows that the term "CBH I expression" refers to transcription and translation of the *cbh I* gene, the products of which include precursor RNA, mRNA, polypeptide, post-translationally processed polypeptides, and derivatives thereof, including CBH I from related species such as *Trichoderma koningii, Hypocrea jecorina (also known as Trichoderma longibrachiatum, Trichoderma reesei or Trichoderma viride) and Hypocrea schweinitzii*. By way of example, assays for CBH I expression include Western blot for CBH I protein, Northern blot analysis and reverse transcriptase polymerase chain reaction (RT-PCR) assays for CBH I mRNA, and *en*doglucanase activity assays as described in Shoemaker S.P. and Brown R.D.Jr. (Biochim. Biophys. Acta, 1978, 523:133-146) and Schulein (Methods Enzymol., 160, 25, pp. 234-243, 1988).

[65] The term "alternative splicing" refers to the process whereby multiple polypeptide isoforms are generated from a single gene, and involves the splicing together of nonconsecutive exons during the processing of some, but not all, transcripts of the gene. Thus a particular exon may be connected to any one of several alternative exons to form messenger RNAs. The alternatively-spliced mRNAs produce polypeptides ("splice variants") in which some parts are common while other parts are different.

[66] The term "signal sequence" refers to a sequence of amino acids at the N-terminal portion of a protein which facilitates the secretion of the mature form of the protein outside the cell. The mature form of the extracellular protein lacks the signal sequence which is cleaved off during the secretion process.

[67] By the term "host cell" is meant a cell that contains a vector and supports the replication, and/or transcription or transcription and translation (expression) of the expression construct. Host cells for use in the present invention can be prokaryotic cells, such as *E. coli*, or eukaryotic cells such as yeast, plant, insect, amphibian, or mammalian cells. In general, host cells are filamentous fungi.

[68] The term "filamentous fungi" means any and all filamentous fungi recognized by those of skill in the art. A preferred fungus is selected from the group consisting of Aspergillus, Trichoderma, Fusarium, Chrysosporium, Penicillium, Humicola, Neurospora, or alternative sexual forms thereof such as Emericella, Hypocrea. It has now been demonstrated that the asexual industrial fungus *Trichoderma reesei* is a clonal derivative of the ascomycete *Hypocrea jecorina*. See Kuhls et al., PNAS (1996) 93:7755-7760.

[69] The term "cellooligosaccharide" refers to oligosaccharide groups containing from 2-8 glucose units and having β-1,4 linkages, *e.g.*, cellobiose.

[70] The term "cellulase" refers to a category of enzymes capable of hydrolyzing cellulose polymers to shorter cello-oligosaccharide oligomers, cellobiose and/or glucose.

Numerous examples of cellulases, such as exoglucanases, exocellobiohydrolases, endoglucanases, and glucosidases have been obtained from cellulolytic organisms, particularly including fungi, plants and bacteria.

[71] CBH I from *Hypocrea jecorina* is a member of the Glycosyl Hydrolase Family 7 (hence Cel 7) and, specifically, was the first member of that family identified in *Hypocrea jecorina* (hence Cel 7A). The Glycosyl Hydrolase Family 7 contains both Endoglucanases and Cellobiohydrolases/exoglucanases, and that CBH I is the latter. Thus, the phrases CBH I, CBH I-type protein and Cel 7 cellobiohydrolases may be used interchangeably herein.

[72] The term "cellulose binding domain" as used herein refers to portion of the amino acid sequence of a cellulase or a region of the enzyme that is involved in the cellulose binding activity of a cellulase or derivative thereof. Cellulose binding domains generally function by non-covalently binding the cellulase to cellulose, a cellulose derivative or other polysaccharide equivalent thereof. Cellulose binding domains permit or facilitate hydrolysis of cellulose fibers by the structurally distinct catalytic core region, and typically function independent of the catalytic core. Thus, a cellulose binding domain will not possess the significant hydrolytic activity attributable to a catalytic core. In other words, a cellulose binding domain is a structural element of the cellulase enzyme protein tertiary structure that is distinct from the structural element which possesses catalytic activity. Cellulose binding domain and cellulose binding module may be used interchangeably herein.

[73] As used herein, the term "surfactant" refers to any compound generally recognized in the art as having surface active qualities. Thus, for example, surfactants comprise anionic, cationic and nonionic surfactants such as those commonly found in detergents. Anionic surfactants include linear or branched alkylbenzenesulfonates; alkyl or alkenyl ether sulfates having linear or branched alkyl groups or alkenyl groups; alkyl or alkenyl sulfates; olefinsulfonates; and alkanesulfonates. Ampholytic surfactants include quaternary ammonium salt sulfonates, and betaine-type ampholytic surfactants. Such ampholytic surfactants have both the positive and negative charged groups in the same molecule. Nonionic surfactants may comprise polyoxyalkylene ethers, as well as higher fatty acid alkanolamides or alkylene oxide adduct thereof, fatty acid glycerine monoesters, and the like.

[74] As used herein, the term "cellulose containing fabric" refers to any sewn or unsewn fabrics, yarns or fibers made of cotton or non-cotton containing cellulose or cotton or non-

cotton containing cellulose blends including natural cellulosics and manmade cellulosics (such as jute, flax, ramie, rayon, and lyocell).

[75] As used herein, the term "cotton-containing fabric" refers to sewn or unsewn fabrics, yarns or fibers made of pure cotton or cotton blends including cotton woven fabrics, cotton knits, cotton denims, cotton yarns, raw cotton and the like.

[76] As used herein, the term "stonewashing composition" refers to a formulation for use in stonewashing cellulose containing fabrics. Stonewashing compositions are used to modify cellulose containing fabrics prior to sale, *i.e.*, during the manufacturing process. In contrast, detergent compositions are intended for the cleaning of soiled garments and are not used during the manufacturing process.

[77] As used herein, the term "detergent composition" refers to a mixture which is intended for use in a wash medium for the laundering of soiled cellulose containing fabrics. In the context of the present invention, such compositions may include, in addition to cellulases and surfactants, additional hydrolytic enzymes, builders, bleaching agents, bleach activators, bluing agents and fluorescent dyes, caking inhibitors, masking agents, cellulase activators, antioxidants, and solubilizers.

[78] As used herein, the term "decrease or elimination in expression of the *cbh1* gene" means that either that the *cbh1* gene has been deleted from the genome and therefore cannot be expressed by the recombinant host microorganism; or that the *cbh1* gene has been modified such that a functional CBH1 enzyme is not produced by the host microorganism.

[79] The term "variant *cbh1 gene*" or "variant CBH1" means, respectively, that the nucleic acid sequence of the *cbh1* gene from *H. jecorina* has been altered by removing, adding, and/or manipulating the coding sequence or the amino acid sequence of the expressed protein has been modified consistent with the invention described herein.

[80] As used herein, the term "purifying" generally refers to subjecting transgenic nucleic acid or protein containing cells to biochemical purification and/or column chromatography.

[81] As used herein, the terms "active" and "biologically active" refer to a biological activity associated with a particular protein and are used interchangeably herein. For example, the enzymatic activity associated with a protease is proteolysis and, thus, an active protease has proteolytic activity. It follows that the biological activity of a given protein refers to any biological activity typically attributed to that protein by those of skill in the art.

[82] As used herein, the term "enriched" means that the CBH is found in a concentration that is greater relative to the CBH concentration found in a wild-type, or naturally occurring, fungal cellulase composition. The terms enriched, elevated and enhanced may be used interchangeably herein.

[83] A wild type fungal cellulase composition is one produced by a naturally occurring fungal source and which comprises one or more BGL, CBH and EG components wherein each of these components is found at the ratio produced by the fungal source. Thus, an enriched CBH composition would have CBH at an altered ratio wherein the ratio of CBH to other cellulase components (i.e., EGs, beta-glucosidases and other endoglucanases) is elevated. This ratio may be increased by either increasing CBH or decreasing (or eliminating) at least one other component by any means known in the art.

[84] Thus, to illustrate, a naturally occurring cellulase system may be purified into substantially pure components by recognized separation techniques well published in the literature, including ion exchange chromatography at a suitable pH, affinity chromatography, size exclusion and the like. For example, in ion exchange chromatography (usually anion exchange chromatography), it is possible to separate the cellulase components by eluting with a pH gradient, or a salt gradient, or both a pH and a salt gradient. The purified CBH may then be added to the enzymatic solution resulting in an enriched CBH solution. It is also possible to elevate the amount of CBH I produced by a microbe using molecular genetics methods to overexpress the gene encoding CBH, possibly in conjunction with deletion of one or more genes encoding other cellulases.

[85] Fungal cellulases may contain more than one CBH component. The different components generally have different isoelectric points which allow for their separation via ion exchange chromatography and the like. Either a single CBH component or a combination of CBH components may be employed in an enzymatic solution.

[86] When employed in enzymatic solutions, the homolog or variant CBH1 component is generally added in an amount sufficient to allow the highest rate of release of soluble sugars from the biomass. The amount of homolog or variant CBH1 component added depends upon the type of biomass to be saccharified which can be readily determined by the skilled artisan. However, when employed, the weight percent of the homolog or variant CBH1 component relative to any EG type components present in the cellulase composition is from preferably about 1, preferably about 5, preferably about 10, preferably about 15, or preferably about 20 weight percent to preferably about 25, preferably about 30, preferably about 35, preferably about 40, preferably about 45 or preferably about 50 weight percent. Furthermore, preferred ranges may be about 0.5 to about 15 weight

percent, about 0.5 to about 20 weight percent, from about 1 to about 10 weight percent, from about 1 to about 15 weight percent, from about 1 to about 20 weight percent, from about 1 to about 25 weight percent, from about 5 to about 20 weight percent, from about 5 to about 25 weight percent, from about 5 to about 30 weight percent, from about 5 to about 35 weight percent, from about 5 to about 40 weight percent, from about 5 to about 45 weight percent, from about 5 to about 50 weight percent, from about 10 to about 20 weight percent, from about 10 to about 25 weight percent, from about 10 to about 30 weight percent, from about 10 to about 35 weight percent, from about 10 to about 40 weight percent, from about 10 to about 45 weight percent, from about 10 to about 50 weight percent, from about 15 to about 20 weight percent, from about 15 to about 25 weight percent, from about 15 to about 30 weight percent, from about 15 to about 35 weight percent, from about 15 to about 30 weight percent, from about 15 to about 45 weight percent, from about 15 to about 50 weight percent.

II. HOST ORGANISMS

[87] Filamentous fungi include all filamentous forms of the subdivision Eumycota and Oomycota. The filamentous fungi are characterized by vegetative mycelium having a cell wall composed of chitin, glucan, chitosan, mannan, and other complex polysaccharides, with vegetative growth by hyphal elongation and carbon catabolism that is obligately aerobic.

[88] In the present invention, the filamentous fungal parent cell may be a cell of a species of, but not limited to, *Trichoderma, e.g., Trichoderma longibrachiatum, Trichoderma viride, Trichoderma koningii, Trichoderma harzianum; Penicillium sp.; Humicola sp.*, including *Humicola insolens* and *Humicola grisea; Chrysosporium sp.,* including *C. lucknowense*; *Gliocladium sp.; Aspergillus sp.*; *Fusarium sp., Neurospora sp., Hypocrea sp., and Emericella sp.* As used herein, the term "*Trichoderma*" or "*Trichoderma sp.*" refers to any fungal strains which have previously been classified as *Trichoderma* or are currently classified as *Trichoderma*.

[89] In one preferred embodiment, the filamentous fungal parent cell is an *Aspergillus niger*, *Aspergillus awamori, Aspergillus aculeatus,* or *Aspergillus nidulans* cell.

[90] In another preferred embodiment, the filamentous fungal parent cell is a *Trichoderma reesei* cell.

III. CELLULASES

[91] Cellulases are known in the art as enzymes that hydrolyze cellulose (beta-1,4-glucan or beta D-glucosidic linkages) resulting in the formation of glucose, cellobiose,

cellooligosaccharides, and the like. As set forth above, cellulases have been traditionally divided into three major classes: endoglucanases (EC 3.2.1.4) ("EG"), exoglucanases or cellobiohydrolases (EC 3.2.1.91) ("CBH") and beta-glucosidases (EC 3.2.1.21) ("BG"). (Knowles, *et al.*, TIBTECH 5, 255-261, 1987; Schulein, 1988).

[92] Certain fungi produce complete cellulase systems which include exo-cellobiohydrolases or CBH-type cellulases, endoglucanases or EG-type cellulases and beta-glucosidases or BG-type cellulases (Schulein, 1988). However, sometimes these systems lack CBH-type cellulases and bacterial cellulases also typically include little or no CBH-type cellulases. In addition, it has been shown that the EG components and CBH components synergistically interact to more efficiently degrade cellulose. See, *e.g.*, Wood, 1985. The different components, *i.e.*, the various endoglucanases and exocellobiohydrolases in a multi-component or complete cellulase system, generally have different properties, such as isoelectric point, molecular weight, degree of glycosylation, substrate specificity and enzymatic action patterns.

[93] It is believed that endoglucanase-type cellulases hydrolyze internal beta -1,4-glucosidic bonds in regions of low crystallinity of the cellulose and exo-cellobiohydrolase-type cellulases hydrolyze cellobiose from the reducing or non-reducing end of cellulose. It follows that the action of endoglucanase components can greatly facilitate the action of exo-cellobiohydrolases by creating new chain ends which are recognized by exo-cellobiohydrolase components. Further, beta-glucosidase-type cellulases have been shown to catalyze the hydrolysis of alkyl and/or aryl β-D-glucosides such as methyl β-D-glucoside and p-nitrophenyl glucoside as well as glycosides containing only carbohydrate residues, such as cellobiose. This yields glucose as the sole product for the microorganism and reduces or eliminates cellobiose which inhibits cellobiohydrolases and endoglucanases.

[94] Cellulases also find a number of uses in detergent compositions including to enhance cleaning ability, as a softening agent and to improve the feel of cotton fabrics (Hemmpel, ITB Dyeing/Printing/Finishing 3:5-14, 1991; Tyndall, Textile Chemist and Colorist 24:23-26, 1992; Kumar *et al.*, Textile Chemist and Colorist, 29:37-42, 1997). While the mechanism is not part of the invention, softening and color restoration properties of cellulase have been attributed to the alkaline endoglucanase components in cellulase compositions, as exemplified by U.S. Patent Nos. 5,648,263, 5,691,178, and 5,776,757, which disclose that detergent compositions containing a cellulase composition enriched in a specified alkaline endoglucanase component impart color restoration and improved softening to treated garments as compared to cellulase compositions not enriched in such

a component. In addition, the use of such alkaline endoglucanase components in detergent compositions has been shown to complement the pH requirements of the detergent composition (*e.g.*, by exhibiting maximal activity at an alkaline pH of 7.5 to 10, as described in U.S. Patent Nos. 5,648,263, 5,691,178, and 5,776,757).

[95] Cellulase compositions have also been shown to degrade cotton-containing fabrics, resulting in reduced strength loss in the fabric (U.S. Patent No. 4,822,516), contributing to reluctance to use cellulase compositions in commercial detergent applications. Cellulase compositions comprising endoglucanase components have been suggested to exhibit reduced strength loss for cotton-containing fabrics as compared to compositions comprising a complete cellulase system.

[96] Cellulases have also been shown to be useful in degradation of cellulase biomass to ethanol (wherein the cellulase degrades cellulose to glucose and yeast or other microbes further ferment the glucose into ethanol), in the treatment of mechanical pulp (Pere *et al.*, 1996), for use as a feed additive (WO 91/04673) and in grain wet milling.

[97] Most CBHs and EGs have a multidomain structure consisting of a core domain separated from a cellulose binding domain (CBD) by a linker peptide (Suurnakki *et al.*, 2000). The core domain contains the active site whereas the CBD interacts with cellulose by binding the enzyme to it (van Tilbeurgh *et al.*, 1986; Tomme *et al.*, Eur. J. Biochem. 170:575-581, 1988). The CBDs are particularly important in the hydrolysis of crystalline cellulose. It has been shown that the ability of cellobiohydrolases to degrade crystalline cellulose clearly decreases when the CBD is absent (Linder and Teeri, J. Biotechnol. 57:15-28, 1997). However, the exact role and action mechanism of CBDs is still a matter of speculation. It has been suggested that the CBD enhances the enzymatic activity merely by increasing the effective enzyme concentration at the surface of cellulose (Stahlberg *et al.*, Bio/Technol. 9:286-290, 1991), and/or by loosening single cellulose chains from the cellulose surface (Tormo *et al.*, EMBO J. vol. 15, no. 21, pp. 5739-5751, 1996). Most studies concerning the effects of cellulase domains on different substrates have been carried out with core proteins of cellobiohydrolases, as their core proteins can easily be produced by limited proteolysis with papain (Tomme *et al.*, 1988). Numerous cellulases have been described in the scientific literature, examples of which include: from *Trichoderma reesei*: Shoemaker, S. et al., Bio/Technology, 1:691-696, 1983, which discloses *CBHI*; Teeri, T. et al., Gene, 51:43-52, 1987, which discloses *CBHII*. Cellulases from species other than Trichoderma have also been described e.g., Ooi *et al.*, Nucleic Acids Research, vol. 18, no. 19, 1990, which discloses the cDNA sequence coding for endoglucanase F1-CMC produced by *Aspergillus aculeatus*; Kawaguchi T *et al.*, Gene

173(2):287-8, 1996, which discloses the cloning and sequencing of the cDNA encoding beta-glucosidase 1 from *Aspergillus aculeatus*; Sakamoto *et al.*, Curr. Genet. 27:435-439, 1995, which discloses the cDNA sequence encoding the endoglucanase CMCase-1 from *Aspergillus kawachii* IFO 4308; Saarilahti *et al.*, Gene 90:9-14, 1990, which discloses an endoglucanase from *Erwinia carotovara*; Spilliaert R, *et al.*, Eur J Biochem. 224(3):923-30, 1994, which discloses the cloning and sequencing of bglA, coding for a thermostable beta-glucanase from *Rhodothermus marinu*; and Halldorsdottir S *et al.*, Appl Microbiol Biotechnol. 49(3):277-84, 1998, which discloses the cloning, sequencing and overexpression of a *Rhodothermus marinus* gene encoding a thermostable cellulase of glycosyl hydrolase family 12. However, there remains a need for identification and characterization of novel cellulases, with improved properties, such as improved performance under conditions of thermal stress or in the presence of surfactants, increased specific activity, altered substrate cleavage pattern, and/or high level expression *in vitro*.

[98] The development of new and improved cellulase compositions that comprise varying amounts CBH-type, EG-type and BG-type cellulases is of interest for use: (1) in detergent compositions that exhibit enhanced cleaning ability, function as a softening agent and/or improve the feel of cotton fabrics (*e.g.*, "stone washing" or "biopolishing"); (2) in compositions for degrading wood pulp or other biomass into sugars (*e.g.*, for bio-ethanol production); and/or (3) in feed compositions.

IV. MOLECULAR BIOLOGY

[99] In one embodiment this invention provides for the expression of variant CBH I genes under control of a promoter functional in a filamentous fungus. Therefore, this invention relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology* (1994)).

A. Methods for Identifying Homologous CBH1 Genes

[100] The nucleic acid sequence for the wild type *H. jecorina* CBH1 is shown in Figure 1 (SEQ ID NO:1). The invention, in one aspect, encompasses a nucleic acid molecule encoding a CBH1 homolog described herein. The nucleic acid may be a DNA molecule.

[101] Techniques that can be used to isolate CBH I encoding DNA sequences are well known in the art and include, but are not limited to, cDNA and/or genomic library screening with a homologous DNA probe and expression screening with activity assays or antibodies against CBH I. Any of these methods can be found in Sambrook, *et al.* or in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, F. Ausubel, *et al.*, ed. Greene Publishing and Wiley-Interscience, New York (1987) ("Ausubel").

B. Methods of Mutating CBH I Nucleic Acid Sequences

[102] Any method known in the art that can introduce mutations is contemplated by the present invention.

[103] The present invention relates to the expression, purification and/or isolation and use of variant CBH1. These enzymes are preferably prepared by recombinant methods utilizing the *cbh* gene from *H. jecorina*.

[104] After the isolation and cloning of the *cbh1* gene from *H. jecorina*, other methods known in the art, such as site directed mutagenesis, are used to make the substitutions, additions or deletions that correspond to substituted amino acids in the expressed CBH1 variant. Again, site directed mutagenesis and other methods of incorporating amino acid changes in expressed proteins at the DNA level can be found in Sambrook, *et al.* and Ausubel, *et al.*

[105] DNA encoding an amino acid sequence variant of the *H. jecorina* CBH1 is prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared DNA encoding the *H. jecorina* CBH1.

[106] Site-directed mutagenesis is a preferred method for preparing substitution variants. This technique is well known in the art (see, e.g.,Carter et al. Nucleic Acids Res. 13:4431-4443 (1985) and Kunkel et al., Proc. Natl. Acad.Sci.USA 82:488 (1987)). Briefly, in carrying out site-directed mutagenesis of DNA, the starting DNA is altered by first hybridizing an oligonucleotide encoding the desired mutation to a single strand of such starting DNA. After hybridization, a DNA polymerase is used to synthesize an entire second strand, using the hybridized oligonucleotide as a primer, and using the single strand of the starting DNA as a template. Thus, the oligonucleotide encoding the desired mutation is incorporated in the resulting double-stranded DNA.

[107] PCR mutagenesis is also suitable for making amino acid sequence variants of the starting polypeptide, i.e., *H. jecorina* CBH1. See Higuchi, in PCR Protocols, pp.177-183 (Academic Press, 1990); and Vallette et al., Nuc. Acids Res. 17:723-733 (1989). See,

also, for example Cadwell et al., PCR Methods and Applications, Vol 2, 28-33 (1992). Briefly, when small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template.

[108] Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al., Gene 34:315-323 (1985). The starting material is the plasmid (or other vector) comprising the starting polypeptide DNA to be mutated. The codon(s) in the starting DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the starting polypeptide DNA. The plasmid DNA is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures, wherein the two strands of the oligonucleotide are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 5' and 3' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated DNA sequence.

[109] Alternatively, or additionally, the desired amino acid sequence encoding a variant CBH I can be determined, and a nucleic acid sequence encoding such amino acid sequence variant can be generated synthetically.

[110] The variant CBH I(s) so prepared may be subjected to further modifications, oftentimes depending on the intended use of the cellulase. Such modifications may involve further alteration of the amino acid sequence, fusion to heterologous polypeptide(s) and/or covalent modifications.

V. *cbh1* Nucleic Acids And CBH1 Polypeptides.

A. Variant *cbh-type* Nucleic acids

[111] The nucleic acid sequence for the wild type *H. jecorina* CBH I is shown in Figure 1 (SEQ ID NO:1). The invention encompasses a nucleic acid molecule encoding the variant cellulases described herein. The nucleic acid may be a DNA molecule.

[112] After the isolation and cloning of the CBH I, other methods known in the art, such as site directed mutagenesis, are used to make the substitutions, additions or deletions

that correspond to substituted amino acids in the expressed CBH I variant. Again, site directed mutagenesis and other methods of incorporating amino acid changes in expressed proteins at the DNA level can be found in Sambrook, *et al.* and Ausubel, *et al.*

[113] After DNA sequences that encode the CBH1 variants have been cloned into DNA constructs, the DNA is used to transform microorganisms. The microorganism to be transformed for the purpose of expressing a variant CBH1 according to the present invention may advantageously comprise a strain derived from *Trichoderma sp.* Thus, a preferred mode for preparing variant CBH1 cellulases according to the present invention comprises transforming a *Trichoderma sp.* host cell with a DNA construct comprising at least a fragment of DNA encoding a portion or all of the variant CBH1. The DNA construct will generally be functionally attached to a promoter. The transformed host cell is then grown under conditions so as to express the desired protein. Subsequently, the desired protein product is purified to substantial homogeneity.

[114] However, it may in fact be that the best expression vehicle for a given DNA encoding a variant CBH1 may differ from *H. jecorina*. Thus, it may be that it will be most advantageous to express a protein in a transformation host that bears phylogenetic similarity to the source organism for the variant CBH1. In an alternative embodiment, *Aspergillus niger* can be used as an expression vehicle. For a description of transformation techniques with *A. niger*, see WO 98/31821.

[115] Accordingly, the present description of a *Trichoderma spp.* expression system is provided for illustrative purposes only and as one option for expressing the variant CBH1 of the invention. One of skill in the art, however, may be inclined to express the DNA encoding variant CBH1 in a different host cell if appropriate and it should be understood that the source of the variant CBH1 should be considered in determining the optimal expression host. Additionally, the skilled worker in the field will be capable of selecting the best expression system for a particular gene through routine techniques utilizing the tools available in the art.

B. Variant CBH1 Polypeptides

[116] The amino acid sequence for the wild type *H. jecorina* CBH I is shown in Figure 1 (SEQ ID NO:2). The variant CBH I polypeptides comprises a substitution or deletion at a position corresponding to one or more of residues S8, Q17, G22, T41, N49, S57, N64, A68, A77, N89, S92, N103, A112, S113, E193, S196, M213, L225, T226, P227, T246, D249, R251, Y252, T255, D257, D259, S278, S279, K286, L288, E295, T296, S297, A299, N301, E325, T332, F338, S342, F352, T356, Y371, T380, Y381, V393, R394, S398, V403, S411.

G430, G440, T445, T462, T484, Q487, and P491 in CBH I from *Hypocrea jecorina*. Furthermore, the variant may further comprises a deletion of residues corresponding to residues 382-393 in CBH I from *Hypocrea jecorina*.

[117] The variant CBH I's of this invention have amino acid sequences that are derived from the amino acid sequence of a precursor CBH I. The amino acid sequence of the CBH I variant differs from the precursor CBH I amino acid sequence by the substitution, deletion or insertion of one or more amino acids of the precursor amino acid sequence. In a preferred embodiment, the precursor CBH I is *Hypocrea jecorina* CBH I. The mature amino acid sequence of *H. jecorina* CBH I is shown in Figure 1 (SEQ ID NO:2). Thus, this invention is directed to CBH I variants which contain amino acid residues at positions which are equivalent to the particular identified residue in *H. jecorina* CBH I. A residue (amino acid) of an CBH I homolog is equivalent to a residue of *Hypocrea jecorina* CBH I if it is either homologous (*i.e.*, corresponding in position in either primary or tertiary structure) or is functionally analogous to a specific residue or portion of that residue in *Hypocrea jecorina* CBH I (*i.e.*, having the same or similar functional capacity to combine, react, or interact chemically or structurally). As used herein, numbering is intended to correspond to that of the mature CBH I amino acid sequence as illustrated in Figure 1 (SEQ ID NO:2). In addition to locations within the precursor CBH I, specific residues in the precursor CBH I corresponding to the amino acid positions that are responsible for instability when the precursor CBH I is under thermal stress are identified herein for substitution or deletion. The amino acid position number (*e.g.*, +51) refers to the number assigned to the mature *Hypocrea jecorina* CBH I sequence presented in Figure 1 (SEQ ID NO:2).

[118] The variant CBH1's of this invention have amino acid sequences that are derived from the amino acid sequence of a precursor *H. jecorina* CBH1. The amino acid sequence of the CBH1 variant differs from the precursor CBH1 amino acid sequence by the substitution, deletion or insertion of one or more amino acids of the precursor amino acid sequence. The mature amino acid sequence of *H. jecorina* CBH1 is shown in Figure 1 (SEQ ID NO:2). Thus, this invention is directed to CBH1 variants which contain amino acid residues at positions which are equivalent to the particular identified residue in *H. jecorina* CBH1. A residue (amino acid) of an CBH1 variant is equivalent to a residue of *Hypocrea jecorina* CBH1 if it is either homologous (*i.e.*, corresponding in position in either primary or tertiary structure) or is functionally analogous to a specific residue or portion of that residue in *Hypocrea jecorina* CBH1 (*i.e.*, having the same or similar functional capacity to combine, react, or interact chemically or structurally). As used herein, numbering is intended to correspond to that of the mature CBH1 amino acid sequence as illustrated in Figure 1 (SEQ ID NO:2). In

addition to locations within the precursor CBH1, specific residues in the precursor CBH1 corresponding to the amino acid positions that are responsible for instability when the precursor CBH1 is under thermal stress are identified herein for substitution or deletion. The amino acid position number (*e.g.*, +51) refers to the number assigned to the mature *Hypocrea jecorina* CBH1 sequence presented in Figure 1 (SEQ ID NO:2).

[119] Alignment of amino acid sequences to determine homology is preferably determined by using a "sequence comparison algorithm." Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* **2**:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* **48**:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l Acad. Sci. USA* **85**:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), by visual inspection or MOE by Chemical Computing Group, Montreal Canada.

[120] An example of an algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul, *et al., J. Mol. Biol.* **215**:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. These initial neighborhood word hits act as starting points to find longer HSPs containing them. The word hits are expanded in both directions along each of the two sequences being compared for as far as the cumulative alignment score can be increased. Extension of the word hits is stopped when: the cumulative alignment score falls off by the quantity X from a maximum achieved value; the cumulative score goes to zero or below; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* **89**:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M'5, N'-4, and a comparison of both strands.

[121] The BLAST algorithm then performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* **90**:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum

probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, an amino acid sequence is considered similar to a protease if the smallest sum probability in a comparison of the test amino acid sequence to a protease amino acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[122] Additional specific strategies for modifying stability of CBH1 cellulases are provided below:

[123] (1) Decreasing the entropy of main-chain unfolding may introduce stability to the enzyme. For example, the introduction of proline residues may significantly stabilize the protein by decreasing the entropy of the unfolding (*see, e.g.,* Watanabe, *et al., Eur. J. Biochem.* **226**:277-283 (1994)). Similarly, glycine residues have no β-carbon, and thus have considerably greater backbone conformational freedom than many other residues. Replacement of glycines, preferably with alanines, may reduce the entropy of unfolding and improve stability (*see, e.g.*, Matthews, *et al.*, *Proc. Natl. Acad. Sci. USA* **84**; 6663-6667 (1987)). Additionally, by shortening external loops it may be possible to improve stability. It has been observed that hyperthermophile produced proteins have shorter external loops than their mesophilic homologues (*see, e.g.,* Russel, *et al., Current Opinions in Biotechnology* **6**:370-374 (1995)). The introduction of disulfide bonds may also be effective to stabilize distinct tertiary structures in relation to each other. Thus, the introduction of cysteines at residues accessible to existing cysteines or the introduction of pairs of cysteines that could form disulfide bonds would alter the stability of a CBH1 variant.

[124] (2) Decreasing internal cavities by increasing side-chain hydrophobicity may alter the stability of an enzyme. Reducing the number and volume of internal cavities increases the stability of enzyme by maximizing hydrophobic interactions and reducing packing defects (*see, e.g.,* Matthews, *Ann. Rev. Biochem.* **62**:139-160 (1993); Burley, *et al., Science* **229**:23-29 (1985); Zuber, *Biophys. Chem.* **29**:171-179 (1988); Kellis, *et al., Nature* **333**:784-786 (1988)). It is known that multimeric proteins from thermophiles often have more hydrophobic sub-unit interfaces with greater surface complementarity than their mesophilic counterparts (Russel, *et al., supra*). This principle is believed to be applicable to domain interfaces of monomeric proteins. Specific substitutions that may improve stability by increasing hydrophobicity include lysine to arginine, serine to alanine and threonine to alanine (Russel, *et al., supra*). Modification by substitution to alanine or proline may increase side-chain size with resultant reduction in cavities, better packing

and increased hydrophobicity. Substitutions to reduce the size of the cavity, increase hydrophobicity and improve the complementarity the interfaces between the domains of CBH1 may improve stability of the enzyme. Specifically, modification of the specific residue at these positions with a different residue selected from any of phenylalanine, tryptophan, tyrosine, leucine and isoleucine may improve performance.

[125] (3) Balancing charge in rigid secondary structure, *i.e.,* α-helices and β-turns may improve stability. For example, neutralizing partial positive charges on a helix N-terminus with negative charge on aspartic acid may improve stability of the structure (*see, e.g.*, Eriksson, *et al., Science* **255**:178-183 (1992)). Similarly, neutralizing partial negative charges on helix C-terminus with positive charge may improve stability. Removing positive charge from interacting with peptide N-terminus in β-turns should be effective in conferring tertiary structure stability. Substitution with a non-positively charged residue could remove an unfavorable positive charge from interacting with an amide nitrogen present in a turn.

[126] (4) Introducing salt bridges and hydrogen bonds to stabilize tertiary structures may be effective. For example, ion pair interactions, *e.g.,* between aspartic acid or glutamic acid and lysine, arginine or histidine, may introduce strong stabilizing effects and may be used to attach different tertiary structure elements with a resultant improvement in thermostability. Additionally, increases in the number of charged residue/non-charged residue hydrogen bonds, and the number of hydrogen-bonds generally, may improve thermostability (*see, e.g.,* Tanner, *et al., Biochemistry* **35**:2597-2609 (1996)). Substitution with aspartic acid, asparagine, glutamic acid or glutamine may introduce a hydrogen bond with a backbone amide. Substitution with arginine may improve a salt bridge and introduce an H-bond into a backbone carbonyl.

[127] (5) Avoiding thermolabile residues in general may increase thermal stability. For example, asparagine and glutamine are susceptible to deamidation and cysteine is susceptible to oxidation at high temperatures. Reducing the number of these residues in sensitive positions may result in improved thermostability (Russel, *et al., supra*). Substitution or deletion by any residue other than glutamine or cysteine may increase stability by avoidance of a thermolabile residue.

[128] (6) Stabilization or destabilization of binding of a ligand that confers modified stability to CBH1 variants. For example, a component of the matrix in which the CBH1 variants of this invention are used may bind to a specific surfactant/thermal sensitivity site of the CBH1 variant. By modifying the site through substitution, binding of the component to the variant may be strengthened or diminished. For example, a non-aromatic residue in

the binding crevice of CBH1 may be substituted with phenylalanine or tyrosine to introduce aromatic side-chain stabilization where interaction of the cellulose substrate may interact favorably with the benzyl rings, increasing the stability of the CBH1 variant.

[129] (7) Increasing the electronegativity of any of the surfactant/ thermal sensitivity ligands may improve stability under surfactant or thermal stress. For example, substitution with phenylalanine or tyrosine may increase the electronegativity of D (aspartate) residues by improving shielding from solvent, thereby improving stability.

C. Anti-CBH Antibodies

[130] The present invention further provides anti-CBH antibodies. The antibodies may be polyclonal, monoclonal, humanized, bispecific or heteroconjugate antibodies.

[131] Methods of preparing polyclonal antibodies are known to the skilled artisan. The immunizing agent may be an CBH polypeptide or a fusion protein thereof. It may be useful to conjugate the antigen to a protein known to be immunogenic in the mammal being immunized. The immunization protocol may be determined by one skilled in the art based on standard protocols or routine experimentation.

[132] Alternatively, the anti-CBH antibodies may be monoclonal antibodies. Monoclonal antibodies may be produced by cells immunized in an animal or using recombinant DNA methods. (See, *e.g.*, Kohler *et al.*, *Nature*, vol. 256, pp. 495-499, August 7, 1975; U.S. Patent No. 4,816,567).

[133] An anti-CBH antibody of the invention may further comprise a humanized or human antibody. The term "humanized antibody" refers to humanized forms of non-human (*e.g.*, murine) antibodies that are chimeric antibodies, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding partial sequences of antibodies) which contain some portion of the sequence derived from non-human antibody. Methods for humanizing non-human antibodies are well known in the art, as further detailed in Jones *et al.*, Nature 321:522-525, 1986; Riechmann *et al.*, Nature, vol. 332, pp. 323-327, 1988; and Verhoeyen *et al.*, Science, vol. 239, pp. 1534-1536, 1988. Methods for producing human antibodies are also known in the art. See, *e.g.*, Jakobovits, A, *et al.*, Annals New York Academy of Sciences, 764:525-535, 1995 and Jakobovits, A, Curr Opin Biotechnol 6(5):561-6, 1995.

VI. Expression Of Recombinant CBH1 Variants

[134] The methods of the invention rely on the use cells to express variant CBH I, with no particular method of CBH I expression required.

[135] The invention provides host cells which have been transduced, transformed or transfected with an expression vector comprising a variant CBH-encoding nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used for the parental host cell prior to transduction, transformation or transfection and will be apparent to those skilled in the art.

[136] In one approach, a filamentous fungal cell or yeast cell is transfected with an expression vector having a promoter or biologically active promoter fragment or one or more (*e.g.*, a series) of enhancers which functions in the host cell line, operably linked to a DNA segment encoding CBH, such that CBH is expressed in the cell line.

A. Nucleic Acid Constructs/Expression Vectors.

[137] Natural or synthetic polynucleotide fragments encoding CBH I ("CBH I-encoding nucleic acid sequences") may be incorporated into heterologous nucleic acid constructs or vectors, capable of introduction into, and replication in, a filamentous fungal or yeast cell. The vectors and methods disclosed herein are suitable for use in host cells for the expression of CBH I. Any vector may be used as long as it is replicable and viable in the cells into which it is introduced. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Cloning and expression vectors are also described in Sambrook *et al.,* 1989, Ausubel FM *et al.,* 1989, and Strathern *et al.*, The Molecular Biology of the Yeast *Saccharomyces*, 1981.

Appropriate expression vectors for fungi are described in van den Hondel, C.A.M.J.J. et al. (1991) In: Bennett, J.W. and Lasure, L.L. (eds.) More Gene Manipulations in Fungi. Academic Press, pp. 396-428. The appropriate DNA sequence may be inserted into a plasmid or vector (collectively referred to herein as "vectors") by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by standard procedures. Such procedures and related sub-cloning procedures are deemed to be within the scope of knowledge of those skilled in the art.

[138] Recombinant filamentous fungi comprising the coding sequence for variant CBH I may be produced by introducing a heterologous nucleic acid construct comprising the variant CBH I coding sequence into the cells of a selected strain of the filamentous fungi.

[139] Once the desired form of a variant *cbh* nucleic acid sequence is obtained, it may be modified in a variety of ways. Where the sequence involves non-coding flanking regions, the flanking regions may be subjected to resection, mutagenesis, etc. Thus, transitions, transversions, deletions, and insertions may be performed on the naturally occurring sequence.

[140] A selected variant *cbh* coding sequence may be inserted into a suitable vector according to well-known recombinant techniques and used to transform filamentous fungi capable of CBH I expression. Due to the inherent degeneracy of the genetic code, other nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used to clone and express variant CBH I. Therefore it is appreciated that such substitutions in the coding region fall within the sequence variants covered by the present invention. Any and all of these sequence variants can be utilized in the same way as described herein for a parent CBH I-encoding nucleic acid sequence.

[141] The present invention also includes recombinant nucleic acid constructs comprising one or more of the variant CBH I-encoding nucleic acid sequences as described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation.

[142] Heterologous nucleic acid constructs may include the coding sequence for variant *cbh*: (i) in isolation; (ii) in combination with additional coding sequences; such as fusion protein or signal peptide coding sequences, where the *cbh* coding sequence is the dominant coding sequence; (iii) in combination with non-coding sequences, such as introns and control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; and/or (iv) in a vector or host environment in which the *cbh* coding sequence is a heterologous gene.

[143] In one aspect of the present invention, a heterologous nucleic acid construct is employed to transfer a variant CBH I-encoding nucleic acid sequence into a cell *in vitro*, with established filamentous fungal and yeast lines preferred. For long-term, production of variant CBH I, stable expression is preferred. It follows that any method effective to generate stable transformants may be used in practicing the invention.

[144] Appropriate vectors are typically equipped with a selectable marker-encoding nucleic acid sequence, insertion sites, and suitable control elements, such as promoter and termination sequences. The vector may comprise regulatory sequences, including, for example, non-coding sequences, such as introns and control elements, *i.e.*, promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in host cells (and/or in a vector or host cell environment in which a modified soluble protein antigen coding sequence is not normally expressed), operably linked to the coding sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, many of which are commercially available and/or are described in Sambrook, *et al.*, (*supra*).

[145] Exemplary promoters include both constitutive promoters and inducible promoters, examples of which include a CMV promoter, an SV40 early promoter, an RSV promoter, an EF-1α promoter, a promoter containing the tet responsive element (TRE) in the tet-on or tet-off system as described (ClonTech and BASF), the beta actin promoter and the metallothionine promoter that can upregulated by addition of certain metal salts. A promoter sequence is a DNA sequence which is recognized by the particular filamentous fungus for expression purposes. It is operably linked to DNA sequence encoding a variant CBH I polypeptide. Such linkage comprises positioning of the promoter with respect to the initiation codon of the DNA sequence encoding the variant CBH I polypeptide in the disclosed expression vectors. The promoter sequence contains transcription and translation control sequence which mediate the expression of the variant CBH I polypeptide. Examples include the promoters from the *Aspergillus niger*, *A awamori* or *A. oryzae* glucoamylase, alpha-amylase, or alpha-glucosidase encoding genes; the *A. nidulans gpdA* or *trpC* Genes; the *Neurospora crassa cbh1* or *trp1* genes; the *A. niger* or *Rhizomucor miehei* aspartic proteinase encoding genes; the *H. jecorina* (*T. reesei) cbh1*, *cbh2*, *egl1*, *egl2*, or other cellulase encoding genes.

[146] The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art. Typical selectable marker genes include argB from *A. nidulans* or *T. reesei*, amdS from *A. nidulans*, pyr4 from *Neurospora crassa* or *T. reesei*, pyrG from *Aspergillus niger or A. nidulans*. Additional exemplary selectable markers include, but are not limited to trpc, trp1, oliC31, niaD or leu2, which are included in heterologous nucleic acid constructs used to transform a mutant strain such as trp-, pyr-, leu- and the like.

[147] Such selectable markers confer to transformants the ability to utilize a metabolite that is usually not metabolized by the filamentous fungi. For example, the amdS gene from *H. jecorina* which encodes the enzyme acetamidase that allows transformant cells to grow on acetamide as a nitrogen source. The selectable marker (e.g. pyrG) may restore the ability of an auxotrophic mutant strain to grow on a selective minimal medium or the selectable marker (e.g. olic31) may confer to transformants the ability to grow in the presence of an inhibitory drug or antibiotic.

[148] The selectable marker coding sequence is cloned into any suitable plasmid using methods generally employed in the art. Exemplary plasmids include pUC18, pBR322, pRAX and pUC100. The pRAX plasmid contains AMA1 sequences from A. nidulans, which make it possible to replicate in A. niger.

[149] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook *et al.*, 1989; Freshney, Animal Cell Culture, 1987; Ausubel, *et al.*, 1993; and Coligan *et al.*, Current Protocols in Immunology, 1991.

B. Host Cells and Culture Conditions For CBH1 Production

(i) Filamentous Fungi

[150] Thus, the present invention provides filamentous fungi comprising cells which have been modified, selected and cultured in a manner effective to result in variant CBH I production or expression relative to the corresponding non-transformed parental fungi.

[151] Examples of species of parental filamentous fungi that may be treated and/or modified for variant CBH I expression include, but are not limited to *Trichoderma, e.g., Trichoderma reesei, Trichoderma longibrachiatum , Trichoderma viride, Trichoderma koningii; Penicillium sp., Humicola sp.*, including *Humicola insolens; Aspergillus sp., Chrysosporium sp., Fusarium sp.*, *Hypocrea* sp., and *Emericella* sp.

[152] CBH I expressing cells are cultured under conditions typically employed to culture the parental fungal line. Generally, cells are cultured in a standard medium containing physiological salts and nutrients, such as described in Pourquie, J. et al., Biochemistry and Genetics of Cellulose Degradation, eds. Aubert, J. P. et al., Academic Press, pp. 71-86, 1988 and Ilmen, M. et al., Appl. Environ. Microbiol. 63:1298-1306, 1997. Culture conditions are also standard, *e.g.*, cultures are incubated at 28°C in shaker cultures or fermenters until desired levels of CBH I expression are achieved.

[153] Preferred culture conditions for a given filamentous fungus may be found in the scientific literature and/or from the source of the fungi such as the American Type Culture Collection. After fungal growth has been established, the cells are exposed to conditions effective to cause or permit the expression of variant CBH I.

[154] In cases where a CBH I coding sequence is under the control of an inducible promoter, the inducing agent, *e.g.*, a sugar, metal salt or antibiotics, is added to the medium at a concentration effective to induce CBH I expression.

[155] In one embodiment, the strain comprises *Aspergillus niger,* which is a useful strain for obtaining overexpressed protein. For example A. niger var awamori dgr246 is known to secrete elevated amounts of secreted cellulases (Goedegebuur et al, Curr. Genet (2002) 41: 89-98). Other strains of Aspergillus niger var awamori such as GCDAP3,

GCDAP4 and GAP3-4 are known Ward et al (Ward, M, Wilson, L.J. and Kodama, K.H., 1993, Appl. Microbiol. Biotechnol. 39:738-743).

[156] In another embodiment, the strain comprises *Trichoderma reesei,* which is a useful strain for obtaining overexpressed protein. For example, RL-P37, described by Sheir-Neiss, *et al., Appl. Microbiol. Biotechnol.* 20:46-53 (1984) is known to secrete elevated amounts of cellulase enzymes. Functional equivalents of RL-P37 include *Trichoderma reesei* strain RUT-C30 (ATCC No. 56765) and strain QM9414 (ATCC No. 26921). It is contemplated that these strains would also be useful in overexpressing variant CBH1.

[157] Where it is desired to obtain the variant CBH I in the absence of potentially detrimental native cellulolytic activity, it is useful to obtain a *Trichoderma* host cell strain which has had one or more cellulase genes deleted prior to introduction of a DNA construct or plasmid containing the DNA fragment encoding the variant CBH I. Such strains may be prepared by the method disclosed in U.S. Patent No. 5,246,853 and WO 92/06209. By expressing a variant CBH I cellulase in a host microorganism that is missing one or more cellulase genes, the identification and subsequent purification procedures are simplified. Any gene from *Trichoderma sp.* which has been cloned can be deleted, for example, the *cbh1*, *cbh2*, *egl1*, and *egl2* genes as well as those encoding EG III and/or EGV protein (*see e.g.*, U.S. Patent No. 5,475,101 and WO 94/28117, respectively).

[158] Gene deletion may be accomplished by inserting a form of the desired gene to be deleted or disrupted into a plasmid by methods known in the art. The deletion plasmid is then cut at an appropriate restriction enzyme site(s), internal to the desired gene coding region, and the gene coding sequence or part thereof replaced with a selectable marker. Flanking DNA sequences from the locus of the gene to be deleted or disrupted, preferably between about 0.5 to 2.0 kb, remain on either side of the selectable marker gene. An appropriate deletion plasmid will generally have unique restriction enzyme sites present therein to enable the fragment containing the deleted gene, including flanking DNA sequences, and the selectable marker gene to be removed as a single linear piece.

[159] A selectable marker must be chosen so as to enable detection of the transformed microorganism. Any selectable marker gene that is expressed in the selected microorganism will be suitable. For example, with *Aspergillus sp.*, the selectable marker is chosen so that the presence of the selectable marker in the transformants will not significantly affect the properties thereof. Such a selectable marker may be a gene that encodes an assayable product. For example, a functional copy of a *Aspergillus sp.* gene

may be used which if lacking in the host strain results in the host strain displaying an auxotrophic phenotype. Similarly, selectable markers exist for *Trichoderma sp.*

[160] In one embodiment, a *pyrG*⁻ derivative strain of *Aspergillus sp.* is transformed with a functional *pyrG* gene, which thus provides a selectable marker for transformation. A *pyrG*⁻ derivative strain may be obtained by selection of *Aspergillus sp.* strains that are resistant to fluoroorotic acid (FOA). The *pyrG* gene encodes orotidine-5'-monophosphate decarboxylase, an enzyme required for the biosynthesis of uridine. Strains with an intact *pyrG* gene grow in a medium lacking uridine but are sensitive to fluoroorotic acid. It is possible to select *pyrG*⁻ derivative strains that lack a functional orotidine monophosphate decarboxylase enzyme and require uridine for growth by selecting for FOA resistance. Using the FOA selection technique it is also possible to obtain uridine-requiring strains which lack a functional orotate pyrophosphoribosyl transferase. It is possible to transform these cells with a functional copy of the gene encoding this enzyme (Berges & Barreau, *Curr. Genet.* 19:359-365 (1991), and van Hartingsveldte et al., (1986) Development of a homologous transformation system for *Aspergillus niger* based on the *pyrG* gene. Mol. Gen. Genet. 206:71-75). Selection of derivative strains is easily performed using the FOA resistance technique referred to above, and thus, the *pyrG* gene is preferably employed as a selectable marker.

[161] In a second embodiment, a *pyr4*⁻ derivative strain of *Hyprocrea sp. (Hyprocrea sp. (Trichoderma sp.))* is transformed with a functional *pyr4* gene, which thus provides a selectable marker for transformation. A *pyr4*⁻ derivative strain may be obtained by selection of *Hyprocrea sp. (Trichoderma sp.)* strains that are resistant to fluoroorotic acid (FOA). The *pyr4* gene encodes orotidine-5'-monophosphate decarboxylase, an enzyme required for the biosynthesis of uridine. Strains with an intact *pyr4* gene grow in a medium lacking uridine but are sensitive to fluoroorotic acid. It is possible to select *pyr4*⁻ derivative strains that lack a functional orotidine monophosphate decarboxylase enzyme and require uridine for growth by selecting for FOA resistance. Using the FOA selection technique it is also possible to obtain uridine-requiring strains which lack a functional orotate pyrophosphoribosyl transferase. It is possible to transform these cells with a functional copy of the gene encoding this enzyme (Berges & Barreau, *Curr. Genet.* **19**:359-365 (1991)). Selection of derivative strains is easily performed using the FOA resistance technique referred to above, and thus, the *pyr4* gene is preferably employed as a selectable marker.

[162] To transform *pyrG*⁻ *Aspergillus sp.* or *pyr4*⁻ *Hyprocrea sp. (Trichoderma sp.)* so as to be lacking in the ability to express one or more cellulase genes, a single DNA fragment

comprising a disrupted or deleted cellulase gene is then isolated from the deletion plasmid and used to transform an appropriate *pyr⁻ Aspergillus* or *pyr⁻ Trichoderma* host. Transformants are then identified and selected based on their ability to express the *pyrG* or *pyr4,* respecitively, gene product and thus compliment the uridine auxotrophy of the host strain. Southern blot analysis is then carried out on the resultant transformants to identify and confirm a double crossover integration event that replaces part or all of the coding region of the genomic copy of the gene to be deleted with the appropriate *pyr* selectable markers.

[163] Although the specific plasmid vectors described above relate to preparation of *pyr⁻* transformants, the present invention is not limited to these vectors. Various genes can be deleted and replaced in the *Aspergillus sp. or Hyprocrea sp. (Trichoderma sp.)* strain using the above techniques. In addition, any available selectable markers can be used, as discussed above. In fact, any host, e.g., *Aspergillus sp. or Hyprocrea sp.,* gene that has been cloned, and thus identified, can be deleted from the genome using the above-described strategy.

[164] As stated above, the host strains used may be derivatives of *Hyprocrea sp. (Trichoderma sp.)* that lack or have a nonfunctional gene or genes corresponding to the selectable marker chosen. For example, if the selectable marker of *pyrG* is chosen for *Aspergillus sp.,* then a specific *pyrG⁻* derivative strain is used as a recipient in the transformation procedure. Also, for example, if the selectable marker of *pyr4* is chosen for a *Hyprocrea sp.*, then a specific *pyr4⁻* derivative strain is used as a recipient in the transformation procedure. Similarly, selectable markers comprising *Hyprocrea sp. (Trichoderma sp.)* genes equivalent to the *Aspergillus nidulans* genes *amdS, argB, trpC, niaD* may be used. The corresponding recipient strain must therefore be a derivative strain such as *argB⁻, trpC⁻, niaD⁻*, respectively.

[165] DNA encoding the CBH I variant is then prepared for insertion into an appropriate microorganism. According to the present invention, DNA encoding a CBH I variant comprises the DNA necessary to encode for a protein that has functional cellulolytic activity. The DNA fragment encoding the CBH I variant may be functionally attached to a fungal promoter sequence, for example, the promoter of the *glaA* gene in *Aspergillus* or the promoter of the *cbh1* or *egl1* genes in *Trichoderma*.

[166] It is also contemplated that more than one copy of DNA encoding a CBH I variant may be recombined into the strain to facilitate overexpression. The DNA encoding the CBH I variant may be prepared by the construction of an expression vector carrying the DNA encoding the variant. The expression vector carrying the inserted DNA fragment

encoding the CBH I variant may be any vector which is capable of replicating autonomously in a given host organism or of integrating into the DNA of the host, typically a plasmid. In preferred embodiments two types of expression vectors for obtaining expression of genes are contemplated. The first contains DNA sequences in which the promoter, gene-coding region, and terminator sequence all originate from the gene to be expressed. Gene truncation may be obtained where desired by deleting undesired DNA sequences (*e.g.*, coding for unwanted domains) to leave the domain to be expressed under control of its own transcriptional and translational regulatory sequences. A selectable marker may also be contained on the vector allowing the selection for integration into the host of multiple copies of the novel gene sequences.

[167] The second type of expression vector is preassembled and contains sequences required for high-level transcription and a selectable marker. It is contemplated that the coding region for a gene or part thereof can be inserted into this general-purpose expression vector such that it is under the transcriptional control of the expression cassettes promoter and terminator sequences.

[168] For example, in *Aspergillus,* pRAX is such a general-purpose expression vector. Genes or part thereof can be inserted downstream of the strong *glaA* promoter.

[169] For example, in *Hypocrea,* pTEX is such a general-purpose expression vector. Genes or part thereof can be inserted downstream of the strong *cbh*1 promoter.

[170] In the vector, the DNA sequence encoding the CBH I variant of the present invention should be operably linked to transcriptional and translational sequences, *i.e.,* a suitable promoter sequence and signal sequence in reading frame to the structural gene. The promoter may be any DNA sequence that shows transcriptional activity in the host cell and may be derived from genes encoding proteins either homologous or heterologous to the host cell. An optional signal peptide provides for extracellular production of the CBH I variant. The DNA encoding the signal sequence is preferably that which is naturally associated with the gene to be expressed, however the signal sequence from any suitable source, for example an exo-cellobiohydrolase or endoglucanase from *Trichoderma*, is contemplated in the present invention.

[171] The procedures used to ligate the DNA sequences coding for the variant CBH I of the present invention with the promoter, and insertion into suitable vectors are well known in the art.

[172] The DNA vector or construct described above may be introduced in the host cell in accordance with known techniques such as transformation, transfection, microinjection, microporation, biolistic bombardment and the like.

[173] In the preferred transformation technique, it must be taken into account that the permeability of the cell wall to DNA in *Hyprocrea sp. (Trichoderma sp.)* is very low. Accordingly, uptake of the desired DNA sequence, gene or gene fragment is at best minimal. There are a number of methods to increase the permeability of the *Hyprocrea sp. (Trichoderma sp.)* cell wall in the derivative strain (*i.e.,* lacking a functional gene corresponding to the used selectable marker) prior to the transformation process.

[174] The preferred method in the present invention to prepare *Aspergillus sp.* or *Hyprocrea sp. (Trichoderma sp.)* for transformation involves the preparation of protoplasts from fungal mycelium. See Campbell *et al*. Improved transformation efficiency of *A.niger* using homologous *nia*D gene for nitrate reductase. Curr. Genet. 16:53-56; 1989. The mycelium can be obtained from germinated vegetative spores. The mycelium is treated with an enzyme that digests the cell wall resulting in protoplasts. The protoplasts are then protected by the presence of an osmotic stabilizer in the suspending medium. These stabilizers include sorbitol, mannitol, potassium chloride, magnesium sulfate and the like. Usually the concentration of these stabilizers varies between 0.8 M and 1.2 M. It is preferable to use about a 1.2 M solution of sorbitol in the suspension medium.

[175] Uptake of the DNA into the host strain, (*Aspergillus sp.* or *Hyprocrea sp. (Trichoderma sp.),* is dependent upon the calcium ion concentration. Generally between about 10 mM $CaCl_2$ and 50 mM $CaCl_2$ is used in an uptake solution. Besides the need for the calcium ion in the uptake solution, other items generally included are a buffering system such as TE buffer (10 Mm Tris, pH 7.4; 1 mM EDTA) or 10 mM MOPS, pH 6.0 buffer (morpholinepropanesulfonic acid) and polyethylene glycol (PEG). It is believed that the polyethylene glycol acts to fuse the cell membranes thus permitting the contents of the medium to be delivered into the cytoplasm of the host cell, by way of example either *Aspergillus sp.* or *Hyprocrea sp.* strain, and the plasmid DNA is transferred to the nucleus. This fusion frequently leaves multiple copies of the plasmid DNA tenderly integrated into the host chromosome.

[176] Usually a suspension containing the *Aspergillus sp.* protoplasts or cells that have been subjected to a permeability treatment at a density of 10^5 to 10^6/mL, preferably 2 x 10^5/mL are used in transformation. Similarly, a suspension containing the *Hyprocrea sp. (Trichoderma sp.)* protoplasts or cells that have been subjected to a permeability treatment at a density of 10^8 to 10^9/mL, preferably 2 x 10^8/mL are used in transformation. A volume of 100 µL of these protoplasts or cells in an appropriate solution (*e.g.,* 1.2 M sorbitol; 50 mM $CaCl_2$) are mixed with the desired DNA. Generally a high concentration of PEG is added to the uptake solution. From 0.1 to 1 volume of 25% PEG 4000 can be

added to the protoplast suspension. However, it is preferable to add about 0.25 volumes to the protoplast suspension. Additives such as dimethyl sulfoxide, heparin, spermidine, potassium chloride and the like may also be added to the uptake solution and aid in transformation.

[177] Generally, the mixture is then incubated at approximately 0°C for a period of between 10 to 30 minutes. Additional PEG is then added to the mixture to further enhance the uptake of the desired gene or DNA sequence. The 25% PEG 4000 is generally added in volumes of 5 to 15 times the volume of the transformation mixture; however, greater and lesser volumes may be suitable. The 25% PEG 4000 is preferably about 10 times the volume of the transformation mixture. After the PEG is added, the transformation mixture is then incubated either at room temperature or on ice before the addition of a sorbitol and $CaCl_2$ solution. The protoplast suspension is then further added to molten aliquots of a growth medium. This growth medium permits the growth of transformants only. Any growth medium can be used in the present invention that is suitable to grow the desired transformants. However, if *Pyr*$^+$ transformants are being selected it is preferable to use a growth medium that contains no uridine. The subsequent colonies are transferred and purified on a growth medium depleted of uridine.

[178] At this stage, stable transformants may be distinguished from unstable transformants by their faster growth rate and the formation of circular colonies with a smooth, rather than ragged outline on solid culture medium lacking uridine. Additionally, in some cases a further test of stability may made by growing the transformants on solid non-selective medium (*i.e.* containing uridine), harvesting spores from this culture medium and determining the percentage of these spores which will subsequently germinate and grow on selective medium lacking uridine.

[179] In a particular embodiment of the above method, the CBH I variant(s) are recovered in active form from the host cell after growth in liquid media either as a result of the appropriate post translational processing of the CBH I variant.

(ii) Yeast

[180] The present invention also contemplates the use of yeast as a host cell for CBH I production. Several other genes encoding hydrolytic enzymes have been expressed in various strains of the yeast *S. cerevisiae*. These include sequences encoding for two endoglucanases (Penttila *et al.*, Yeast vol. 3, pp 175-185, 1987), two cellobiohydrolases (Penttila *et al.*, Gene, 63: 103-112, 1988) and one beta-glucosidase from *Trichoderma reesei* (Cummings and Fowler, Curr. Genet. 29:227-233, 1996), a xylanase from *Aureobasidlium pullulans* (Li and Ljungdahl, Appl. Environ. Microbiol. 62, no. 1, pp. 209-

213, 1996), an alpha-amylase from wheat (Rothstein *et al.*, Gene 55:353-356, 1987), etc. In addition, a cellulase gene cassette encoding the *Butyrivibrio fibrisolvens* endo- [beta] -1,4-glucanase (END1), *Phanerochaete chrysosporium* cellobiohydrolase (CBH1), the *Ruminococcus flavefaciens* cellodextrinase (CEL1) and the *Endomyces fibrilizer* cellobiase (Bgl1) was successfully expressed in a laboratory strain of *S. cerevisiae* (Van Rensburg *et al.*, Yeast, vol. 14, pp. 67-76, 1998).

C. Introduction of an CBH I-Encoding Nucleic Acid Sequence into Host Cells.

[181] The invention further provides cells and cell compositions which have been genetically modified to comprise an exogenously provided variant CBH I -encoding nucleic acid sequence. A parental cell or cell line may be genetically modified (*i.e.*, transduced, transformed or transfected) with a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc, as further described above.

[182] The methods of transformation of the present invention may result in the stable integration of all or part of the transformation vector into the genome of the filamentous fungus. However, transformation resulting in the maintenance of a self-replicating extra-chromosomal transformation vector is also contemplated.

[183] Many standard transfection methods can be used to produce *Trichoderma reesei* cell lines that express large quantities of the heterologus protein. Some of the published methods for the introduction of DNA constructs into cellulase-producing strains of Trichoderma include Lorito, Hayes, DiPietro and Harman, 1993, Curr. Genet. 24: 349-356; Goldman, VanMontagu and Herrera-Estrella, 1990, Curr. Genet. 17:169-174; Penttila, Nevalainen, Ratto, Salminen and Knowles, 1987, Gene 6: 155-164, for *Aspergillus* Yelton, Hamer and Timberlake, 1984, Proc. Natl. Acad. Sci. USA 81: 1470-1474, for Fusarium Bajar, Podila and Kolattukudy, 1991, Proc. Natl. Acad. Sci. USA 88: 8202-8212, for Streptomyces Hopwood et al., 1985, The John Innes Foundation, Norwich, UK and for Bacillus Brigidi, DeRossi, Bertarini, Riccardi and Matteuzzi, 1990, FEMS Microbiol. Lett. 55: 135-138).

[184] Other methods for introducing a heterologous nucleic acid construct (expression vector) into filamentous fungi (*e.g.*, *H. jecorina*) include, but are not limited to the use of a particle or gene gun, permeabilization of filamentous fungi cells walls prior to the transformation process (*e.g.*, by use of high concentrations of alkali, *e.g.*, 0.05 M to 0.4 M $CaC1_2$ or lithium acetate), protoplast fusion or agrobacterium mediated transformation. An exemplary method for transformation of filamentous fungi by treatment of protoplasts or

spheroplasts with polyethylene glycol and $CaCl_2$ is described in Campbell, E.I. et al., Curr. Genet. 16:53-56, 1989 and Penttila, M. et al., Gene, 63:11-22, 1988.

[185] Any of the well-known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, biolistics, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (*see, e.g.*, Sambrook *et al., supra*). Also of use is the Agrobacterium-mediated transfection method described in U.S. Patent No. 6,255,115. It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing the heterologous gene.

[186] In addition, heterologous nucleic acid constructs comprising a variant CBH I-encoding nucleic acid sequence can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, *e.g.*, by injection.

[187] The invention further includes novel and useful transformants of filamentous fungi such as *H. jecorina* and *A. niger* for use in producing fungal cellulase compositions. The invention includes transformants of filamentous fungi especially fungi comprising the variant CBH I coding sequence, or deletion of the endogenous *cbh* coding sequence.

[188] Following introduction of a heterologous nucleic acid construct comprising the coding sequence for a variant *cbh 1*, the genetically modified cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying expression of a variant CBH I-encoding nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used for the host cell selected for expression, and will be apparent to those skilled in the art.

[189] The progeny of cells into which such heterologous nucleic acid constructs have been introduced are generally considered to comprise the variant CBH I-encoding nucleic acid sequence found in the heterologous nucleic acid construct.

[190] The invention further includes novel and useful transformants of filamentous fungi such as *H. jecorina* for use in producing fungal cellulase compositions. The invention includes transformants of filamentous fungi especially fungi comprising the variant *cbh 1* coding sequence, or deletion of the endogenous *cbh* coding sequence.

[191] Stable transformants of filamentous fungi can generally be distinguished from unstable transformants by their faster growth rate and the formation of circular colonies with a smooth rather than ragged outline on solid culture medium. Additionally, in some

cases, a further test of stability can be made by growing the transformants on solid non-selective medium, harvesting the spores from this culture medium and determining the percentage of these spores which will subsequently germinate and grow on selective medium.

VII. Analysis For CBH1 Nucleic Acid Coding Sequences and/or Protein Expression.

[192] In order to evaluate the expression of a variant CBH I by a cell line that has been transformed with a variant CBH I-encoding nucleic acid construct, assays can be carried out at the protein level, the RNA level or by use of functional bioassays particular to cellobiohydrolase activity and/or production.

[193] In one exemplary application of the variant *cbh 1* nucleic acid and protein sequences described herein, a genetically modified strain of filamentous fungi, *e.g.*, *Trichoderma reesei*, is engineered to produce an increased amount of CBH I. Such genetically modified filamentous fungi would be useful to produce a cellulase product with greater increased cellulolytic capacity. In one approach, this is accomplished by introducing the coding sequence for *cbh 1* into a suitable host, *e.g.*, a filamentous fungi such as *Aspergillus niger*.

[194] Accordingly, the invention includes methods for expressing variant CBH I in a filamentous fungus or other suitable host by introducing an expression vector containing the DNA sequence encoding variant CBH I into cells of the filamentous fungus or other suitable host.

[195] In another aspect, the invention includes methods for modifying the expression of CBH I in a filamentous fungus or other suitable host. Such modification includes a decrease or elimination in expression of the endogenous CBH.

[196] In general, assays employed to analyze the expression of variant CBH I include, Northern blotting, dot blotting (DNA or RNA analysis), RT-PCR (reverse transcriptase polymerase chain reaction), or *in situ* hybridization, using an appropriately labeled probe (based on the nucleic acid coding sequence) and conventional Southern blotting and autoradiography.

[197] In addition, the production and/or expression of variant CBH I may be measured in a sample directly, for example, by assays for cellobiohydrolase activity, expression and/or production. Such assays are described, for example, in Becker et al., Biochem J. (2001) 356:19-30 and Mitsuishi et al., FEBS (1990) 275:135-138.

The ability of CBH I to hydrolyze isolated soluble and insoluble substrates can be measured using assays described in Srisodsuk et al., J.

Biotech. (1997) 57:49-57 and Nidetzky and Claeyssens Biotech. Bioeng. (1994) 44:961-966. Substrates useful for assaying cellobiohydrolase, endoglucanase or β-glucosidase activities include crystalline cellulose, filter paper, phosphoric acid swollen cellulose, cellooligosaccharides, methylumbelliferyl lactoside, methylumbelliferyl cellobioside, orthonitrophenyl lactoside, paranitrophenyl lactoside, orthonitrophenyl cellobioside, paranitrophenyl cellobioside.

[198] In addition, protein expression, may be evaluated by immunological methods, such as immunohistochemical staining of cells, tissue sections or immunoassay of tissue culture medium, *e.g.*, by Western blot or ELISA. Such immunoassays can be used to qualitatively and quantitatively evaluate expression of a CBH I variant. The details of such methods are known to those of skill in the art and many reagents for practicing such methods are commercially available.

[199] A purified form of a variant CBH I may be used to produce either monoclonal or polyclonal antibodies specific to the expressed protein for use in various immunoassays. (See, *e.g.*, Hu *et al.*, Mol Cell Biol. vol.11, no. 11, pp. 5792-5799, 1991). Exemplary assays include ELISA, competitive immunoassays, radioimmunoassays, Western blot, indirect immunofluorescent assays and the like. In general, commercially available antibodies and/or kits may be used for the quantitative immunoassay of the expression level of cellobiohydrolase proteins.

VIII. Isolation And Purification Of Recombinant CBH1 Protein.

[200] In general, a variant CBH I protein produced in cell culture is secreted into the medium and may be purified or isolated, *e.g.*, by removing unwanted components from the cell culture medium. However, in some cases, a variant CBH I protein may be produced in a cellular form necessitating recovery from a cell lysate. In such cases the variant CBH I protein is purified from the cells in which it was produced using techniques routinely employed by those of skill in the art. Examples include, but are not limited to, affinity chromatography (Tilbeurgh *et al.*, FEBS Lett. 16:215, 1984), ion-exchange chromatographic methods (Goyal *et al.*, Bioresource Technol. 36:37-50, 1991; Fliess *et al.*, Eur. J. Appl. Microbiol. Biotechnol. 17:314-318, 1983; Bhikhabhai *et al.*, J. Appl. Biochem. 6:336-345, 1984; Ellouz *et al.*, J. Chromatography 396:307-317, 1987), including ion-exchange using materials with high resolution power (Medve *et al.*, J. Chromatography A 808:153-165, 1998), hydrophobic interaction chromatography (Tomaz and Queiroz, J. Chromatography A 865:123-128, 1999), and two-phase partitioning (Brumbauer, *et al.*, Bioseparation 7:287-295, 1999).

[201] Typically, the variant CBH I protein is fractionated to segregate proteins having selected properties, such as binding affinity to particular binding agents, *e.g.*, antibodies or receptors; or which have a selected molecular weight range, or range of isoelectric points.

[202] Once expression of a given variant CBH I protein is achieved, the CBH I protein thereby produced is purified from the cells or cell culture. Exemplary procedures suitable for such purification include the following: antibody-affinity column chromatography, ion exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; and gel filtration using, *e.g.*, Sephadex G-75. Various methods of protein purification may be employed and such methods are known in the art and described *e.g.* in Deutscher, Methods in Enzymology, vol. 182, no. 57, pp. 779, 1990; Scopes, Methods Enzymol. 90: 479-91, 1982. The purification step(s) selected will depend, *e.g.*, on the nature of the production process used and the particular protein produced.

IX. Utility of *cbh1* and CBH1

[203] **It can be appreciated that** the variant *cbh* nucleic acids, the variant CBH I protein and compositions comprising variant CBH I protein activity find utility in a wide variety applications, some of which are described below.

[204] New and improved cellulase compositions that comprise varying amounts BG-type, EG-type and variant CBH-type cellulases find utility in detergent compositions that exhibit enhanced cleaning ability, function as a softening agent and/or improve the feel of cotton fabrics (*e.g.*, "stone washing" or "biopolishing"), in compositions for degrading wood pulp into sugars (*e.g.*, for bio-ethanol production), and/or in feed compositions. The isolation and characterization of cellulase of each type provides the ability to control the aspects of such compositions.

[205] Variant (or mutant) CBHs with increased thermostability find uses in all of the above areas due to their ability to retain activity at elevated temperatures.

[206] Variant (or mutant) CBHs with decreased thermostability find uses, for example, in areas where the enzyme activity is required to be neutralized at lower temperatures so that other enzymes that may be present are left unaffected. In addition, the enzymes may find utility in the limited conversion of cellulosics, for example, in controlling the degree of crystallinity or of cellulosic chain-length. After reaching the desired extent of conversion the saccharifying temperature can be raised above the survival temperature of the de-stabilized CBH I. As the CBH I activity is essential for hydrolysis of crystalline cellulose, conversion of crystalline cellulose will cease at the elevated temperature.

[207] Variant (or mutant) CBHs with increased reversibility, i.e., enhanced refolding and retention of activity, also find use in similar areas. Depending upon the conditions of thermal inactivation, reversible denaturation can compete with, or dominate over, the irreversible process. Variants with increased reversibility would, under these conditions, exhibit increased resistance to thermal inactivation. Increased reversibility would also be of potential benefit in any process in which an inactivation event was followed by a treatment under non-inactivating conditions. For instance, in a Hybrid Hydrolysis and Fermentation (HHF) process for biomass conversion to ethanol, the biomass would first be incompletely saccharified by cellulases at elevated temperature (say 50°C or higher), then the temperature would be dropped (to 30°C, for instance) to allow a fermentative organism to be introduced to convert the sugars to ethanol. If, upon decrease of process temperature, thermally inactivated cellulase reversibly re-folded and recovered activity then saccharification could continue to higher levels of conversion during the low temperature fermentation process.

[208] In one approach, the cellulase of the invention finds utility in detergent compositions or in the treatment of fabrics to improve the feel and appearance.

[209] Since the rate of hydrolysis of cellulosic products may be increased by using a transformant having at least one additional copy of the *cbh* gene inserted into the genome, products that contain cellulose or heteroglycans can be degraded at a faster rate and to a greater extent. Products made from cellulose such as paper, cotton, cellulosic diapers and the like can be degraded more efficiently in a landfill. Thus, the fermentation product obtainable from the transformants or the transformants alone may be used in compositions to help degrade by liquefaction a variety of cellulose products that add to the overcrowded landfills.

[210] Separate saccharification and fermentation is a process whereby cellulose present in biomass, e.g., corn stover, is converted to glucose and subsequently yeast strains convert glucose into ethanol. Simultaneous saccharification and fermentation is a process whereby cellulose present in biomass, e.g., corn stover, is converted to glucose and, at the same time and in the same reactor, yeast strains convert glucose into ethanol. Thus, in another approach, the variant CBH type cellulase of the invention finds utility in the degradation of biomass to ethanol. Ethanol production from readily available sources of cellulose provides a stable, renewable fuel source.

[211] Cellulose-based feedstocks are comprised of agricultural wastes, grasses and woods and other low-value biomass such as municipal waste (e.g., recycled paper, yard clippings, etc.). Ethanol may be produced from the fermentation of any of these cellulosic

feedstocks. However, the cellulose must first be converted to sugars before there can be conversion to ethanol.

[212] A large variety of feedstocks may be used with the inventive variant CBH and the one selected for use may depend on the region where the conversion is being done. For example, in the Midwestern United States agricultural wastes such as wheat straw, corn stover and bagasse may predominate while in California rice straw may predominate. However, it should be understood that any available cellulosic biomass may be used in any region.

[213] A cellulase composition containing an enhanced amount of cellobiohydrolase finds utility in ethanol production. Ethanol from this process can be further used as an octane enhancer or directly as a fuel in lieu of gasoline which is advantageous because ethanol as a fuel source is more environmentally friendly than petroleum derived products. It is known that the use of ethanol will improve air quality and possibly reduce local ozone levels and smog. Moreover, utilization of ethanol in lieu of gasoline can be of strategic importance in buffering the impact of sudden shifts in non-renewable energy and petro-chemical supplies.

[214] Ethanol can be produced via saccharification and fermentation processes from cellulosic biomass such as trees, herbaceous plants, municipal solid waste and agricultural and forestry residues. However, the ratio of individual cellulase enzymes within a naturally occurring cellulase mixture produced by a microbe may not be the most efficient for rapid conversion of cellulose in biomass to glucose. It is known that endoglucanases act to produce new cellulose chain ends which themselves are substrates for the action of cellobiohydrolases and thereby improve the efficiency of hydrolysis of the entire cellulase system. Therefore, the use of increased or optimized cellobiohydrolase activity may greatly enhance the production of ethanol.

[215] Thus, the inventive cellobiohydrolase finds use in the hydrolysis of cellulose to its sugar components. In one embodiment, a variant cellobiohydrolase is added to the biomass prior to the addition of a fermentative organism. In a second embodiment, a variant cellobiohydrolase is added to the biomass at the same time as a fermentative organism. Optionally, there may be other cellulase components present in either embodiment.

[216] In another embodiment the cellulosic feedstock may be pretreated. Pretreatment may be by elevated temperature and the addition of either of dilute acid, concentrated acid or dilute alkali solution. The pretreatment solution is added for a time sufficient to at least partially hydrolyze the hemicellulose components and then neutralized.

[217] The major product of CBHI action on cellulose is cellobiose which is available for conversion to glucose by BG activity (for instance in a fungal cellulase product). Either by the pretreatment of the cellulosic biomass or by the enzymatic action on the biomass, other sugars, in addition to glucose and cellobiose, can be made available from the biomass. The hemi-cellulose content of the biomass can be converted (by hemi-cellulases) to sugars such as xylose, galactose, mannose and arabinose. Thus, in a biomass conversion process, enzymatic saccharification can produce sugars that are made available for biological or chemical conversions to other intermediates or end-products. Therefore, the sugars generated from biomass find use in a variety of processes in addition to the generation of ethanol. Examples of such conversions are fermentation of glucose to ethanol (as reviewed by M.E. Himmel *et al.* pp2-45, in "Fuels and Chemicals from Biomass", ACS Symposium Series 666, ed B.C. Saha and J. Woodward, 1997) and other biological conversions of glucose to 2,5-diketo-D-gluconate (US Patent No. 6,599,722), lactic acid (R. Datta and S-P. Tsai pp224-236, *ibid*), succinate (R.R. Gokarn, M.A. Eiteman and J. Sridhar pp237-263, *ibid*), 1,3-propanediol (A-P. Zheng, H. Biebl and W-D. Deckwer pp264-279, *ibid*), 2,3-butanediol (C.S. Gong, N. Cao and G.T. Tsao pp280-293, *ibid*), and the chemical and biological conversions of xylose to xylitol (B.C. Saha and R.J. Bothast pp307-319, *ibid*). See also, for example, WO 98/21339.

[218] The detergent compositions of this invention may employ besides the cellulase composition (irrespective of the cellobiohydrolase content, i.e., cellobiohydrolase -free, substantially cellobiohydrolase -free, or cellobiohydrolase enhanced), a surfactant, including anionic, non-ionic and ampholytic surfactants, a hydrolase, building agents, bleaching agents, bluing agents and fluorescent dyes, caking inhibitors, solubilizers, cationic surfactants and the like. All of these components are known in the detergent art. The cellulase composition as described above can be added to the detergent composition either in a liquid diluent, in granules, in emulsions, in gels, in pastes, and the like. Such forms are well known to the skilled artisan. When a solid detergent composition is employed, the cellulase composition is preferably formulated as granules. Preferably, the granules can be formulated so as to contain a cellulase protecting agent. For a more thorough discussion, see US Patent Number 6,162,782 entitled "Detergent compositions containing cellulase compositions deficient in CBH I type components".

[219] Preferably the cellulase compositions are employed from about 0.00005 weight percent to about 5 weight percent relative to the total detergent composition. More

preferably, the cellulase compositions are employed from about 0.0002 weight percent to about 2 weight percent relative to the total detergent composition.

[220] In addition the variant *cbh I* nucleic acid sequence finds utility in the identification and characterization of related nucleic acid sequences. A number of techniques useful for determining (predicting or confirming) the function of related genes or gene products include, but are not limited to, (A) DNA/RNA analysis, such as (1) overexpression, ectopic expression, and expression in other species; (2) gene knock-out (reverse genetics, targeted knock-out, viral induced gene silencing (VIGS, see Baulcombe, 100 Years of Virology, Calisher and Horzinek eds., Springer-Verlag, New York, NY 15:189-201, 1999); (3) analysis of the methylation status of the gene, especially flanking regulatory regions; and (4) in situ hybridization; (B) gene product analysis such as (1) recombinant protein expression; (2) antisera production, (3) immunolocalization; (4) biochemical assays for catalytic or other activity; (5) phosphorylation status; and (6) interaction with other proteins via yeast two-hybrid analysis; (C) pathway analysis, such as placing a gene or gene product within a particular biochemical or signaling pathway based on its overexpression phenotype or by sequence homology with related genes; and (D) other analyses which may also be performed to determine or confirm the participation of the isolated gene and its product in a particular metabolic or signaling pathway, and help determine gene function.

EXAMPLES

[222] The present invention is described in further detain in the following examples which are not in any way intended to limit the scope of the invention as claimed. The attached Figures are meant to be considered as integral parts of the specification and description of the invention.

EXAMPLE 1
Alignment of known Cel7A cellulases

[223] The choice of several of the mutations was determined by first aligning *Hypocrea jecorina* Cel7A to its 41 family members using structural information and a modeling program. The alignment of the primary amino acid sequence of all 42 family members is shown in Figure 8 (SEQ ID NO:32-74).

[224] For four of the members (i.e., 20VW.1, 1A39, 6CEL and 1EG1.1), the crystal structure had been previously determined. The 4 aligned proteins for which there were published structures had their alignment locked for all residues whose backbone atoms were within a specific RMS deviation (RMS less than or equal to 2.0 A). The tertiary structural alignment of the four sequences was performed using MOE version 2001.01 by Chemical Computing Group, Montreal Canada. The overlapping structural elements were used to freeze the primary structures of the four sequences. The remaining 38 sequences then had their primary amino acid structure aligned with the frozen four using MOE with secondary structure prediction on and other parameters set to their default settings.

[225] Based on the alignments, various single and multiple amino acid mutations were made in the protein by site mutagenesis.

[226] Single amino acid mutations were based on the following rationale (see also Table 1): After examining the conservation of amino acids between the homologues, sites were picked in the *H. jecorina* sequence where a statistical preference for another amino acid was seen amongst the other 41 sequences (e.g.: at position 77 the Ala, only present in *H. jecorina* and 3 other homologues, was changed to Asp, present in 22 others). The effect of each substitution on the structure was then modeled.

Table 1: Cel7A Variants and Rationale for Change

Cel7A Variants and Rationale for Change		Tm	ΔTm
Wild Type *H. jecorina*		62.5	
(4)A77D(22)	3 possible H-bonds to Q7 and I80	62.2	-0.3
(7)S113D(18)	numerous new H-bonds to backbone to stabilize turn	62.8	0.3
(8)L225F(13)	better internal packing	61.6	-0.9
(5)L288F(17)	better internal packing	62.4	-0.1
(1)A299E(24)	extra ligand to cobalt atom observed in crystal structure	61.2	-1.3
(4)N301K(11)	salt bridges to E295 and E325	63.5	1.0
(5)T356L(20)	better internal packing	62.6	0.1
(2)G430F(17)	better surface packing	61.7	-0.8

[227] Multiple amino acid mutations were based on a desire to affect the stability, processivity, and product inhibition of the enzyme. The following multiple site changes in the *H. jecorina* sequence were constructed:

1) Thr 246 Cys + Tyr 371 Cys
2) Thr 246 Ala + Arg 251 Ala + Tyr 252 Ala
3) Thr 380 Gly + Tyr 381 Asp + Arg 394 Ala + deletion of Residues 382 to 393, inclusive
4) Thr 380 Gly + Tyr 381 Asp + Arg 394 Ala
5) Tyr 252 Gln + Asp 259 Trp + Ser 342 Tyr

[228] The T246A/R251A/Y252A and the other triple + deletion mutant are both predicted to decrease the product inhibition of the enzyme. The Thr246Cys + Tyr371Cys is predicted to increase the stability of the enzyme and increase the processitivity of it. The D259W/Y252Q/S342Y variant is predicted to affect the product inhibition of the enzyme.

[229] Other single and multiple mutations were constructed using methods well known in the art (see references above) and are presented in Table 2.

Table 2: *H. jecorina* CBH I variants

Mutations
S8P
N49S
A68T
A77D
N89D
S92T
S113N
S113D
L225F
P227A
P227L
D249K
T255P
D257E
S279N
L288F
E295K
S297T
A299E
N301K
T332K
T332Y
T332H
T356L
F338Y
V393G
G430F
T41I (plus deletion of Thr @ 445)
V403D/T462I
S196T/S411F
E295K/S398T
A112E/T226A
T246C/Y371C
G22D/S278P/T296P
S8P/N103I/S113N
S113T/T255P/K286M
P227L/E325K/Q487L
P227T/T484S/F352L
T246A/R251A/Y252A

Mutations
T380G/Y381D/R394A
Y252Q/D259W/S342Y
A68T/G440R/P491L
Q17L/E193V/M213I/F352L
S8P/N49S/A68T/S113N
A112E/P227L/S278P/T296P
S8P/N49S/A68T/N103I/S113N
S8P/N49S/A68T/S278P/T296P
G22D/N49S/A68T/S278P/T296P
G22D/N103I/S113N/S278P/T296P
S8P/N49S/A68T/S113N/P227L
S8P/N49S/A68T/A112E/T226A
S8P/N49S/A68T/A112E/P227L
T41I/A112E/P227L/S278P/T296P
S8P/T41I/N49S/A68T/S113N/P227L
S8P/T41I/N49S/A68T/A112E/P227L
G22D/N49S/A68T/P227L/S278P/T296P
G22D/N49S/A68T/N103I/S113N/S278P/T296P
G22D/N49S/A68T/N103I/S113N/P227L/S278P/ T296P
G22D/N49S/A68T/N103I/A112E/P227L/S278P/ T296P
G22D/N49S/N64D/A68T/N103I/S113N/S278P/ T296P
S8P/T41I/N49S/A68T/S92T/S113N/P227L/D249K/S411F
S8P/G22D/T41I/N49S/A68T/N103I/S113N/S278P/T296P
S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/S278P/T296P
S8P/G22D/T41I/N49S/A68T/S113N/P227L/D249K/S278P/N301R
S8P/G22D/T41I/N49S/A68T/S92T/S113N/P227L/D249K/S411F
S8P/T41I/N49S/A68T/S92T/S113N/P227L/D249K/V403D/T462I
S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/D249K/S278P/T296P
S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/S278P/T296P/N301R
S8P/G22D/T41I/N49S/A68T/S113N/P227L/D249K/S278P/T296P/N301R
S8P/T41I/N49S/S57N/A68T/S113N/P227L/D249K/S278P/T296P/N301R
S8P/G22D/T41I/N49S/A68T/S92T/S113N/P227L/D249K/V403D/T462I
S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/D249K/S278P/T296P/N301R
S8P/G22D/T41I/N49S/A68T/S92T/S113N/S196T/P227L/D249K/T255P/S278P/T296P/N301R/ E325K/S411F
S8P/T41I/N49S/A68T/S92T/S113N/S196T/P227L/D249K/T255P/S278P/T296P/N301R/E325K/ V403D/S411F/T462I
S8P/G22D/T41I/N49S/A68T/S92T/S113N/S196T/P227L/D249K/T255P/S278P/T296P/N301R/ E325K/V403D/S411F/T462I

EXAMPLE 2

Cloning and Expression of CBHI variants in *H. jecorina*

A. Construction of the *H. jecorina* general-purpose expression plasmid-PTEX.

[230] The plasmid, pTEX was constructed following the methods of Sambrook et al. (1989), *supra*, and is illustrated in FIG. 7. This plasmid has been designed as a multi-purpose expression vector for use in the filamentous fungus *Trichoderma longibrachiatum*.

The expression cassette has several unique features that make it useful for this function. Transcription is regulated using the strong CBH I gene promoter and terminator sequences for *T. longibrachiatum*. Between the CBHI promoter and terminator there are unique PmeI and SstI restriction sites that are used to insert the gene to be expressed. The *T. longibrachiatum* pyr4 selectable marker gene has been inserted into the CBHI terminator and the whole expression cassette (CBHI promoter-insertion sites-CBHI terminator-pyr4 gene-CBHI terminator) can be excised utilizing the unique NotI restriction site or the unique NotI and NheI restriction sites.

[231] This vector is based on the bacterial vector, pSL1180 (Pharmacia Inc., Piscataway, N.J.), which is a PUC-type vector with an extended multiple cloning site. One skilled in the art would be able to construct this vector based on the flow diagram illustrated in FIG. 7.

[232] The vector pTrex2L was constructed from pTrex2, a derivative of pTEX. The sequence for pTrex2 is given in Figure 6 (SEQ ID NO:31).

[233] The exact plasmid used is not that important as long as the variant protein is expressed at a useful level. However, maximizing the expression level by forcing integration at the cbh1 locus is advantageous.

B. Cloning

[234] Using methods known in the art a skilled person can clone the desired CBH I variant into an appropriate vector. As noted above, the exact plasmid used is not that important as long as the variant protein is expressed at a useful level. The following description of the preparation of one of the inventive variant CBH I enzymes can be utilized to prepare any of the inventive variants described herein.

[235] The variant *cbh 1* genes were cloned into the pTrex2L vector.

[236] Construction of plasmid pTrex2L was done as follows: The 6 nucleotides between the unique Sac II (SEQ ID NO:78) and Asc I sites of pTrex2 were replaced with a synthetic linker (SEQ ID NO:79) containing a BstE II and BamH I sites to produce plasmid Trex2L. The complementary synthetic linkers

21-mer synthetic oligo CBHlink1+: GGTTT**GGATCCGGTCACC**AGG (SEQ ID NO:75)

and

27-mer synthetic oligo CBHlink-: CGCGCCT**GGTGACCGGATCCA**AACCGC (SEQ ID NO:76)

were annealed.

[237] The pTrex2 was digested with Sac II and Asc I. The annealed linker (SEQ ID NO:80) was then ligated into pTrex2 to create pTrex2L. The plasmid was then digested with an appropriate restriction enzyme(s) and a wild type CBH I gene was ligated into the plasmid.

[238] Primers were used to introduce the desired mutations into the wild-type gene. It will be understood that any method that results in the introduction of a desired alteration or mutation in the gene may be used. Synthetic DNA primers were used as PCR templates for mutant constructions. It is well within the knowledge of the skilled artisan to design the primers based on the desired mutation to be introduced.

[239] The mutagenic templates were extended and made double stranded by PCR using the synthetic DNA oligonucleotides. After 25 PCR cycles the final product was primarily a 58 bp double stranded product comprising the desired mutation. The mutagenic fragments were subsequently attached to wild-type CBH I fragments and ligated into the plasmid using standard techniques.

C. Transformation and Expression

[240] The prepared vector for the desired variant was transformed into the uridine auxotroph version of the double or quad deleted Trichoderma strains (see Table 3; see also U.S. Patent Nos. 5,861,271 and 5,650,322) and stable transformants were identified.

Table 3: Transformation/Expression strain

CBH I Variant	**Expression Strain**
A77D	quad-delete strain (1A52)
S113D	double-delete strain
L225F	double-delete strain
L288F	double-delete strain
A299E	quad-delete strain (1A52)
N301K	quad-delete strain (1A52)
T356L	double-delete strain
G430F	quad-delete strain (1A52)
T246C/Y371C	quad-delete strain (1A52)
T246A/R251A/Y252A	quad-delete strain (1A52)
Y252Q/D259W/S342Y	quad-delete strain (1A52)
T380G/Y381D/R394A	quad-delete strain (1A52)
T380G/Y381D/R394A plus deletion of 382-393	quad-delete strain (1A52)

"double-delete" (Δ CBHI & Δ CBHII) and the "quad-delete" (Δ CBHI & Δ CBHII, Δ EGI & Δ EGII) T.reesei host strains

[241] To select which transformants expressed variant CBH I, DNA was isolated from strains following growth on Vogels+1% glucose and Southern blot experiments performed using an isolated DNA fragment containing only the variant CBH I. Transformants were isolated having a copy of the variant CBH I expression cassette integrated into the genome of the host cell. Total mRNA was isolated from the strains following growth for 1 day on Vogels+1% lactose. The mRNA was subjected to Northern analysis using the variant CBH I coding region as a probe. Transformants expressing variant CBH I mRNA were identified.

[242] One may obtain any other novel variant CBH I cellulases or derivative thereof by employing the methods described above.

EXAMPLE 3

Expression of CBH1 variants in *A. niger*

[243] The PCR fragments were obtained using the following primers and protocols

[244] The following DNA primers were constructed for use in amplification of homologous CBH1 genes from genomic DNA's isolated from various microorganisms. All symbols used herein for protein and DNA sequences correspond to IUPAC IUB Biochemical Nomenclature Commission codes.

[245] Homologous 5' (FRG192) and 3' (FRG193) primers were developed based on the sequence of CBH1 from *Trichoderma reesei*. Both primers contained Gateway cloning sequences from Invitrogen® at the 5' of the primer. Primer FRG192 contained attB1 sequence and primer FRG193 contained attB2 sequence.

Sequence of FRG192 without the attB1:
ATGTATCGGAAGTTGGCCG (signal sequence of CBH1 *H. jecorina*) (SEQ ID NO: 3)

Sequence of FRG193 without the attB2:
TTACAGGCACTGAGAGTAG (cellulose binding module of CBH1 *H. jecorina*) (SEQ ID NO: 4)

[246] The *H. jecorina* CBH I cDNA clone served as template.

[247] PCR conditions were as follows: 10 µL of 10X reaction buffer (10X reaction buffer comprising 100mM Tris HCl, pH 8-8.5; 250 mM KCl; 50 mM $(NH_4)_2SO_4$; 20 mM $MgSO_4$); 0.2 mM each of dATP, dTTP, dGTP, dCTP (final concentration), 1 µL of 100 ng/µL genomic DNA, 0.5 µL of PWO polymerase (Boehringer Mannheim, Cat # 1644-947) at 1 unit per µL, 0.2µM of each primer, FRG192 and FRG193, (final concentration), 4µl DMSO and water to 100 µL.

[248] Various sites in *H. jecorina* CBH1 may be involved in the thermostability of the variants and the *H. jecorina* CBH1 gene was therefore subjected to mutagenesis.

[249] The fragments encoding the variants were purified from an agarose gel using the Qiagen Gel extraction KIT. The purified fragments were used to perform a clonase reaction with the pDONR™201 vector from Invitrogen® using the Gateway™ Technology instruction manual (version C) from Invitrogen®.

Genes were then transferred from this ENTRY vector to the destination vector (pRAXdes2) to obtain the expression vector pRAXCBH1.

[250] Cells were transformed with an expression vector comprising a variant CBH I cellulase encoding nucleic acid. The constructs were transformed into *A. niger var. awamori* according to the method described by Cao *et al* (Cao Q-N, Stubbs M, Ngo KQP, Ward M, Cunningham A, Pai EF, Tu G-C and Hofmann T (2000) Penicillopepsin-JT2 a recombinant enzyme from *Penicillium janthinellum* and contribution of a hydrogen bond in subsite S3 to *kcat Protein Science* 9:991-1001).

[251] Transformants were streaked on minimal medium plates (Ballance DJ, Buxton FP, and Turner G (1983) Transformation of *Aspergillus nidulans* by the orotidine-5'-phosphate decarboxylase gene of *Neurospora crassa Biochem Biophys Res Commun* 112:284-289) and grown for 4 days at 30°C. Spores were collected using methods well known in the art. *A. nidulans* conidia are harvested in water (by rubbing the surface of a conidiating culture with a sterile bent glass rod to dislodge the spores) and can be stored for weeks to months at 4°C without a serious loss of viability. However, freshly harvested spores germinate more reproducibly. For long-term storage, spores can be stored in 50% glycerol at –20°C, or in 15-20% glycerol at –80°C. Glycerol is more easily pipetted as an 80% solution in water. 800µl of aqueous conidial suspension (as made for 4°C storage) added to 200µl 80% glycerol is used for a –80°C stock; 400 µl suspension added to 600 µl 80% glycerol is used for a –20°C stock. Vortex before freezing. For mutant collections, small pieces of conidiating cultures can be excised and placed in 20% glycerol, vortexed, and frozen as –80°C stocks. In our case we store them in 50% glycerol at –80°C.

[252] *A. niger* var *awamori* transformants were grown on minimal medium lacking uridine (Ballance et al. 1983). Transformants were screened for cellulase activity by inoculating 1cm^2 of spore suspension from the sporulated grown agar plate into 100ml shake flasks for 3 days at 37°C as described by Cao et al. (2000).

[253] The CBHI activity assay is based on the hydrolysis of the nonfluorescent 4-methylumbelliferyl-ß-lactoside to the products lactose and 7-hydroxy-4-methylcoumarin,

the latter product is responsible for the fluorescent signal. Pipette 170 µl 50 mM NaAc buffer pH 4.5 in a 96-well microtiter plate (MTP) (Greiner, Fluotrac 200, art. nr. 655076) suitable for fluorescence. Add 10 µl of supernatant and then add 10 µl of MUL (1 mM 4-methylumbelliferyl-ß-lactoside (MUL) in milliQ water) and put the MTP in the Fluostar Galaxy (BMG Labtechnologies; D-77656 Offenburg). Measure the kinetics for 16 min. (8 cycles of 120s each) using $\lambda_{320\ nm}$ (excitation) and $\lambda_{460\ nm}$ (emission) at 50°C. Supernatents having CBH activity were then subjected to Hydrophobic Interaction Chromatography.

EXAMPLE 4

Stability of CBH 1 variants

[254] CBH I cellulase variants were cloned and expressed as above (see Examples 2 and 3). Cel7A wild type and variants were then purified from cell-free supernatants of these cultures by column chromatography. Proteins were purified using hydrophobic interaction chromatography (HIC). Columns were run on a BioCAD® Sprint Perfusion Chromatography System using Poros® 20 HP2 resin both made by Applied Biosystems.

[255] HIC columns were equilibrated with 5 column volumes of 0.020 M sodium phosphate, 0.5 M ammonium sulfate at pH 6.8. Ammonium sulfate was added to the supernatants to a final concentration of approximately 0.5 M and the pH was adjusted to 6.8. After filtration, the supernatant was loaded onto the column. After loading, the column was washed with 10 column volumes of equilibration buffer and then eluted with a 10 column volume gradient from 0.5 M ammonium sulfate to zero ammonium sulfate in 0.02 M sodium phosphate pH 6.8. Cel7A eluted approximately mid-gradient. Fractions were collected and pooled on the basis of reduced, SDS-PAGE gel analysis.

[256] The melting points were determined according to the methods of Luo, *et al., Biochemistry* **34**:10669 and Gloss, *et al., Biochemistry* **36**:5612. See also Sandgren at al. (2003) Protein Science 12(4) pp848.

[257] Data was collected on the Aviv 215 circular dichroism spectrophotometer. The native spectra of the variants between 210 and 260 nanometers were taken at 25°C. Buffer conditions were 50 mM Bis Tris Propane/50 mM ammonium acetate/glacial acetic acid at pH 5.5. The protein concentration was kept between 0.25 and 0.5 mgs/mL. After determining the optimal wavelength to monitor unfolding, the samples were thermally denatured by ramping the temperature from 25°C to 75°C under the same buffer conditions. Data was collected for 5 seconds every 2 degrees. Partially reversible unfolding was monitored at 230 nanometers in a 0.1 centimeter path length cell. While at 75°C, an unfolded spectra was collected as described above. The sample was then

cooled to 25°C to collect a refolded spectra. The difference between the three spectra at 230nm was used to assess the variants reversibility.

[258] The thermal denaturation profiles are shown in Figure 9A and 9B for wildtype CBH I and various variant CBH I's. See also Table 4.

Table 4: Thermal Stability of Variant CBH I cellulases

H. jecorina CBH I Residue Substitution	Tm	delta Tm	% rev 230nm
Wild type	**62.5**		23
S8P	63.1	0.6	
N49S	63.7	1.2	
A68T	63.7	1.2	32
A77D	62.2	-0.3	
N89D	63.6	1.1	50
S92T	64.4	1.9	25
S113D	62.8	0.3	
S113N	64.0	1.5	
L225F	61.6	-0.9	
P227A	64.8	2.3	49
P227L	65.2	2.7	45
D249K	64.0	1.5	39
T255P	64.4	1.9	35
S279N	62.4	-0.1	~95
E295K	64.0	1.5	~95
T332K	63.3	0.8	37
T332Y	63.3	0.8	37
T332H	62.7	0.2	64
F338Y	60.8	-1.7	~95
G430F	61.7	-0.8	
L288F	62.4	-0.1	
A299E	61.2	-1.3	
N301K	63.5	1.0	
T356L	62.6	0.1	
D257E	61.8	-0.7	45
V393G	61.7	-0.8	43
S297T	63.3	0.8	31
T41I plus deletion @ T445	64.2	1.7	
T246C/Y371C	65.0	2.5	
S196T/S411F	65.3	2.8	27
E295K/S398T	63.9	1.4	36
V403D/T462I	64.5	2	53
A112E/T226A	63.5	1.0	
A68T/G440R/P491L	63.1	0.6	32
G22D/S278P/T296P	63.6	1.1	
T246A/R251A/Y252A	63.5	1.0	
T380G/Y381D/R394A	58.1	-4.4	
Y252Q/D259W/S342Y	59.9	-2.6	50
S113T/T255P/K286M	63.8	1.3	16

H. jecorina CBH I Residue Substitution	Tm	delta Tm	% rev 230nm
P227L/E325K/Q487L	64.5	2.0	22
P227T/T484S/F352L	64.2	1.7	45
Q17L/E193V/M213I/F352L	64.0	1.5	34
S8P/N49S/A68T/S113N	64.5	2.0	90
S8P/N49S/A68T/S113N/P227L	66.0	3.5	86
T41I/A112E/P227L/S278P/T296P	66.1	3.6	48
S8P/N49S/A68T/A112E/T226A	64.6	2.1	46
S8P/N49S/A68T/A112E/P227L	65.2	2.7	32
S8P/T41I/N49S/A68T/A112E/P227L	67.6	5.1	40
G22D/N49S/A68T/P227L/S278P/T296P	65.9	3.4	26
G22D/N49S/A68T/N103I/S113N/P227L/S278P/T296P	65.3	2.8	72
G22D/N49S/A68T/N103I/A112E/P227L/S278P/T296P	65.1	2.6	20
G22D/N49S/N64D/A68T/N103I/S113N/S278P/T296P	61.4	-1.1	75
S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/S278P/ T296P	68.8	6.3	56
S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/D249K/ S278P/T296P	69.0	6.5	71
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S8P/G22D/T41I/N49S/A68T/S113N/P227L/D249K/S278P/ T296P/N301R	69.9	7.4	88
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S8P/G22D/T41I/N49S/A68T/S92T/S113N/P227L/D249K/ S411F	69.5	7.0	~100
S8P/G22D/T41I/N49S/A68T/S92T/S113N/S196T/P227L/ D249K/T255P/S278P/T296P/N301R/E325K/S411F	70.7	8.2	~100
S8P/T41I/N49S/A68T/S92T/S113N/S196T/P227L/D249K/ T255P/S278P/T296P/N301R/E325K/V403D/S411F/T462I	71.0	8.5	~100
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[259] Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed

should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the claims.

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accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg 1080
gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 8
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 8
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Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
 1               5                  10                  15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
            20                  25                  30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
        35                  40                  45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
    50                  55                  60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
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65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Asp Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
245 250 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
275 280 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
290 295 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305 310 315 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
325 330 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
340 345 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
355 360 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
370 375 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385 390 395 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
405 410 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
420 425 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
435 440 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
450 455 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465 470 475 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
485 490 495
Leu

<210> 9
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 9
cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg 60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccga ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
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gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 10
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 10
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Asp Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220

Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
245 250 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
275 280 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
290 295 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305 310 315 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
325 330 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
340 345 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
355 360 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
370 375 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385 390 395 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
405 410 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
420 425 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
435 440 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
450 455 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465 470 475 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
485 490 495
Leu

<210> 11
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 11
cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg 60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa atttaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg 1080

gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 12
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 12
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
245 250 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Phe
275 280 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
290 295 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305 310 315 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
325 330 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
340 345 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
355 360 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn

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    370                 375                 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385                 390                 395                 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
                405                 410                 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
            420                 425                 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
        435                 440                 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
    450                 455                 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465                 470                 475                 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
                485                 490                 495
Leu
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<210> 13
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 13

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cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg      60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg     120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta     180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc     240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg     300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc     360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg     420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc     480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg     540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac     600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc     660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt     720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc     780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt     840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgagatc     900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt     960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga    1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg    1080
gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc    1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc    1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac    1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga    1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga    1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc    1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g              1491
```

<210> 14
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 14

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Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
 1               5                  10                  15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
            20                  25                  30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
```

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         35                  40                  45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
    50                  55                  60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65                  70                  75                  80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
                85                  90                  95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
            100                 105                 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
        115                 120                 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
    130                 135                 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145                 150                 155                 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
                165                 170                 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
            180                 185                 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
        195                 200                 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
    210                 215                 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225                 230                 235                 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
                245                 250                 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
            260                 265                 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
        275                 280                 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Glu Ile Asn Arg Tyr Tyr
    290                 295                 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305                 310                 315                 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
                325                 330                 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
            340                 345                 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
        355                 360                 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
    370                 375                 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385                 390                 395                 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
                405                 410                 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
            420                 425                 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
        435                 440                 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
    450                 455                 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465                 470                 475                 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
                485                 490                 495
Leu
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<210> 15
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 15
cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg 60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aagcgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg 1080
gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
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aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 16
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 16
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175

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Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
            180                 185                 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
        195                 200                 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
    210                 215                 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225                 230                 235                 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
                245                 250                 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
            260                 265                 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
        275                 280                 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Lys Arg Tyr Tyr
    290                 295                 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305                 310                 315                 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
                325                 330                 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
            340                 345                 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
        355                 360                 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
    370                 375                 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385                 390                 395                 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
                405                 410                 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
            420                 425                 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
        435                 440                 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
    450                 455                 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465                 470                 475                 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
                485                 490                 495
Leu
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<210> 17
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 17

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cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg      60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg     120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta     180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc     240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg     300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc     360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg     420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc     480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg     540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac     600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc     660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt     720
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gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctctctc tggcggcatg 1080
gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 18
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 18
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
245 250 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
275 280 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
290 295 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305 310 315 320

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Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
                325                 330                 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
            340                 345                 350
Lys Lys Ala Leu Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
        355                 360                 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
    370                 375                 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385                 390                 395                 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
                405                 410                 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
            420                 425                 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
        435                 440                 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
    450                 455                 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465                 470                 475                 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
                485                 490                 495
Leu
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<210> 19
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 19
cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg 60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg 1080
gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccttc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 20
<211> 510
<212> PRT
<213> Hypocrea jecorina

<400> 20

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Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
 1               5                  10                  15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
            20                  25                  30
Val Ile Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser
        35                  40                  45
Thr Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp
    50                  55                  60
Asn Glu Thr Cys Ile Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr
65                  70                  75                  80
Ala Ser Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly
                85                  90                  95
Phe Val Thr Gln Ser Ala Ile Gln Lys Asn Val Gly Ala Arg Leu Tyr
            100                 105                 110
Leu Met Ala Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn
        115                 120                 125
Glu Phe Ser Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn
    130                 135                 140
Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys
145                 150                 155                 160
Tyr Pro Thr Asn Thr Ala Gly Ala Lys Tyr Ile Gly Thr Gly Tyr Cys
                165                 170                 175
Asp Ser Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn
            180                 185                 190
Val Glu Gly Trp Glu Pro Ser Ser Asn Asn Ala Asn Ile Thr Gly Ile
        195                 200                 205
Gly Gly His Gly Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn
    210                 215                 220
Ser Ile Ser Glu Ala Leu Thr Pro His Pro Cys Thr Thr Val Gly Ile
225                 230                 235                 240
Gln Glu Ile Cys Glu Gly Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn
                245                 250                 255
Arg Tyr Gly Gly Thr Cys Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr
            260                 265                 270
Arg Ile Leu Gly Asn Thr Ser Phe Tyr Gly Pro Gly Ser Ser Phe Thr
        275                 280                 285
Leu Asp Thr Thr Lys Lys Leu Thr Val Val Thr Gln Phe Glu Thr Ser
    290                 295                 300
Gly Ala Ile Ile Asn Arg Tyr Tyr Val Gln Asn Gly Val Thr Phe Gln
305                 310                 315                 320
Gln Pro Asn Ala Glu Leu Gly Ser Tyr Ser Gly Asn Glu Leu Asn Asp
                325                 330                 335
Asp Tyr Cys Thr Ala Glu Ile Glu Ala Glu Phe Gly Gly Ser Ser Phe
            340                 345                 350
Ser Asp Lys Gly Gly Leu Thr Gln Phe Lys Lys Ala Thr Ser Gly Gly
        355                 360                 365
Met Val Leu Val Met Ser Leu Trp Ile Asp Asp Tyr Tyr Ala Asn Met
    370                 375                 380
Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn Glu Thr Ser Ser Thr Pro
385                 390                 395                 400
Gly Ala Val Arg Gly Ser Cys Ser Thr Ser Ile Ser Gly Val Pro Ala
                405                 410                 415
Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val Thr Phe Ser Asn Ile
            420                 425                 430
Lys Phe Gly Pro Ile Gly Ser Thr Phe Asn Pro Ser Gly Ile Gly Asn
        435                 440                 445
Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr Arg Arg Pro Ala
    450                 455                 460
Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser His Tyr Gly Ile
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465 470 475 480
Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys Ala Ser Gly
485 490 495
Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys Leu
500 505 510

<210> 21
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 21
cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg 60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggatgtta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg 1080
gttctggtca tgagtctgtg ggatgattac tgcgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 22
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 22
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu

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    130                 135                 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145                 150                 155                 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
                165                 170                 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
            180                 185                 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
        195                 200                 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
    210                 215                 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225                 230                 235                 240
Asp Gly Cys Gly Gly Cys Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
                245                 250                 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
            260                 265                 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
        275                 280                 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
    290                 295                 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305                 310                 315                 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
                325                 330                 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
            340                 345                 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
        355                 360                 365
Asp Tyr Cys Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
    370                 375                 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385                 390                 395                 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
                405                 410                 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
            420                 425                 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
        435                 440                 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
    450                 455                 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465                 470                 475                 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
                485                 490                 495
Leu
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<210> 23
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 23
cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg 60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420

aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggagctta ctccgataac gcagctggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctctttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg 1080
gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 24
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 24
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240
Asp Gly Cys Gly Gly Ala Tyr Ser Asp Asn Ala Ala Gly Gly Thr Cys
245 250 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
275 280 285

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Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
    290                 295                 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305                 310                 315                 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
                325                 330                 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
            340                 345                 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
        355                 360                 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
    370                 375                 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385                 390                 395                 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
                405                 410                 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
            420                 425                 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
        435                 440                 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
    450                 455                 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465                 470                 475                 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
                485                 490                 495
Leu
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<210> 25
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 25
cagtcggcct gcactctcca atcggagact cacccgcctc tgacatggca gaaatgctcg 60
tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccaccccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
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gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccggc 1140
gacccgacaa acgagacctc ctccacaccc ggtgccgtgg ccggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 26
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 26
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
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Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
245 250 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
275 280 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
290 295 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305 310 315 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
325 330 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
340 345 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
355 360 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Gly Asp Pro Thr Asn
370 375 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Ala Gly Ser Cys Ser Thr Ser
385 390 395 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
405 410 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
420 425 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr

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          435                 440                 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
    450                 455                 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465                 470                 475                 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
                485                 490                 495
Leu
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<210> 27
<211> 1455
<212> DNA
<213> Hypocrea jecorina

<400> 27
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tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300
cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccaccccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agatatggcg gcacttgcga tcccgatggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
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gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccggc 1140
gacgccggaa gctgctccac cagctccggt gtccctgctc aggtcgaatc tcagtctccc 1200
aacgccaagg tcaccttctc caacatcaag ttcggaccca ttggcagcac cggcaaccct 1260
agcggcggca accctcccgg cggaaacccg cctggcacca ccaccacccg ccgcccagcc 1320
actaccactg gaagctctcc cggacctacc cagtctcact acggccagtg cggcggtatt 1380
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tactctcagt gcctg 1455

<210> 28
<211> 486
<212> PRT
<213> Hypocrea jecorina

<400> 28
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Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
 1               5                  10                  15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
            20                  25                  30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
        35                  40                  45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
    50                  55                  60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65                  70                  75                  80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
                85                  90                  95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
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                100                 105                 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
        115                 120                 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
    130                 135                 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145                 150                 155                 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
                165                 170                 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
            180                 185                 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
        195                 200                 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
    210                 215                 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225                 230                 235                 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
                245                 250                 255
Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
            260                 265                 270
Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
        275                 280                 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
    290                 295                 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305                 310                 315                 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
                325                 330                 335
Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
            340                 345                 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
        355                 360                 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Gly Asp Ala Gly Ser
    370                 375                 380
Cys Ser Thr Ser Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro
385                 390                 395                 400
Asn Ala Lys Val Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser
                405                 410                 415
Thr Gly Asn Pro Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly
            420                 425                 430
Thr Thr Thr Thr Thr Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro
        435                 440                 445
Gly Pro Thr Gln Ser His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser
    450                 455                 460
Gly Pro Thr Val Cys Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro
465                 470                 475                 480
Tyr Tyr Ser Gln Cys Leu
                485
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<210> 29
<211> 1491
<212> DNA
<213> Hypocrea jecorina

<400> 29
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tctggtggca cttgcactca acagacaggc tccgtggtca tcgacgccaa ctggcgctgg 120
actcacgcta cgaacagcag cacgaactgc tacgatggca acacttggag ctcgacccta 180
tgtcctgaca acgagacctg cgcgaagaac tgctgtctgg acggtgccgc ctacgcgtcc 240
acgtacggag ttaccacgag cggtaacagc ctctccattg gctttgtcac ccagtctgcg 300

cagaagaacg ttggcgctcg cctttacctt atggcgagcg acacgaccta ccaggaattc 360
accctgcttg gcaacgagtt ctctttcgat gttgatgttt cgcagctgcc gtgcggcttg 420
aacggagctc tctacttcgt gtccatggac gcggatggtg gcgtgagcaa gtatcccacc 480
aacaccgctg gcgccaagta cggcacgggg tactgtgaca gccagtgtcc ccgcgatctg 540
aagttcatca atggccaggc caacgttgag ggctgggagc cgtcatccaa caacgcgaac 600
acgggcattg gaggacacgg aagctgctgc tctgagatgg atatctggga ggccaactcc 660
atctccgagg ctcttacccc ccacccttgc acgactgtcg gccaggagat ctgcgagggt 720
gatgggtgcg gcggaactta ctccgataac agacagggcg gcacttgcga tccctggggc 780
tgcgactgga acccataccg cctgggcaac accagcttct acggccctgg ctcaagcttt 840
accctcgata ccaccaagaa attgaccgtt gtcacccagt tcgagacgtc gggtgccatc 900
aaccgatact atgtccagaa tggcgtcact ttccagcagc ccaacgccga gcttggtagt 960
tactctggca acgagctcaa cgatgattac tgcacagctg aggaggcaga attcggcgga 1020
tcctatttct cagacaaggg cggcctgact cagttcaaga aggctacctc tggcggcatg 1080
gttctggtca tgagtctgtg ggatgattac tacgccaaca tgctgtggct ggactccacc 1140
tacccgacaa acgagacctc ctccacaccc ggtgccgtgc gcggaagctg ctccaccagc 1200
tccggtgtcc ctgctcaggt cgaatctcag tctcccaacg ccaaggtcac cttctccaac 1260
atcaagttcg gacccattgg cagcaccggc aaccctagcg gcggcaaccc tcccggcgga 1320
aacccgcctg gcaccaccac cacccgccgc ccagccacta ccactggaag ctctcccgga 1380
cctacccagt ctcactacgg ccagtgcggc ggtattggct acagcggccc cacggtctgc 1440
gccagcggca caacttgcca ggtcctgaac ccttactact ctcagtgcct g 1491

<210> 30
<211> 497
<212> PRT
<213> Hypocrea jecorina

<400> 30
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65 70 75 80
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
85 90 95
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
100 105 110
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125
Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140
Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160
Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175
Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190
Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205
Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240
Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Gln Gly Gly Thr Cys
245 250 255
Asp Pro Trp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270

Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
275 280 285
Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
290 295 300
Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305 310 315 320
Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
325 330 335
Glu Phe Gly Gly Ser Tyr Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
340 345 350
Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
355 360 365
Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
370 375 380
Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385 390 395 400
Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
405 410 415
Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
420 425 430
Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr
435 440 445
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
450 455 460
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
465 470 475 480
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
485 490 495
Leu

<210> 31
<211> 8970
<212> DNA
<213> Artificial Sequence

<220>
<223> pTrex2 vector

<400> 31
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cccgtggaat tctcacggtg aatgtaggcc ttttgtaggg taggaattgt cactcaagca 180
cccccaacct ccattacgcc tcccccatag agttcccaat cagtgagtca tggcactgtt 240
ctcaaataga ttggggagaa gttgacttcc gcccagagct gaaggtcgca caaccgcatg 300
atatagggtc ggcaacggca aaaaagcacg tggctcaccg aaaagcaaga tgtttgcgat 360
ctaacatcca ggaacctgga tacatccatc atcacgcacg accactttga tctgctggta 420
aactcgtatt cgccctaaac cgaagtgcgt ggtaaatcta cacgtgggcc cctttcggta 480
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cgtgcacctg catcatgtat ataatagtga tcctgagaag gggggtttgg agcaatgtgg 660
gactttgatg gtcatcaaac aaagaacgaa gacgcctctt ttgcaaagtt ttgtttcggc 720
tacggtgaag aactggatac ttgttgtgtc ttctgtgtat ttttgtggca acaagaggcc 780
agagacaatc tattcaaaca ccaagcttgc tcttttgagc tacaagaacc tgtggggtat 840
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gactgaccgg acgtgttttg cccttcattt ggagaaataa tgtcattgcg atgtgtaatt 1320
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gcttgcatgc aaagatacac atcaatcgca gctggggtac aatcatccat catcccaact 2760
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ctgctggtcc tcgtcttcgg ggggcagctg gcagccgggc gtcatgtgga taaaggcatc 3180
gtcgggctcg gtgttgagcg tctcctgcga gatgaagccc atgacaaagt ccttgtgctc 3240
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<210> 32
<211> 397
<212> PRT
<213> Fusarium oxysporum

<400> 32

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Cys	Thr	Lys	Ala	Ser	Gly	Cys	Lys	Lys	Gln	Thr	Asn	Tyr	Ile	Val	Ala
			20					25				30			
Asp	Ala	Gly	Ile	His	Gly	Ile	Arg	Gln	Lys	Asn	Gly	Ala	Gly	Cys	Gly
		35					40					45			
Asp	Trp	Gly	Gln	Lys	Pro	Asn	Ala	Thr	Ala	Cys	Pro	Asp	Glu	Ala	Ser
	50					55					60				
Cys	Ala	Lys	Asn	Cys	Ile	Leu	Ser	Gly	Met	Asp	Ser	Asn	Ala	Tyr	Lys
65					70					75					80
Asn	Ala	Gly	Ile	Thr	Thr	Ser	Gly	Asn	Lys	Leu	Arg	Leu	Gln	Gln	Leu
				85					90					95	
Ile	Asn	Asn	Gln	Leu	Val	Ser	Pro	Arg	Val	Tyr	Leu	Leu	Glu	Glu	Asn
			100					105					110		
Lys	Lys	Lys	Tyr	Glu	Met	Leu	His	Leu	Thr	Gly	Thr	Glu	Phe	Ser	Phe
		115					120					125			
Asp	Val	Glu	Met	Glu	Lys	Leu	Pro	Cys	Gly	Met	Asn	Gly	Ala	Leu	Tyr
	130					135					140				
Leu	Ser	Glu	Met	Pro	Gln	Asp	Gly	Gly	Lys	Ser	Thr	Ser	Arg	Asn	Ser
145					150					155					160
Lys	Ala	Gly	Ala	Tyr	Tyr	Gly	Ala	Gly	Tyr	Cys	Asp	Ala	Gln	Cys	Tyr
				165					170					175	
Val	Thr	Pro	Phe	Ile	Asn	Gly	Val	Gly	Asn	Ile	Lys	Gly	Gln	Gly	Val
			180					185					190		
Cys	Cys	Asn	Glu	Leu	Asp	Ile	Trp	Glu	Ala	Asn	Ser	Arg	Ala	Thr	His
		195					200					205			
Ile	Ala	Pro	His	Pro	Cys	Ser	Lys	Pro	Gly	Leu	Tyr	Gly	Cys	Thr	Gly
	210					215					220				
Asp	Glu	Cys	Gly	Ser	Ser	Gly	Ile	Cys	Asp	Lys	Ala	Gly	Cys	Gly	Trp
225					230					235					240
Asn	His	Asn	Arg	Ile	Asn	Val	Thr	Asp	Phe	Tyr	Gly	Arg	Gly	Lys	Gln
				245					250					255	
Tyr	Lys	Val	Asp	Ser	Thr	Arg	Lys	Phe	Thr	Val	Thr	Ser	Gln	Phe	Val
			260					265					270		
Ala	Asn	Lys	Gln	Gly	Asp	Leu	Ile	Glu	Leu	His	Arg	His	Tyr	Ile	Gln
		275					280					285			
Asp	Asn	Lys	Val	Ile	Glu	Ser	Ala	Val	Val	Asn	Ile	Ser	Gly	Pro	Pro
	290					295					300				
Lys	Ile	Asn	Phe	Ile	Asn	Asp	Lys	Tyr	Cys	Ala	Ala	Thr	Gly	Ala	Asn
305					310					315					320
Glu	Tyr	Met	Arg	Leu	Gly	Gly	Thr	Lys	Gln	Met	Gly	Asp	Ala	Met	Ser
				325					330					335	
Arg	Gly	Met	Val	Leu	Ala	Met	Ser	Val	Trp	Trp	Ser	Glu	Gly	Asp	Phe
			340					345					350		
Met	Ala	Trp	Leu	Asp	Gln	Gly	Val	Ala	Gly	Pro	Cys	Asp	Ala	Thr	Glu

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        355                 360                 365
Gly Asp Pro Lys Asn Ile Val Lys Val Gln Pro Asn Pro Glu Val Thr
    370                 375                 380
Phe Ser Asn Ile Arg Ile Gly Glu Ile Gly Ser Thr Ser
385                 390                 395
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<210> 33
<211> 401
<212> PRT
<213> Humicola insolens

<400> 33

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Lys Pro Gly Glu Thr Lys Glu Val His Pro Gln Leu Thr Thr Phe Arg
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Cys Thr Lys Arg Gly Gly Cys Lys Pro Ala Thr Asn Phe Ile Val Leu
            20                  25                  30
Asp Ser Leu Trp His Trp Ile His Arg Ala Glu Gly Leu Gly Pro Gly
        35                  40                  45
Gly Cys Gly Asp Trp Gly Asn Pro Pro Pro Lys Asp Val Cys Pro Asp
    50                  55                  60
Val Glu Ser Cys Ala Lys Asn Cys Ile Met Glu Gly Ile Pro Asp Tyr
65                  70                  75                  80
Ser Gln Tyr Gly Val Thr Thr Asn Gly Thr Ser Leu Arg Leu Gln His
                85                  90                  95
Ile Leu Pro Asp Gly Arg Val Pro Ser Pro Arg Val Tyr Leu Leu Asp
            100                 105                 110
Lys Thr Lys Arg Arg Tyr Glu Met Leu His Leu Thr Gly Phe Glu Phe
        115                 120                 125
Thr Phe Asp Val Asp Ala Thr Lys Leu Pro Cys Gly Met Asn Ser Ala
    130                 135                 140
Leu Tyr Leu Ser Glu Met His Pro Thr Gly Ala Lys Ser Lys Tyr Asn
145                 150                 155                 160
Pro Gly Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys Asp Ala Gln Cys Phe
                165                 170                 175
Val Thr Pro Phe Ile Asn Gly Leu Gly Asn Ile Glu Gly Lys Gly Ser
            180                 185                 190
Cys Cys Asn Glu Met Asp Ile Trp Glu Ala Asn Ser Arg Ala Ser His
        195                 200                 205
Val Ala Pro His Thr Cys Asn Lys Lys Gly Leu Tyr Leu Cys Glu Gly
    210                 215                 220
Glu Glu Cys Ala Phe Glu Gly Val Cys Asp Lys Asn Gly Cys Gly Trp
225                 230                 235                 240
Asn Asn Tyr Arg Val Asn Val Thr Asp Tyr Tyr Gly Arg Gly Glu Glu
                245                 250                 255
Phe Lys Val Asn Thr Leu Lys Pro Phe Thr Val Val Thr Gln Phe Leu
            260                 265                 270
Ala Asn Arg Arg Gly Lys Leu Glu Lys Ile His Arg Phe Tyr Val Gln
        275                 280                 285
Asp Gly Lys Val Ile Glu Ser Phe Tyr Thr Asn Lys Glu Gly Val Pro
    290                 295                 300
Tyr Thr Asn Met Ile Asp Asp Glu Phe Cys Glu Ala Thr Gly Ser Arg
305                 310                 315                 320
Lys Tyr Met Glu Leu Gly Ala Thr Gln Gly Met Gly Glu Ala Leu Thr
                325                 330                 335
Arg Gly Met Val Leu Ala Met Ser Ile Trp Trp Asp Gln Gly Gly Asn
            340                 345                 350
Met Glu Trp Leu Asp His Gly Glu Ala Gly Pro Cys Ala Lys Gly Glu
        355                 360                 365
Gly Ala Pro Ser Asn Ile Val Gln Val Glu Pro Phe Pro Glu Val Thr
    370                 375                 380
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Tyr Thr Asn Leu Arg Trp Gly Glu Ile Gly Ser Thr Tyr Gln Glu Leu
385 390 395 400
Gln

<210> 34
<211> 433
<212> PRT
<213> Hypocrea jecorina

<400> 34
Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp Gln
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Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val Val
20 25 30
Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr Asn
35 40 45
Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn Glu
50 55 60
Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser Thr
65 70 75 80
Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Asp Phe Val Thr
85 90 95
Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala Ser
100 105 110
Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser Phe
115 120 125
Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr
130 135 140
Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr Asn
145 150 155 160
Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
165 170 175
Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp Glu
180 185 190
Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser Cys
195 200 205
Cys Ser Gln Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala Leu
210 215 220
Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly Asp
225 230 235 240
Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys Asp
245 250 255
Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser Phe
260 265 270
Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu Thr
275 280 285
Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr Val
290 295 300
Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser Tyr
305 310 315 320
Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala Glu
325 330 335
Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe Lys
340 345 350
Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp Asp
355 360 365
Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn Glu
370 375 380
Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser Ser
385 390 395 400

Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val Thr
405 410 415
Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro Ser
420 425 430
Gly

<210> 35
<211> 370
<212> PRT
<213> Hypocrea jecorina

<400> 35
Gln Pro Gly Thr Ser Thr Pro Glu Val His Pro Lys Leu Thr Thr Tyr
1 5 10 15
Lys Cys Thr Lys Ser Gly Gly Cys Val Ala Gln Asp Thr Ser Val Val
20 25 30
Leu Asp Trp Asn Tyr Arg Trp Met His Asp Ala Asn Tyr Asn Ser Cys
35 40 45
Thr Val Asn Gly Gly Val Asn Thr Thr Leu Cys Pro Asp Glu Ala Thr
50 55 60
Cys Gly Lys Asn Cys Phe Ile Glu Gly Val Asp Tyr Ala Ala Ser Gly
65 70 75 80
Val Thr Thr Ser Gly Ser Ser Leu Thr Met Asn Gln Tyr Met Pro Ser
85 90 95
Ser Ser Gly Gly Tyr Ser Ser Val Ser Pro Arg Leu Tyr Leu Leu Asp
100 105 110
Ser Asp Gly Glu Tyr Val Met Leu Lys Leu Asn Gly Gln Glu Leu Ser
115 120 125
Phe Asp Val Asp Leu Ser Ala Leu Pro Cys Gly Glu Asn Gly Ser Leu
130 135 140
Tyr Leu Ser Gln Met Asp Glu Asn Gly Gly Ala Asn Gln Tyr Asn Thr
145 150 155 160
Ala Gly Ala Asn Tyr Gly Ser Gly Tyr Cys Asp Ala Gln Cys Pro Val
165 170 175
Gln Thr Trp Arg Asn Gly Thr Leu Asn Thr Ser His Gln Gly Phe Cys
180 185 190
Cys Asn Glu Met Asp Ile Leu Glu Gly Asn Ser Arg Ala Asn Ala Leu
195 200 205
Thr Pro His Ser Cys Thr Ala Thr Ala Cys Asp Ser Ala Gly Cys Gly
210 215 220
Phe Asn Pro Tyr Gly Ser Gly Tyr Lys Ser Tyr Tyr Gly Pro Gly Asp
225 230 235 240
Thr Val Asp Thr Ser Lys Thr Phe Thr Ile Ile Thr Gln Phe Asn Thr
245 250 255
Asp Asn Gly Ser Pro Ser Gly Asn Leu Val Ser Ile Thr Arg Lys Tyr
260 265 270
Gln Gln Asn Gly Val Asp Ile Pro Ser Ala Gln Pro Gly Gly Asp Thr
275 280 285
Ile Ser Ser Cys Pro Ser Ala Ser Ala Tyr Gly Gly Leu Ala Thr Met
290 295 300
Gly Lys Ala Leu Ser Ser Gly Met Val Leu Val Phe Ser Ile Trp Asn
305 310 315 320
Asp Asn Ser Gln Tyr Met Asn Trp Leu Asp Ser Gly Asn Ala Gly Pro
325 330 335
Cys Ser Ser Thr Glu Gly Asn Pro Ser Asn Ile Leu Ala Asn Asn Pro
340 345 350
Asn Thr His Val Val Phe Ser Asn Ile Arg Trp Gly Asp Ile Gly Ser
355 360 365
Thr Thr
370

<210> 36
<211> 504
<212> PRT
<213> Phanerochaete chrysosporium

<400> 36
Met Arg Thr Ala Leu Ala Leu Ile Leu Ala Leu Ala Ala Phe Ser Ala
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Val Ser Ala Gln Gln Ala Gly Thr Ile Thr Ala Glu Thr His Pro Thr
20 25 30
Leu Thr Ile Gln Gln Cys Thr Gln Ser Gly Gly Cys Ala Pro Leu Thr
35 40 45
Thr Lys Val Val Leu Asp Val Asn Trp Arg Trp Ile His Ser Thr Thr
50 55 60
Gly Tyr Thr Asn Cys Tyr Ser Gly Asn Thr Trp Asp Ala Ile Leu Cys
65 70 75 80
Pro Asp Pro Val Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp
85 90 95
Tyr Thr Gly Thr Phe Gly Ile Leu Pro Ser Gly Thr Ser Val Thr Leu
100 105 110
Arg Pro Val Asp Gly Leu Gly Leu Arg Leu Phe Leu Leu Ala Asp Asp
115 120 125
Ser His Tyr Gln Met Phe Gln Leu Leu Asn Lys Glu Phe Thr Phe Asp
130 135 140
Val Glu Met Pro Asn Met Arg Cys Gly Ser Ser Gly Ala Ile His Leu
145 150 155 160
Thr Ala Met Asp Ala Asp Gly Gly Leu Ala Lys Tyr Pro Gly Asn Gln
165 170 175
Ala Gly Ala Lys Tyr Gly Thr Gly Phe Cys Ser Ala Gln Cys Pro Lys
180 185 190
Gly Val Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp Leu Gly
195 200 205
Thr Thr Ala Thr Thr Gly Thr Gly Phe Phe Gly Ser Cys Cys Thr Asp
210 215 220
Ile Ala Leu Trp Glu Ala Asn Asp Asn Ser Ala Ser Phe Ala Pro His
225 230 235 240
Pro Cys Thr Thr Asn Ser Gln Thr Arg Cys Ser Gly Ser Asp Cys Thr
245 250 255
Ala Asp Ser Gly Leu Cys Asp Ala Asp Gly Cys Asn Phe Asn Ser Phe
260 265 270
Arg Met Gly Asn Thr Thr Phe Phe Gly Ala Gly Met Ser Val Asp Thr
275 280 285
Thr Lys Leu Phe Thr Val Val Thr Gln Phe Ile Thr Ser Asp Asn Thr
290 295 300
Ser Met Gly Ala Leu Val Glu Ile His Arg Leu Tyr Ile Gln Asn Gly
305 310 315 320
Gln Val Ile Gln Asn Ser Val Val Asn Ile Pro Gly Ile Asn Pro Ala
325 330 335
Thr Ser Ile Thr Asp Asp Leu Cys Ala Gln Glu Asn Ala Ala Phe Gly
340 345 350
Gly Thr Ser Ser Phe Ala Gln His Gly Gly Leu Ala Gln Val Gly Glu
355 360 365
Ala Leu Arg Ser Gly Met Val Leu Ala Leu Ser Ile Val Asn Ser Ala
370 375 380
Ala Asp Thr Leu Trp Leu Asp Ser Asn Tyr Pro Ala Asp Ala Asp Pro
385 390 395 400
Ser Ala Pro Gly Val Ala Arg Gly Thr Cys Pro Gln Asp Ser Ala Ser
405 410 415
Ile Pro Glu Ala Pro Thr Pro Ser Val Val Phe Ser Asn Ile Lys Leu
420 425 430

Gly Asp Ile Gly Thr Thr Phe Gly Ala Gly Ser Ala Leu Phe Ser Gly
435 440 445
Arg Ser Pro Pro Gly Pro Val Pro Gly Ser Ala Pro Ala Ser Ser Ala
450 455 460
Thr Ala Thr Ala Pro Pro Phe Gly Ser Gln Cys Gly Gly Leu Gly Tyr
465 470 475 480
Ala Gly Pro Thr Gly Val Cys Pro Ser Pro Tyr Thr Cys Gln Ala Leu
485 490 495
Asn Ile Tyr Tyr Ser Gln Cys Ile
500

<210> 37
<211> 451
<212> PRT
<213> Phanerochaete chrysosporium

<400> 37
Met Arg Thr Ala Leu Ala Leu Ile Leu Ala Leu Ala Ala Phe Ser Ala
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Val Ser Ala Gln Gln Ala Gly Thr Ile Thr Ala Glu Thr His Pro Thr
20 25 30
Leu Thr Ile Gln Gln Cys Thr Gln Ser Gly Gly Cys Ala Pro Leu Thr
35 40 45
Thr Lys Val Val Leu Asp Val Asn Trp Arg Trp Ile His Ser Thr Thr
50 55 60
Gly Tyr Thr Asn Cys Tyr Ser Gly Asn Thr Trp Asp Ala Ile Leu Cys
65 70 75 80
Pro Asp Pro Val Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp
85 90 95
Tyr Thr Gly Thr Phe Gly Ile Leu Pro Ser Gly Thr Ser Val Thr Leu
100 105 110
Arg Pro Val Asp Gly Leu Gly Leu Arg Leu Phe Leu Leu Ala Asp Asp
115 120 125
Ser His Tyr Gln Met Phe Gln Leu Leu Asn Lys Glu Phe Thr Phe Asp
130 135 140
Val Glu Met Pro Asn Met Arg Cys Gly Ser Ser Gly Ala Ile His Leu
145 150 155 160
Thr Ala Met Asp Ala Asp Gly Gly Leu Ala Lys Tyr Pro Gly Asn Gln
165 170 175
Ala Gly Ala Lys Tyr Gly Thr Gly Phe Cys Ser Ala Gln Cys Pro Lys
180 185 190
Gly Val Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp Leu Gly
195 200 205
Thr Thr Ala Thr Thr Gly Thr Gly Phe Phe Gly Ser Cys Cys Thr Asp
210 215 220
Ile Ala Leu Trp Glu Ala Asn Asp Asn Ser Ala Ser Phe Ala Pro His
225 230 235 240
Pro Cys Thr Thr Asn Ser Gln Thr Arg Cys Ser Gly Ser Asp Cys Thr
245 250 255
Ala Asp Ser Gly Leu Cys Asp Ala Asp Gly Cys Asn Phe Asn Ser Phe
260 265 270
Arg Met Gly Asn Thr Thr Phe Phe Gly Ala Gly Met Ser Val Asp Thr
275 280 285
Thr Lys Leu Phe Thr Val Val Thr Gln Phe Ile Thr Ser Asp Asn Thr
290 295 300
Ser Met Gly Ala Leu Val Glu Ile His Arg Leu Tyr Ile Gln Asn Gly
305 310 315 320
Gln Val Ile Gln Asn Ser Val Val Asn Ile Pro Gly Ile Asn Pro Ala
325 330 335
Thr Ser Ile Thr Asp Asp Leu Cys Ala Gln Glu Asn Ala Ala Phe Gly
340 345 350

```
Gly Thr Ser Ser Phe Ala Gln His Gly Gly Leu Ala Gln Val Gly Glu
        355                 360                 365
Ala Leu Arg Ser Gly Met Val Leu Ala Leu Ser Ile Val Asn Ser Ala
    370                 375                 380
Ala Asp Thr Leu Trp Leu Asp Ser Asn Tyr Pro Ala Asp Ala Asp Pro
385                 390                 395                 400
Ser Ala Pro Gly Val Ala Arg Gly Thr Cys Pro Gln Asp Ser Ala Ser
                405                 410                 415
Ile Pro Glu Ala Pro Thr Pro Ser Val Val Phe Ser Asn Ile Lys Leu
            420                 425                 430
Gly Asp Ile Gly Thr Thr Phe Gly Ala Gly Ser Ala Leu Phe Pro Ser
        435                 440                 445
Gly Arg Ser
    450
```

<210> 38
<211> 449
<212> PRT
<213> Phanerochaete chrysosporium

<400> 38

```
Met Val Asp Ile Gln Ile Ala Thr Phe Leu Leu Leu Gly Val Val Gly
 1               5                  10                  15
Val Ala Ala Gln Gln Val Gly Thr Tyr Ile Pro Glu Asn His Pro Leu
            20                  25                  30
Leu Ala Thr Gln Ser Cys Thr Ala Ser Gly Gly Cys Thr Thr Ser Ser
        35                  40                  45
Ser Lys Ile Val Leu Asp Ala Asn Arg Arg Trp Ile His Ser Thr Leu
    50                  55                  60
Gly Thr Thr Ser Cys Leu Thr Ala Asn Gly Trp Asp Pro Thr Leu Cys
65                  70                  75                  80
Pro Asp Gly Ile Thr Cys Ala Asn Tyr Cys Ala Leu Asp Gly Val Ser
                85                  90                  95
Tyr Ser Ser Thr Tyr Gly Ile Thr Thr Ser Gly Ser Ala Leu Arg Leu
            100                 105                 110
Gln Phe Val Thr Gly Thr Asn Ile Gly Ser Arg Val Phe Leu Met Ala
        115                 120                 125
Asp Asp Thr His Tyr Arg Thr Phe Gln Leu Leu Asn Gln Glu Leu Ala
    130                 135                 140
Phe Asp Val Asp Val Ser Lys Leu Pro Cys Gly Leu Asn Gly Ala Leu
145                 150                 155                 160
Tyr Phe Val Ala Met Asp Ala Asp Gly Gly Lys Ser Lys Tyr Pro Gly
                165                 170                 175
Asn Arg Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
            180                 185                 190
Pro Arg Asp Val Gln Phe Ile Asn Gly Gln Ala Asn Val Gln Gly Trp
        195                 200                 205
Asn Ala Thr Ser Ala Thr Thr Gly Thr Gly Ser Tyr Gly Ser Cys Cys
    210                 215                 220
Thr Glu Leu Asp Ile Trp Glu Ala Asn Ser Asn Ala Ala Ala Leu Thr
225                 230                 235                 240
Pro His Thr Cys Thr Asn Asn Ala Gln Thr Arg Cys Ser Gly Ser Asn
                245                 250                 255
Cys Thr Ser Asn Thr Gly Phe Cys Asp Ala Asp Gly Cys Asp Phe Asn
            260                 265                 270
Ser Phe Arg Leu Gly Asn Thr Thr Phe Leu Gly Ala Gly Met Ser Val
        275                 280                 285
Asp Thr Thr Lys Thr Phe Thr Val Val Thr Gln Phe Ile Thr Ser Asp
    290                 295                 300
Asn Thr Ser Thr Gly Asn Leu Thr Glu Ile Arg Arg Phe Tyr Val Gln
```

305 310 315 320
Asn Gly Asn Val Ile Pro Asn Ser Val Val Asn Val Thr Gly Ile Gly
325 330 335
Ala Val Asn Ser Ile Thr Asp Pro Phe Cys Ser Gln Gln Lys Lys Ala
340 345 350
Phe Ile Glu Thr Asn Tyr Phe Ala Gln His Gly Gly Leu Ala Gln Leu
355 360 365
Gly Gln Ala Leu Arg Thr Gly Met Val Leu Ala Phe Ser Ile Ser Asp
370 375 380
Asp Pro Ala Asn His Met Leu Trp Leu Asp Ser Asn Phe Pro Pro Ser
385 390 395 400
Ala Asn Pro Ala Val Pro Gly Val Ala Arg Gly Met Cys Ser Ile Thr
405 410 415
Ser Gly Asn Pro Ala Asp Val Gly Ile Leu Asn Pro Ser Pro Tyr Val
420 425 430
Ser Phe Leu Asn Ile Lys Phe Gly Ser Ile Gly Thr Thr Phe Arg Pro
435 440 445
Ala

<210> 39
<211> 516
<212> PRT
<213> Phanerochaete chrysosporium

<400> 39
Met Phe Arg Thr Ala Thr Leu Leu Ala Phe Thr Met Ala Ala Met Val
1 5 10 15
Phe Gly Gln Gln Val Gly Thr Asn Thr Ala Glu Asn His Arg Thr Leu
20 25 30
Thr Ser Gln Lys Cys Thr Lys Ser Gly Gly Cys Ser Asn Leu Asn Thr
35 40 45
Lys Ile Val Leu Asp Ala Asn Trp Arg Trp Leu His Ser Thr Ser Gly
50 55 60
Tyr Thr Asn Cys Tyr Thr Gly Asn Gln Trp Asp Ala Thr Leu Cys Pro
65 70 75 80
Asp Gly Lys Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr
85 90 95
Thr Gly Thr Tyr Gly Ile Thr Ala Ser Gly Ser Ser Leu Lys Leu Gln
100 105 110
Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr Leu Met Ala Asp
115 120 125
Asp Thr His Tyr Gln Met Phe Gln Leu Leu Asn Gln Glu Phe Thr Phe
130 135 140
Asp Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr
145 150 155 160
Leu Ser Ala Met Asp Ala Asp Gly Gly Met Ala Lys Tyr Pro Thr Asn
165 170 175
Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
180 185 190
Arg Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Glu Gly Trp Asn
195 200 205
Ala Thr Ser Ala Asn Ala Gly Thr Gly Asn Tyr Gly Thr Cys Cys Thr
210 215 220
Glu Met Asp Ile Trp Glu Ala Asn Asn Asp Ala Ala Ala Tyr Thr Pro
225 230 235 240
His Pro Cys Thr Thr Asn Ala Gln Thr Arg Cys Ser Gly Ser Asp Cys
245 250 255
Thr Arg Asp Thr Gly Leu Cys Asp Ala Asp Gly Cys Asp Phe Asn Ser
260 265 270
Phe Arg Met Gly Asp Gln Thr Phe Leu Gly Lys Gly Leu Thr Val Asp

```
          275                 280                 285
Thr Ser Lys Pro Phe Thr Val Val Thr Gln Phe Ile Thr Asn Asp Gly
    290                 295                 300
Thr Ser Ala Gly Thr Leu Thr Glu Ile Arg Arg Leu Tyr Val Gln Asn
305                 310                 315                 320
Gly Lys Val Ile Gln Asn Ser Ser Val Lys Ile Pro Gly Ile Asp Pro
                325                 330                 335
Val Asn Ser Ile Thr Asp Asn Phe Cys Ser Gln Gln Lys Thr Ala Phe
            340                 345                 350
Gly Asp Thr Asn Tyr Phe Ala Gln His Gly Gly Leu Lys Gln Val Gly
        355                 360                 365
Glu Ala Leu Arg Thr Gly Met Val Leu Ala Leu Ser Ile Trp Asp Asp
    370                 375                 380
Tyr Ala Ala Asn Met Leu Trp Leu Asp Ser Asn Tyr Pro Thr Asn Lys
385                 390                 395                 400
Asp Pro Ser Thr Pro Gly Val Ala Arg Gly Thr Cys Ala Thr Thr Ser
                405                 410                 415
Gly Val Pro Ala Gln Ile Glu Ala Gln Ser Pro Asn Ala Tyr Val Val
            420                 425                 430
Phe Ser Asn Ile Lys Phe Gly Asp Leu Asn Thr Thr Tyr Thr Gly Thr
        435                 440                 445
Val Ser Ser Ser Ser Val Ser Ser Ser His Ser Ser Thr Ser Thr Ser
    450                 455                 460
Ser Ser His Ser Ser Ser Ser Thr Pro Pro Thr Gln Pro Thr Gly Val
465                 470                 475                 480
Thr Val Pro Gln Trp Gly Gln Cys Gly Gly Ile Gly Tyr Thr Gly Ser
                485                 490                 495
Thr Thr Cys Ala Ser Pro Tyr Thr Cys His Val Leu Asn Pro Tyr Tyr
            500                 505                 510
Ser Gln Cys Tyr
        515
```

<210> 40
<211> 516
<212> PRT
<213> Phanerochaete chrysosporium

<400> 40

```
Met Phe Arg Thr Ala Thr Leu Leu Ala Phe Thr Met Ala Ala Met Val
 1               5                  10                  15
Phe Gly Gln Gln Val Gly Thr Asn Thr Ala Glu Asn His Arg Thr Leu
            20                  25                  30
Thr Ser Gln Lys Cys Thr Lys Ser Gly Gly Cys Ser Asn Leu Asn Thr
        35                  40                  45
Lys Ile Val Leu Asp Ala Asn Trp Arg Trp Leu His Ser Thr Ser Gly
    50                  55                  60
Tyr Thr Asn Cys Tyr Thr Gly Asn Gln Trp Asp Ala Thr Leu Cys Pro
65                  70                  75                  80
Asp Gly Lys Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr
                85                  90                  95
Thr Gly Thr Tyr Gly Ile Thr Ala Ser Gly Ser Ser Leu Lys Leu Gln
            100                 105                 110
Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr Leu Met Ala Asp
        115                 120                 125
Asp Thr His Tyr Gln Met Phe Gln Leu Leu Asn Gln Glu Phe Thr Phe
    130                 135                 140
Asp Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr
145                 150                 155                 160
Leu Ser Ala Met Asp Ala Asp Gly Gly Met Ala Lys Tyr Pro Thr Asn
                165                 170                 175
Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
```

```
                180                 185                 190
Arg Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Glu Gly Trp Asn
        195                 200                 205
Ala Thr Ser Ala Asn Ala Gly Thr Gly Asn Tyr Gly Thr Cys Cys Thr
    210                 215                 220
Glu Met Asp Ile Trp Glu Ala Asn Asn Asp Ala Ala Ala Tyr Thr Pro
225                 230                 235                 240
His Pro Cys Thr Thr Asn Ala Gln Thr Arg Cys Ser Gly Ser Asp Cys
                245                 250                 255
Thr Arg Asp Thr Gly Leu Cys Asp Ala Asp Gly Cys Asp Phe Asn Ser
            260                 265                 270
Phe Arg Met Gly Asp Gln Thr Phe Leu Gly Lys Gly Leu Thr Val Asp
        275                 280                 285
Thr Ser Lys Pro Phe Thr Val Val Thr Gln Phe Ile Thr Asn Asp Gly
    290                 295                 300
Thr Ser Ala Gly Thr Leu Thr Glu Ile Arg Arg Leu Tyr Val Gln Asn
305                 310                 315                 320
Gly Lys Val Ile Gln Asn Ser Ser Val Lys Ile Pro Gly Ile Asp Leu
                325                 330                 335
Val Asn Ser Ile Thr Asp Asn Phe Cys Ser Gln Gln Lys Thr Ala Phe
            340                 345                 350
Gly Asp Thr Asn Tyr Phe Ala Gln His Gly Gly Leu Lys Gln Val Gly
        355                 360                 365
Glu Ala Leu Arg Thr Gly Met Val Leu Ala Leu Ser Ile Trp Asp Asp
    370                 375                 380
Tyr Ala Ala Asn Met Leu Trp Leu Asp Ser Asn Tyr Pro Thr Asn Lys
385                 390                 395                 400
Asp Pro Ser Thr Pro Gly Val Ala Arg Gly Thr Cys Ala Thr Thr Ser
                405                 410                 415
Gly Val Pro Ala Gln Ile Glu Ala Gln Ser Pro Asn Ala Tyr Val Val
            420                 425                 430
Phe Ser Asn Ile Lys Phe Gly Asp Leu Asn Thr Thr Tyr Thr Gly Thr
        435                 440                 445
Val Ser Ser Ser Ser Val Ser Ser Ser His Ser Ser Thr Ser Thr Ser
    450                 455                 460
Ser Ser His Ser Ser Ser Ser Thr Pro Pro Thr Gln Pro Thr Gly Val
465                 470                 475                 480
Thr Val Pro Gln Trp Gly Gln Cys Gly Gly Ile Gly Tyr Thr Gly Ser
                485                 490                 495
Thr Thr Cys Ala Ser Pro Tyr Thr Cys His Val Leu Asn Pro Tyr Tyr
            500                 505                 510
Ser Gln Cys Tyr
        515
```

<210> 41
<211> 516
<212> PRT
<213> Phanerochaete chrysosporium

<400> 41

```
Met Phe Arg Thr Ala Thr Leu Leu Ala Phe Thr Met Ala Ala Met Val
 1               5                  10                  15
Phe Gly Gln Gln Val Gly Thr Asn Thr Ala Arg Ser His Pro Ala Leu
            20                  25                  30
Thr Ser Gln Lys Cys Thr Lys Ser Gly Gly Cys Ser Asn Leu Asn Thr
        35                  40                  45
Lys Ile Val Leu Asp Ala Asn Trp Arg Trp Leu His Ser Thr Ser Gly
    50                  55                  60
Tyr Thr Asn Cys Tyr Thr Gly Asn Gln Trp Asp Ala Thr Leu Cys Pro
65                  70                  75                  80
Asp Gly Lys Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr
```

85 90 95
Thr Gly Thr Tyr Gly Ile Thr Ala Ser Gly Ser Ser Leu Lys Leu Gln
100 105 110
Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr Leu Met Ala Asp
115 120 125
Asp Thr His Tyr Gln Met Phe Gln Leu Leu Asn Gln Glu Phe Thr Phe
130 135 140
Asp Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr
145 150 155 160
Leu Ser Ala Met Asp Ala Asp Gly Gly Met Ala Lys Tyr Pro Thr Asn
165 170 175
Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
180 185 190
Arg Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Glu Gly Trp Asn
195 200 205
Ala Thr Ser Ala Asn Ala Gly Thr Gly Asn Tyr Gly Thr Cys Cys Thr
210 215 220
Glu Met Asp Ile Trp Glu Ala Asn Asn Asp Ala Ala Ala Tyr Thr Pro
225 230 235 240
His Pro Cys Thr Thr Asn Ala Gln Thr Arg Cys Ser Gly Ser Asp Cys
245 250 255
Thr Arg Asp Thr Gly Leu Cys Asp Ala Asp Gly Cys Asp Phe Asn Ser
260 265 270
Phe Arg Met Gly Asp Gln Thr Phe Leu Gly Lys Gly Leu Thr Val Asp
275 280 285
Thr Ser Lys Pro Phe Thr Val Val Thr Gln Phe Ile Thr Asn Asp Gly
290 295 300
Thr Ser Ala Gly Thr Leu Thr Glu Ile Arg Arg Leu Tyr Val Gln Asn
305 310 315 320
Gly Lys Val Ile Gln Asn Ser Ser Val Lys Ile Pro Gly Ile Asp Pro
325 330 335
Val Asn Ser Ile Thr Asp Asn Phe Cys Ser Gln Gln Lys Thr Ala Phe
340 345 350
Gly Asp Thr Asn Tyr Phe Ala Gln His Gly Gly Leu Lys Gln Val Gly
355 360 365
Glu Ala Leu Arg Thr Gly Met Val Leu Ala Leu Ser Ile Trp Asp Asp
370 375 380
Tyr Ala Ala Asn Met Leu Trp Leu Asp Ser Asn Tyr Pro Thr Asn Lys
385 390 395 400
Asp Pro Ser Thr Pro Gly Val Ala Arg Gly Thr Cys Ala Thr Thr Ser
405 410 415
Gly Val Pro Ala Gln Ile Glu Ala Gln Ser Pro Asn Ala Tyr Val Val
420 425 430
Phe Ser Asn Ile Lys Phe Gly Asp Leu Asn Thr Thr Tyr Thr Gly Thr
435 440 445
Val Ser Ser Ser Ser Val Ser Ser Ser His Ser Ser Thr Ser Thr Ser
450 455 460
Ser Ser His Ser Ser Ser Ser Thr Pro Pro Thr Gln Pro Thr Gly Val
465 470 475 480
Thr Val Pro Gln Trp Gly Gln Cys Gly Gly Ile Gly Tyr Thr Gly Ser
485 490 495
Thr Thr Cys Ala Ser Pro Tyr Thr Cys His Val Leu Asn Pro Tyr Tyr
500 505 510
Ser Gln Cys Tyr
515

<210> 42
<211> 511
<212> PRT
<213> Phanerochaete chrysosporium

<400> 42

```
Met Phe Arg Ala Ala Ala Leu Leu Ala Phe Thr Cys Leu Ala Met Val
 1               5                  10                  15
Ser Gly Gln Gln Ala Gly Thr Asn Thr Ala Glu Asn His Pro Gln Leu
            20                  25                  30
Gln Ser Gln Gln Cys Thr Thr Ser Gly Gly Cys Lys Pro Leu Ser Thr
        35                  40                  45
Lys Val Val Leu Asp Ser Asn Trp Arg Trp Val His Ser Thr Ser Gly
    50                  55                  60
Tyr Thr Asn Cys Tyr Thr Gly Asn Glu Trp Asn Thr Ser Leu Cys Pro
65                  70                  75                  80
Asp Gly Lys Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr
                85                  90                  95
Ser Gly Thr Tyr Gly Ile Thr Ser Thr Gly Thr Ala Leu Thr Leu Lys
            100                 105                 110
Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr Leu Met Ala Asp
        115                 120                 125
Asp Thr His Tyr Gln Leu Leu Lys Leu Leu Asn Gln Glu Phe Thr Phe
    130                 135                 140
Asp Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr
145                 150                 155                 160
Leu Ser Ala Met Asp Ala Asp Gly Gly Met Ser Lys Tyr Pro Gly Asn
                165                 170                 175
Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
            180                 185                 190
Lys Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Gly Asn Trp Thr
        195                 200                 205
Glu Thr Gly Ser Asn Thr Gly Thr Gly Ser Tyr Gly Thr Cys Cys Ser
    210                 215                 220
Glu Met Asp Ile Trp Glu Ala Asn Asn Asp Ala Ala Ala Phe Thr Pro
225                 230                 235                 240
His Pro Cys Thr Thr Thr Gly Gln Thr Arg Cys Ser Gly Asp Asp Cys
                245                 250                 255
Ala Arg Asn Thr Gly Leu Cys Asp His Gly Asp Gly Cys Asp Phe Asn
            260                 265                 270
Ser Phe Arg Met Gly Asp Lys Thr Phe Leu Gly Lys Gly Met Thr Val
        275                 280                 285
Asp Thr Ser Lys Pro Phe Thr Asp Val Thr Gln Phe Leu Thr Asn Asp
    290                 295                 300
Asn Thr Ser Thr Gly Thr Leu Ser Glu Ile Arg Arg Ile Tyr Ile Gln
305                 310                 315                 320
Asn Gly Lys Val Ile Gln Asn Ser Val Ala Asn Ile Pro Gly Val Asp
                325                 330                 335
Pro Val Asn Ser Ile Thr Asp Asn Phe Cys Ala Gln Gln Lys Thr Ala
            340                 345                 350
Phe Gly Asp Thr Asn Trp Phe Ala Gln Lys Gly Gly Leu Lys Gln Met
        355                 360                 365
Gly Glu Ala Leu Gly Asn Gly Met Val Leu Ala Leu Ser Ile Trp Asp
    370                 375                 380
Asp His Ala Ala Asn Met Leu Trp Leu Asp Ser Asp Tyr Pro Thr Asp
385                 390                 395                 400
Lys Asp Pro Ser Ala Pro Gly Val Ala Arg Gly Thr Cys Ala Thr Thr
                405                 410                 415
Ser Gly Val Pro Ser Asp Val Glu Ser Gln Val Pro Asn Ser Gln Val
            420                 425                 430
Val Phe Ser Asn Ile Lys Phe Gly Asp Ile Gly Ser Thr Phe Ser Gly
        435                 440                 445
Thr Ser Ser Pro Asn Pro Pro Gly Gly Ser Thr Thr Ser Ser Pro Val
    450                 455                 460
Thr Thr Ser Pro Thr Pro Pro Pro Thr Gly Pro Thr Val Pro Gln Trp
465                 470                 475                 480
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```
Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Ser Thr Thr Cys Ala Ser
                485                 490                 495
Pro Tyr Thr Cys His Val Leu Asn Pro Tyr Tyr Ser Gln Cys Tyr
            500                 505                 510
```

<210> 43
<211> 510
<212> PRT
<213> Phanerochaete chrysosporium

<400> 43

```
Met Phe Arg Ala Ala Ala Leu Leu Ala Phe Thr Cys Leu Ala Met Val
 1               5                  10                  15
Ser Gly Gln Gln Ala Gly Thr Asn Thr Ala Glu Asn His Pro Gln Leu
            20                  25                  30
Gln Ser Gln Gln Cys Thr Thr Ser Gly Gly Cys Lys Pro Leu Ser Thr
        35                  40                  45
Lys Val Val Leu Asp Ser Asn Trp Arg Trp Val His Ser Thr Ser Gly
    50                  55                  60
Tyr Thr Asn Cys Tyr Thr Gly Asn Glu Trp Asp Thr Ser Leu Cys Pro
65                  70                  75                  80
Asp Gly Lys Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr
                85                  90                  95
Ser Gly Thr Tyr Gly Ile Thr Ser Thr Gly Thr Ala Leu Thr Leu Lys
            100                 105                 110
Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr Leu Met Ala Asp
        115                 120                 125
Asp Thr His Tyr Gln Leu Leu Lys Leu Leu Asn Gln Glu Phe Thr Phe
    130                 135                 140
Asp Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr
145                 150                 155                 160
Leu Ser Ala Met Asp Ala Asp Gly Gly Met Ser Lys Tyr Pro Gly Asn
                165                 170                 175
Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
            180                 185                 190
Lys Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Gly Asn Trp Thr
        195                 200                 205
Glu Thr Gly Ser Asn Thr Gly Thr Gly Ser Tyr Gly Thr Cys Cys Ser
    210                 215                 220
Glu Met Asp Ile Trp Glu Ala Asn Asn Asp Ala Ala Ala Phe Thr Pro
225                 230                 235                 240
His Pro Cys Thr Thr Thr Gly Gln Thr Arg Cys Ser Gly Asp Asp Cys
                245                 250                 255
Ala Arg Asn Thr Gly Leu Cys Asp Gly Asp Gly Cys Asp Phe Asn Ser
            260                 265                 270
Phe Arg Met Gly Asp Lys Thr Phe Leu Gly Lys Gly Met Thr Val Asp
        275                 280                 285
Thr Ser Lys Pro Phe Thr Val Val Thr Gln Phe Leu Thr Asn Asp Asn
    290                 295                 300
Thr Ser Thr Gly Thr Leu Ser Glu Ile Arg Arg Ile Tyr Ile Gln Asn
305                 310                 315                 320
Gly Lys Val Ile Gln Asn Ser Val Ala Asn Ile Pro Gly Val Asp Pro
                325                 330                 335
Val Asn Ser Ile Thr Asp Asn Phe Cys Ala Gln Gln Lys Thr Ala Phe
            340                 345                 350
Gly Asp Thr Asn Trp Phe Ala Gln Lys Gly Gly Leu Lys Gln Met Gly
        355                 360                 365
Glu Ala Leu Gly Asn Gly Met Val Leu Ala Leu Ser Ile Trp Asp Asp
    370                 375                 380
His Ala Ala Asn Met Leu Trp Leu Asp Ser Asp Tyr Pro Thr Asp Lys
385                 390                 395                 400
```

Asp Pro Ser Ala Pro Gly Val Ala Arg Gly Thr Cys Ala Thr Thr Ser
405 410 415
Gly Val Pro Ser Asp Val Glu Ser Gln Val Pro Asn Ser Gln Val Val
420 425 430
Phe Ser Asn Ile Lys Phe Gly Asp Ile Gly Ser Thr Phe Ser Gly Thr
435 440 445
Ser Ser Pro Asn Pro Pro Gly Gly Ser Thr Thr Ser Ser Pro Val Thr
450 455 460
Thr Ser Pro Thr Pro Pro Pro Thr Gly Pro Thr Val Pro Gln Trp Gly
465 470 475 480
Gln Cys Gly Gly Ile Gly Tyr Ser Gly Ser Thr Thr Cys Ala Ser Pro
485 490 495
Tyr Thr Cys His Val Leu Asn Pro Tyr Tyr Ser Gln Cys Tyr
500 505 510

<210> 44
<211> 540
<212> PRT
<213> Phanerochaete chrysosporium

<400> 44
Met Phe Arg Ala Ala Ala Leu Leu Ala Phe Thr Cys Leu Ala Met Val
1 5 10 15
Ser Gly Gln Gln Ala Gly Thr Asn Thr Ala Glu Asn His Pro Gln Leu
20 25 30
Gln Ser Gln Gln Cys Thr Thr Ser Gly Gly Cys Lys Pro Leu Ser Thr
35 40 45
Lys Val Val Leu Asp Ser Asn Trp Arg Trp Val His Ser Thr Ser Gly
50 55 60
Tyr Thr Asn Cys Tyr Thr Gly Asn Glu Trp Asp Thr Ser Leu Cys Pro
65 70 75 80
Asp Gly Lys Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr
85 90 95
Ser Gly Thr Tyr Gly Ile Thr Ser Thr Gly Thr Ala Leu Thr Leu Lys
100 105 110
Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr Leu Met Ala Asp
115 120 125
Asp Thr His Tyr Gln Leu Leu Lys Leu Leu Asn Gln Glu Phe Thr Phe
130 135 140
Asp Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr
145 150 155 160
Leu Ser Ala Met Asp Ala Asp Gly Gly Met Ser Lys Tyr Pro Gly Asn
165 170 175
Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
180 185 190
Lys Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Gly Asn Trp Thr
195 200 205
Glu Thr Gly Ser Asn Thr Gly Thr Gly Ser Tyr Gly Thr Cys Cys Ser
210 215 220
Glu Met Asp Ile Trp Glu Ala Asn Asn Asp Ala Ala Ala Phe Thr Pro
225 230 235 240
His Pro Cys Thr Thr Thr Gly Gln Thr Arg Cys Ser Gly Asp Asp Cys
245 250 255
Ala Arg Asn Thr Gly Leu Cys Asp Gly Asp Gly Cys Asp Phe Asn Ser
260 265 270
Phe Arg Met Gly Asp Lys Thr Phe Leu Gly Lys Gly Met Thr Val Asp
275 280 285
Thr Ser Lys Pro Phe Thr Val Val Thr Gln Phe Leu Thr Asn Asp Asn
290 295 300
Thr Ser Thr Gly Thr Leu Ser Glu Ile Arg Arg Ile Tyr Ile Gln Asn
305 310 315 320

Gly Lys Val Ile Gln Asn Ser Val Ala Asn Ile Pro Gly Val Asp Pro
325 330 335
Val Asn Ser Ile Thr Asp Asn Phe Cys Ala Gln Gln Lys Thr Ala Phe
340 345 350
Gly Asp Thr Asn Trp Phe Ala Gln Lys Gly Gly Leu Lys Gln Met Gly
355 360 365
Glu Ala Leu Gly Asn Gly Met Val Leu Ala Leu Ser Ile Trp Asp Asp
370 375 380
His Ala Ala Asn Met Leu Trp Leu Asp Ser Asp Tyr Pro Thr Asp Lys
385 390 395 400
Asp Pro Ser Ala Pro Gly Val Ala Arg Gly Thr Cys Ala Thr Thr Ser
405 410 415
Gly Val Pro Ser Asp Val Glu Ser Gln Val Pro Asn Ser Gln Val Val
420 425 430
Phe Ser Asn Ile Lys Phe Gly Asp Ile Gly Ser Thr Phe Ser Gly Thr
435 440 445
Ser Ser Pro Asn Pro Pro Gly Gly Ser Thr Thr Ser Ser Pro Val Thr
450 455 460
Thr Ser Pro Thr Pro Pro Pro Thr Gly Pro Thr Val Pro Gln Trp Gly
465 470 475 480
Gln Cys Gly Gly Ile Gly Tyr Ser Gly Ser Thr Thr Cys Ala Ser Pro
485 490 495
Tyr Thr Cys His Val Leu Asn Pro Cys Glu Ser Ile Leu Ser Leu Gln
500 505 510
Arg Ser Ser Asn Ala Asp Gln Tyr Leu Gln Thr Thr Arg Ser Ala Thr
515 520 525
Lys Arg Arg Leu Asp Thr Ala Leu Gln Pro Arg Lys
530 535 540

<210> 45
<211> 523
<212> PRT
<213> Irpex lacteus

<400> 45
Met Phe Arg Lys Ala Ala Leu Leu Ala Phe Ser Phe Leu Ala Ile Ala
1 5 10 15
His Gly Gln Gln Val Gly Thr Asn Gln Ala Glu Asn His Pro Ser Leu
20 25 30
Pro Ser Gln Lys Cys Thr Ala Ser Gly Cys Thr Thr Ser Ser Thr Ser
35 40 45
Val Val Leu Asp Ala Asn Trp Arg Trp Val His Thr Thr Thr Gly Tyr
50 55 60
Thr Asn Cys Tyr Thr Gly Gln Thr Trp Asp Ala Ser Ile Cys Pro Asp
65 70 75 80
Gly Val Thr Cys Ala Lys Ala Cys Ala Leu Asp Gly Ala Asp Tyr Ser
85 90 95
Gly Thr Tyr Gly Ile Thr Thr Ser Gly Asn Ala Leu Thr Leu Gln Phe
100 105 110
Val Lys Gly Thr Asn Val Gly Ser Arg Val Tyr Leu Leu Gln Asp Ala
115 120 125
Ser Asn Tyr Gln Met Phe Gln Leu Ile Asn Gln Glu Phe Thr Phe Asp
130 135 140
Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Val Tyr Leu
145 150 155 160
Ser Gln Met Asp Gln Asp Gly Gly Val Ser Arg Phe Pro Thr Asn Thr
165 170 175
Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro Arg
180 185 190
Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Glu Gly Trp Thr Gly
195 200 205

```
Ser Ser Thr Asp Ser Asn Ser Gly Thr Gly Asn Tyr Gly Thr Cys Cys
    210                 215                 220
Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Val Ala Ala Ala Tyr Thr
225                 230                 235                 240
Pro His Pro Cys Ser Val Asn Gln Gln Thr Arg Cys Thr Gly Ala Asp
                245                 250                 255
Cys Gly Gln Gly Asp Asp Arg Tyr Asp Gly Val Cys Asp Pro Asp Gly
            260                 265                 270
Cys Asp Phe Asn Ser Phe Arg Met Gly Asp Gln Thr Phe Leu Gly Lys
        275                 280                 285
Gly Leu Thr Val Asp Thr Ser Arg Lys Phe Thr Ile Val Thr Gln Phe
    290                 295                 300
Ile Ser Asp Asp Gly Thr Thr Ser Gly Asn Leu Ala Glu Ile Arg Arg
305                 310                 315                 320
Phe Tyr Val Gln Asp Gly Asn Val Ile Pro Asn Ser Lys Val Ser Ile
                325                 330                 335
Ala Gly Ile Asp Ala Val Asn Ser Ile Thr Asp Asp Phe Cys Thr Gln
            340                 345                 350
Gln Lys Thr Ala Phe Gly Asp Thr Asn Arg Phe Ala Ala Gln Gly Gly
        355                 360                 365
Leu Lys Gln Met Gly Ala Ala Leu Lys Ser Gly Met Val Leu Ala Leu
    370                 375                 380
Ser Leu Trp Asp Asp His Ala Ala Asn Met Leu Trp Leu Asp Ser Asp
385                 390                 395                 400
Tyr Pro Thr Thr Ala Asp Ala Ser Asn Pro Gly Val Ala Arg Gly Thr
                405                 410                 415
Cys Pro Thr Thr Ser Gly Phe Pro Arg Asp Val Glu Ser Gln Ser Gly
            420                 425                 430
Ser Ala Thr Val Thr Tyr Ser Asn Ile Lys Trp Gly Asp Leu Asn Ser
        435                 440                 445
Thr Phe Thr Gly Thr Leu Thr Thr Pro Ser Gly Ser Ser Ser Pro Ser
    450                 455                 460
Ser Pro Ala Ser Thr Ser Gly Ser Ser Thr Ser Ala Ser Ser Ser Ala
465                 470                 475                 480
Ser Val Pro Thr Gln Ser Gly Thr Val Ala Gln Trp Ala Gln Cys Gly
                485                 490                 495
Gly Ile Gly Tyr Ser Gly Ala Thr Thr Cys Val Ser Pro Tyr Thr Cys
            500                 505                 510
His Val Val Asn Ala Tyr Tyr Ser Gln Cys Tyr
        515                 520
```

<210> 46
<211> 526
<212> PRT
<213> Irpex lacteus

<400> 46

```
Met Phe His Lys Ala Val Leu Val Ala Phe Ser Leu Val Thr Ile Val
 1                   5                  10                  15
His Gly Gln Gln Ala Gly Thr Gln Thr Ala Glu Asn His Pro Gln Leu
            20                  25                  30
Ser Ser Gln Lys Cys Thr Ala Gly Gly Ser Cys Thr Ser Ala Ser Thr
        35                  40                  45
Ser Val Val Leu Asp Ser Asn Trp Arg Trp Val His Thr Thr Ser Gly
    50                  55                  60
Tyr Thr Asn Cys Tyr Thr Gly Asn Thr Trp Asp Ala Ser Ile Cys Ser
65                  70                  75                  80
Asp Pro Val Ser Cys Ala Gln Asn Cys Ala Leu Asp Gly Ala Asp Tyr
                85                  90                  95
Ala Gly Thr Tyr Gly Ile Thr Thr Ser Gly Asp Ala Leu Thr Leu Lys
            100                 105                 110
```

Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr Leu Met Glu Asp
115 120 125
Glu Thr Asn Tyr Gln Met Phe Lys Leu Met Asn Gln Glu Phe Thr Phe
130 135 140
Asp Val Asp Val Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Val Tyr
145 150 155 160
Phe Val Gln Met Asp Gln Asp Gly Gly Thr Ser Lys Phe Pro Asn Asn
165 170 175
Lys Ala Gly Ala Lys Phe Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
180 185 190
Gln Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Ile Val Asp Trp Thr
195 200 205
Ala Ser Ala Gly Asp Ala Asn Ser Gly Thr Gly Ser Phe Gly Thr Cys
210 215 220
Cys Gln Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Ala Ala Tyr
225 230 235 240
Thr Pro His Pro Cys Thr Val Thr Glu Gln Thr Arg Cys Ser Gly Ser
245 250 255
Asp Cys Gly Gln Gly Ser Asp Arg Phe Asn Gly Ile Cys Asp Pro Asp
260 265 270
Gly Cys Asp Phe Asn Ser Phe Arg Met Gly Asn Thr Glu Phe Tyr Gly
275 280 285
Lys Gly Leu Thr Val Asp Thr Ser Gln Lys Phe Thr Ile Val Thr Gln
290 295 300
Phe Ile Ser Asp Asp Gly Thr Ala Asp Gly Asn Leu Ala Glu Ile Arg
305 310 315 320
Arg Phe Tyr Val Gln Asn Gly Lys Val Ile Pro Asn Ser Val Val Gln
325 330 335
Ile Thr Gly Ile Asp Pro Val Asn Ser Ile Thr Glu Asp Phe Cys Thr
340 345 350
Gln Gln Lys Thr Val Phe Gly Asp Thr Asn Asn Phe Ala Ala Lys Gly
355 360 365
Gly Leu Lys Gln Met Gly Glu Ala Val Lys Asn Gly Met Val Leu Ala
370 375 380
Leu Ser Leu Trp Asp Asp Tyr Ala Ala Gln Met Leu Trp Leu Asp Ser
385 390 395 400
Asp Tyr Pro Thr Thr Ala Asp Pro Ser Gln Pro Gly Val Ala Arg Gly
405 410 415
Thr Cys Pro Thr Thr Ser Gly Val Pro Ser Gln Val Glu Gly Gln Glu
420 425 430
Gly Ser Ser Ser Val Ile Tyr Ser Asn Ile Lys Phe Gly Asp Leu Asn
435 440 445
Ser Thr Phe Thr Gly Thr Leu Thr Asn Pro Ser Ser Pro Ala Gly Pro
450 455 460
Pro Val Thr Ser Ser Pro Ser Glu Pro Ser Gln Ser Thr Gln Pro Ser
465 470 475 480
Gln Pro Ala Gln Pro Thr Gln Pro Ala Gly Thr Ala Ala Gln Trp Ala
485 490 495
Gln Cys Gly Gly Met Gly Phe Thr Gly Pro Thr Val Cys Ala Ser Pro
500 505 510
Phe Thr Cys His Val Leu Asn Pro Tyr Tyr Ser Gln Cys Tyr
515 520 525

<210> 47
<211> 517
<212> PRT
<213> Irpex lacteus

<400> 47
Met Phe Pro Lys Ala Ser Leu Ile Ala Leu Ser Phe Ile Ala Ala Val
1 5 10 15

Tyr Gly Gln Gln Val Gly Thr Gln Met Ala Glu Val His Pro Lys Leu
20 25 30
Pro Ser Gln Leu Cys Thr Lys Ser Gly Cys Thr Asn Gln Asn Thr Ala
35 40 45
Val Val Leu Asp Ala Asn Trp Arg Trp Leu His Thr Thr Ser Gly Tyr
50 55 60
Thr Asn Cys Tyr Thr Gly Asn Ser Trp Asp Ala Thr Leu Cys Pro Asp
65 70 75 80
Ala Thr Thr Cys Ala Gln Asn Cys Ala Val Asp Gly Ala Asp Tyr Ser
85 90 95
Gly Thr Tyr Gly Ile Thr Thr Ser Gly Asn Ala Leu Thr Leu Lys Phe
100 105 110
Lys Thr Gly Thr Asn Val Gly Ser Arg Val Tyr Leu Met Gln Thr Asp
115 120 125
Thr Ala Tyr Gln Met Phe Gln Leu Leu Asn Gln Glu Phe Thr Phe Asp
130 135 140
Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr Leu
145 150 155 160
Ser Gln Met Asp Gln Asp Gly Gly Leu Ser Lys Phe Pro Thr Asn Lys
165 170 175
Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro His
180 185 190
Asp Ile Lys Phe Ile Asn Gly Met Ala Asn Val Ala Gly Trp Ala Gly
195 200 205
Ser Ala Ser Asp Pro Asn Ala Gly Ser Gly Thr Leu Gly Thr Cys Cys
210 215 220
Ser Glu Met Asp Ile Trp Glu Ala Asn Asn Asp Ala Ala Ala Phe Thr
225 230 235 240
Pro His Pro Cys Ser Val Asp Gly Gln Thr Gln Cys Ser Gly Thr Gln
245 250 255
Cys Gly Asp Asp Asp Glu Arg Tyr Ser Gly Leu Cys Asp Lys Asp Gly
260 265 270
Cys Asp Phe Asn Ser Phe Arg Met Gly Asp Lys Ser Phe Leu Gly Lys
275 280 285
Gly Met Thr Val Asp Thr Ser Arg Lys Phe Thr Val Val Thr Gln Phe
290 295 300
Val Thr Thr Asp Gly Thr Thr Asn Gly Asp Leu His Glu Ile Arg Arg
305 310 315 320
Leu Tyr Val Gln Asp Gly Lys Val Ile Gln Asn Ser Val Val Ser Ile
325 330 335
Pro Gly Ile Asp Ala Val Asp Ser Ile Thr Asp Asn Phe Cys Ala Gln
340 345 350
Gln Lys Ser Val Phe Gly Asp Thr Asn Tyr Phe Ala Thr Leu Gly Gly
355 360 365
Leu Lys Lys Met Gly Ala Ala Leu Lys Ser Gly Met Val Leu Ala Met
370 375 380
Ser Val Trp Asp Asp His Ala Ala Ser Met Gln Trp Leu Asp Ser Asn
385 390 395 400
Tyr Pro Ala Asp Gly Asp Ala Thr Lys Pro Gly Val Ala Arg Gly Thr
405 410 415
Cys Ser Ala Asp Ser Gly Leu Pro Thr Asn Val Glu Ser Gln Ser Ala
420 425 430
Ser Ala Ser Val Thr Phe Ser Asn Ile Lys Trp Gly Asp Ile Asn Thr
435 440 445
Thr Phe Thr Gly Thr Gly Ser Thr Ser Pro Ser Ser Pro Ala Gly Pro
450 455 460
Val Ser Ser Ser Thr Ser Val Ala Ser Gln Pro Thr Gln Pro Ala Gln
465 470 475 480
Gly Thr Val Ala Gln Trp Gly Gln Cys Gly Gly Thr Gly Phe Thr Gly
485 490 495
Pro Thr Val Cys Ala Ser Pro Phe Thr Cys His Val Val Asn Pro Tyr

500 505 510
Tyr Ser Gln Cys Tyr
515

<210> 48
<211> 423
<212> PRT
<213> Alternaria alternata

<400> 48
Met Thr Trp Gln Ser Cys Thr Ala Lys Gly Ser Cys Thr Asn Lys Asn
1 5 10 15
Gly Lys Ile Val Ile Asp Ala Asn Trp Arg Trp Leu His Lys Lys Glu
20 25 30
Gly Tyr Asp Asn Cys Tyr Thr Gly Asn Glu Trp Asp Ala Thr Ala Cys
35 40 45
Pro Asp Asn Lys Ala Cys Ala Ala Asn Cys Ala Val Asp Gly Ala Asp
50 55 60
Tyr Ser Gly Thr Tyr Gly Ile Thr Ala Gly Ser Asn Ser Leu Lys Leu
65 70 75 80
Lys Phe Ile Thr Lys Gly Ser Tyr Ser Thr Asn Ile Gly Ser Arg Thr
85 90 95
Tyr Leu Met Lys Asp Asp Thr Thr Tyr Glu Met Phe Lys Phe Thr Gly
100 105 110
Asn Gln Glu Phe Thr Phe Asp Val Asp Val Ser Asn Leu Pro Cys Gly
115 120 125
Phe Asn Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Leu
130 135 140
Lys Lys Tyr Ser Thr Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr
145 150 155 160
Cys Asp Ala Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Glu Gly
165 170 175
Asn Val Glu Gly Trp Lys Pro Ser Ser Asn Asp Ala Asn Ala Gly Val
180 185 190
Gly Gly His Gly Ser Cys Cys Ala Glu Met Asp Ile Trp Glu Ala Asn
195 200 205
Ser Val Ser Thr Ala Val Thr Pro His Ser Cys Ser Thr Ile Glu Gln
210 215 220
Ser Arg Cys Asp Gly Asp Gly Cys Gly Gly Thr Tyr Ser Ala Asp Arg
225 230 235 240
Tyr Ala Gly Val Cys Asp Pro Asp Gly Cys Asp Phe Asn Ser Tyr Arg
245 250 255
Met Gly Val Lys Asp Phe Tyr Gly Lys Gly Lys Thr Val Asp Thr Ser
260 265 270
Lys Lys Phe Thr Val Val Thr Gln Phe Ile Gly Thr Gly Asp Ala Met
275 280 285
Glu Ile Lys Arg Phe Tyr Val Gln Asn Gly Lys Thr Ile Ala Gln Pro
290 295 300
Ala Ser Ala Val Pro Gly Val Glu Gly Asn Ser Ile Thr Thr Lys Phe
305 310 315 320
Cys Asp Gln Gln Lys Ala Val Phe Gly Asp Thr Tyr Thr Phe Lys Asp
325 330 335
Lys Gly Gly Met Ala Asn Met Ala Lys Ala Leu Ala Asn Gly Met Val
340 345 350
Leu Val Met Ser Leu Trp Asp Asp His Tyr Ser Asn Met Leu Trp Leu
355 360 365
Asp Ser Thr Tyr Pro Thr Asp Lys Asn Pro Asp Thr Asp Leu Gly Thr
370 375 380
Gly Arg Gly Glu Cys Glu Thr Ser Ser Gly Val Pro Ala Asp Val Glu
385 390 395 400
Ser Gln His Ala Asp Ala Thr Val Val Tyr Ser Asn Ile Lys Phe Gly

405 410 415
Pro Leu Asn Ser Thr Phe Gly
420

<210> 49
<211> 438
<212> PRT
<213> Leptosphaeria maculans

<400> 49
Met Tyr Arg Ser Leu Ile Phe Ala Thr Ser Leu Leu Ser Leu Ala Lys
1 5 10 15
Gly Gln Leu Val Gly Asn Leu Tyr Cys Lys Gly Ser Cys Thr Ala Lys
20 25 30
Asn Gly Lys Val Val Ile Asp Ala Asn Trp Arg Trp Leu His Val Lys
35 40 45
Gly Gly Tyr Thr Asn Cys Tyr Thr Gly Asn Glu Trp Asn Ala Thr Ala
50 55 60
Cys Pro Asp Asn Lys Ser Cys Ala Thr Asn Cys Ala Ile Asp Gly Ala
65 70 75 80
Asp Tyr Arg Arg Leu Arg His Tyr Cys Glu Arg Gln Leu Leu Gly Thr
85 90 95
Glu Val His His Gln Gly Leu Tyr Ser Thr Asn Ile Gly Ser Arg Thr
100 105 110
Tyr Leu Met Gln Asp Asp Ser Thr Tyr Gln Leu Phe Lys Phe Thr Gly
115 120 125
Ser Gln Glu Phe Thr Phe Asp Val Asp Leu Ser Asn Leu Pro Cys Gly
130 135 140
Leu Asn Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Leu
145 150 155 160
Lys Lys Tyr Pro Thr Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr
165 170 175
Cys Asp Ala Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Glu Gly
180 185 190
Asn Val Glu Gly Trp Gln Pro Ser Lys Asn Asp Gln Asn Ala Gly Val
195 200 205
Gly Gly His Gly Ser Cys Cys Ala Glu Met Asp Ile Trp Glu Ala Asn
210 215 220
Ser Val Ser Thr Ala Val Thr Pro His Ser Cys Ser Thr Ile Glu Gln
225 230 235 240
Ser Arg Cys Asp Gly Asp Gly Cys Gly Gly Thr Tyr Ser Ala Asp Arg
245 250 255
Tyr Ala Gly Val Cys Asp Pro Asp Gly Cys Asp Phe Asn Ser Tyr Arg
260 265 270
Met Gly Val Lys Asp Phe Tyr Gly Lys Gly Lys Thr Val Asp Thr Ser
275 280 285
Lys Lys Phe Thr Val Val Thr Gln Phe Ile Gly Ser Gly Asp Ala Met
290 295 300
Glu Ile Lys Arg Phe Tyr Val Gln Asn Gly Lys Thr Ile Pro Gln Pro
305 310 315 320
Asp Ser Thr Ile Pro Gly Val Thr Gly Asn Ser Ile Thr Thr Phe Phe
325 330 335
Cys Asp Ala Gln Lys Lys Ala Phe Gly Asp Lys Tyr Thr Phe Lys Asp
340 345 350
Lys Gly Gly Met Ala Asn Met Pro Ser Thr Cys Asn Gly Met Val Leu
355 360 365
Val Met Ser Leu Trp Asp Asp His Tyr Ser Asn Met Leu Trp Leu Asp
370 375 380
Ser Thr Tyr Pro Thr Asp Lys Asn Pro Asp Thr Asp Ala Gly Ser Gly
385 390 395 400
Arg Gly Glu Cys Ala Ile Thr Ser Gly Val Pro Ala Asp Val Glu Ser

405 410 415
Gln His Pro Asp Ala Ser Val Ile Tyr Ser Asn Ile Lys Phe Gly Pro
420 425 430
Ile Asn Thr Thr Phe Gly
435

<210> 50
<211> 452
<212> PRT
<213> Cryphonectria parasitica

<400> 50
Met Phe Ser Lys Phe Ala Leu Thr Gly Ser Leu Leu Ala Gly Ala Val
1 5 10 15
Asn Ala Gln Gly Val Gly Thr Gln Gln Thr Glu Thr His Pro Gln Met
20 25 30
Thr Trp Gln Ser Cys Thr Ser Pro Ser Ser Cys Thr Thr Asn Gln Gly
35 40 45
Glu Val Val Ile Asp Ser Asn Trp Arg Trp Val His Asp Lys Asp Gly
50 55 60
Tyr Val Asn Cys Tyr Thr Gly Asn Thr Trp Asn Thr Thr Leu Cys Pro
65 70 75 80
Asp Asp Lys Thr Cys Ala Ala Asn Cys Val Leu Asp Gly Ala Asp Tyr
85 90 95
Ser Ser Thr Tyr Gly Ile Thr Thr Ser Gly Asn Ala Leu Ser Leu Gln
100 105 110
Phe Val Thr Gln Ser Ser Gly Lys Asn Ile Gly Ser Arg Thr Tyr Leu
115 120 125
Met Glu Ser Ser Thr Lys Tyr His Leu Phe Asp Leu Ile Gly Asn Glu
130 135 140
Phe Ala Phe Asp Val Asp Leu Ser Lys Leu Pro Cys Gly Leu Asn Gly
145 150 155 160
Ala Leu Tyr Phe Val Thr Met Asp Ala Asp Gly Gly Met Ala Lys Tyr
165 170 175
Ser Thr Asn Thr Ala Gly Ala Glu Tyr Gly Thr Gly Tyr Cys Asp Ser
180 185 190
Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Gly Asn Val Glu
195 200 205
Gly Trp Thr Pro Ser Thr Asn Asp Ala Asn Ala Gly Val Gly Gly Leu
210 215 220
Gly Ser Cys Cys Ser Glu Met Asp Val Trp Glu Ala Asn Ser Met Asp
225 230 235 240
Met Ala Tyr Thr Pro His Pro Cys Glu Thr Ala Ala Gln His Ser Cys
245 250 255
Asn Ala Asp Glu Cys Gly Gly Thr Tyr Ser Ser Ser Arg Tyr Ala Gly
260 265 270
Asp Cys Asp Pro Asp Gly Cys Asp Trp Asn Pro Phe Arg Met Gly Asn
275 280 285
Lys Asp Phe Tyr Gly Ser Gly Asp Thr Val Asp Thr Ser Gln Lys Phe
290 295 300
Thr Val Val Thr Gln Phe His Gly Ser Gly Ser Ser Leu Thr Glu Ile
305 310 315 320
Ser Gln Tyr Tyr Ile Gln Gly Gly Thr Lys Ile Gln Gln Pro Asn Ser
325 330 335
Thr Trp Pro Thr Leu Thr Gly Tyr Asn Ser Ile Thr Asp Asp Phe Cys
340 345 350
Lys Ala Gln Lys Val Glu Phe Asn Asp Thr Asp Val Phe Ser Glu Lys
355 360 365
Gly Gly Leu Ala Gln Met Gly Ala Gly Met Ala Asp Gly Met Val Leu
370 375 380
Val Met Ser Leu Trp Asp Asp His Tyr Ala Asn Met Leu Trp Leu Asp

385 390 395 400
Ser Thr Tyr Pro Val Asp Ala Asp Ala Ser Ser Pro Gly Lys Gln Arg
405 410 415
Gly Thr Cys Ala Thr Thr Ser Gly Val Pro Ala Asp Val Glu Ser Ser
420 425 430
Asp Ala Ser Ala Thr Val Ile Tyr Ser Asn Ile Lys Phe Gly Pro Ile
435 440 445
Gly Ala Thr Tyr
450

<210> 51
<211> 456
<212> PRT
<213> Cochliobolus carbonum

<400> 51
Met Tyr Arg Thr Leu Ala Phe Ala Ser Leu Ser Leu Tyr Gly Ala Ala
1 5 10 15
Arg Ala Gln Gln Val Gly Thr Ser Thr Ala Glu Asn His Pro Lys Leu
20 25 30
Thr Trp Gln Thr Cys Thr Gly Thr Gly Gly Thr Asn Cys Ser Asn Lys
35 40 45
Ser Gly Ser Val Val Leu Asp Ser Asn Trp Arg Trp Ala His Asn Val
50 55 60
Gly Gly Tyr Thr Asn Cys Tyr Thr Gly Asn Ser Trp Ser Thr Gln Tyr
65 70 75 80
Cys Pro Asp Gly Asp Ser Cys Thr Lys Asn Cys Ala Ile Asp Gly Ala
85 90 95
Asp Tyr Ser Gly Thr Tyr Gly Ile Thr Thr Ser Asn Asn Ala Leu Ser
100 105 110
Leu Lys Phe Val Thr Lys Gly Ser Phe Ser Ser Asn Ile Gly Ser Arg
115 120 125
Thr Tyr Leu Met Glu Thr Asp Thr Lys Tyr Gln Met Phe Asn Leu Ile
130 135 140
Asn Lys Glu Phe Thr Phe Asp Val Asp Val Ser Lys Leu Pro Cys Gly
145 150 155 160
Leu Asn Gly Ala Leu Tyr Phe Val Glu Met Ala Ala Asp Gly Gly Ile
165 170 175
Gly Lys Gly Asn Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys
180 185 190
Asp Ser Gln Cys Pro His Asp Ile Lys Phe Ile Asn Gly Lys Ala Asn
195 200 205
Val Glu Gly Trp Asn Pro Ser Asp Ala Asp Pro Asn Gly Gly Ala Gly
210 215 220
Lys Ile Gly Ala Cys Cys Pro Glu Met Asp Ile Trp Glu Ala Asn Ser
225 230 235 240
Ile Ser Thr Ala Tyr Thr Pro His Pro Cys Arg Gly Val Gly Leu Gln
245 250 255
Glu Cys Ser Asp Ala Ala Ser Cys Gly Asp Gly Ser Asn Arg Tyr Asp
260 265 270
Gly Gln Cys Asp Lys Asp Gly Cys Asp Phe Asn Ser Tyr Arg Met Gly
275 280 285
Val Lys Asp Phe Tyr Gly Pro Gly Ala Thr Leu Asp Thr Thr Lys Lys
290 295 300
Met Thr Val Ile Thr Gln Phe Leu Gly Ser Gly Ser Ser Leu Ser Glu
305 310 315 320
Ile Lys Arg Phe Tyr Val Gln Asn Gly Lys Val Tyr Lys Asn Ser Gln
325 330 335
Ser Ala Val Ala Gly Val Thr Gly Asn Ser Ile Thr Glu Ser Phe Cys
340 345 350
Thr Ala Gln Lys Lys Ala Phe Gly Asp Thr Ser Ser Phe Ala Ala Leu

```
          355                 360                 365
Gly Gly Leu Asn Glu Met Gly Ala Ser Leu Ala Arg Gly His Val Leu
    370                 375                 380
Ile Met Ser Leu Trp Gly Asp His Ala Val Asn Met Leu Trp Leu Asp
385                 390                 395                 400
Ser Thr Tyr Pro Thr Asp Ala Asp Pro Ser Lys Pro Gly Ala Ala Arg
                405                 410                 415
Gly Thr Cys Pro Thr Thr Ser Gly Lys Pro Glu Asp Val Glu Lys Asn
            420                 425                 430
Ser Pro Asp Ala Thr Val Val Phe Ser Asn Ile Lys Phe Gly Pro Ile
        435                 440                 445
Gly Ser Thr Phe Ala Gln Pro Ala
    450                 455
```

<210> 52
<211> 525
<212> PRT
<213> Humicola grisea

<400> 52

```
Met Arg Thr Ala Lys Phe Ala Thr Leu Ala Ala Leu Val Ala Ser Ala
 1               5                  10                  15
Ala Ala Gln Gln Ala Cys Ser Leu Thr Thr Glu Arg His Pro Ser Leu
            20                  25                  30
Ser Trp Asn Lys Cys Thr Ala Gly Gly Gln Cys Gln Thr Val Gln Ala
        35                  40                  45
Ser Ile Thr Leu Asp Ser Asn Trp Arg Trp Thr His Gln Val Ser Gly
    50                  55                  60
Ser Thr Asn Cys Tyr Thr Gly Asn Lys Trp Asp Thr Ser Ile Cys Thr
65                  70                  75                  80
Asp Ala Lys Ser Cys Ala Gln Asn Cys Cys Val Asp Gly Ala Asp Tyr
                85                  90                  95
Thr Ser Thr Tyr Gly Ile Thr Thr Asn Gly Asp Ser Leu Ser Leu Lys
            100                 105                 110
Phe Val Thr Lys Gly Gln His Ser Thr Asn Val Gly Ser Arg Thr Tyr
        115                 120                 125
Leu Met Asp Gly Glu Asp Lys Tyr Gln Thr Phe Glu Leu Leu Gly Asn
    130                 135                 140
Glu Phe Thr Phe Asp Val Asp Val Ser Asn Ile Gly Cys Gly Leu Asn
145                 150                 155                 160
Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Leu Ser Arg
                165                 170                 175
Tyr Pro Gly Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp
            180                 185                 190
Ala Gln Cys Pro Arg Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Ile
        195                 200                 205
Glu Gly Trp Thr Gly Ser Thr Asn Asp Pro Asn Ala Gly Ala Gly Arg
    210                 215                 220
Tyr Gly Thr Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Asn Met
225                 230                 235                 240
Ala Thr Ala Phe Thr Pro His Pro Cys Thr Ile Ile Gly Gln Ser Arg
                245                 250                 255
Cys Glu Gly Asp Ser Cys Gly Gly Thr Tyr Ser Asn Glu Arg Tyr Ala
            260                 265                 270
Gly Val Cys Asp Pro Asp Gly Cys Asp Phe Asn Ser Tyr Arg Gln Gly
        275                 280                 285
Asn Lys Thr Phe Tyr Gly Lys Gly Met Thr Val Asp Thr Thr Lys Lys
    290                 295                 300
Ile Thr Val Val Thr Gln Phe Leu Lys Asp Ala Asn Gly Asp Leu Gly
305                 310                 315                 320
Glu Ile Lys Arg Phe Tyr Val Gln Asp Gly Lys Ile Ile Pro Asn Ser
```

```
                325                 330                 335
Glu Ser Thr Ile Pro Gly Val Glu Gly Asn Ser Ile Thr Gln Asp Trp
            340                 345                 350
Cys Asp Arg Gln Lys Val Ala Phe Gly Asp Ile Asp Asp Phe Asn Arg
        355                 360                 365
Lys Gly Gly Met Lys Gln Met Gly Lys Ala Leu Ala Gly Pro Met Val
    370                 375                 380
Leu Val Met Ser Ile Trp Asp Asp His Ala Ser Asn Met Leu Trp Leu
385                 390                 395                 400
Asp Ser Thr Phe Pro Val Asp Ala Ala Gly Lys Pro Gly Ala Glu Arg
                405                 410                 415
Gly Ala Cys Pro Thr Thr Ser Gly Val Pro Ala Glu Val Glu Ala Glu
            420                 425                 430
Ala Pro Asn Ser Asn Val Val Phe Ser Asn Ile Arg Phe Gly Pro Ile
        435                 440                 445
Gly Ser Thr Val Ala Gly Leu Pro Gly Ala Gly Asn Gly Gly Asn Asn
    450                 455                 460
Gly Gly Asn Pro Pro Pro Pro Thr Thr Thr Thr Ser Ser Ala Pro Ala
465                 470                 475                 480
Thr Thr Thr Thr Ala Ser Ala Gly Pro Lys Ala Gly Arg Trp Gln Gln
                485                 490                 495
Cys Gly Gly Ile Gly Phe Thr Gly Pro Thr Gln Cys Glu Glu Pro Tyr
            500                 505                 510
Ile Cys Thr Lys Leu Asn Asp Trp Tyr Ser Gln Cys Leu
        515                 520                 525
```

<210> 53
<211> 525
<212> PRT
<213> Humicola grisea

<400> 53

```
Met Arg Thr Ala Lys Phe Ala Thr Leu Ala Ala Leu Val Ala Ser Ala
 1               5                  10                  15
Ala Ala Gln Gln Ala Cys Ser Leu Thr Thr Glu Arg His Pro Ser Leu
            20                  25                  30
Ser Trp Lys Lys Cys Thr Ala Gly Gly Gln Cys Gln Thr Val Gln Ala
        35                  40                  45
Ser Ile Thr Leu Asp Ser Asn Trp Arg Trp Thr His Gln Val Ser Gly
    50                  55                  60
Ser Thr Asn Cys Tyr Thr Gly Asn Lys Trp Asp Thr Ser Ile Cys Thr
65                  70                  75                  80
Asp Ala Lys Ser Cys Ala Gln Asn Cys Cys Val Asp Gly Ala Asp Tyr
                85                  90                  95
Thr Ser Thr Tyr Gly Ile Thr Thr Asn Gly Asp Ser Leu Ser Leu Lys
            100                 105                 110
Phe Val Thr Lys Gly Gln Tyr Ser Thr Asn Val Gly Ser Arg Thr Tyr
        115                 120                 125
Leu Met Asp Gly Glu Asp Lys Tyr Gln Thr Phe Glu Leu Leu Gly Asn
    130                 135                 140
Glu Phe Thr Phe Asp Val Asp Val Ser Asn Ile Gly Cys Gly Leu Asn
145                 150                 155                 160
Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Leu Ser Arg
                165                 170                 175
Tyr Pro Gly Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp
            180                 185                 190
Ala Gln Cys Pro Arg Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Ile
        195                 200                 205
Glu Gly Trp Thr Gly Ser Thr Asn Asp Pro Asn Ala Gly Ala Gly Arg
    210                 215                 220
Tyr Gly Thr Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Asn Met
```

225 230 235 240
Ala Thr Ala Phe Thr Pro His Pro Cys Thr Ile Ile Gly Gln Ser Arg
245 250 255
Cys Glu Gly Asp Ser Cys Gly Gly Thr Tyr Ser Asn Glu Arg Tyr Ala
260 265 270
Gly Val Cys Asp Pro Asp Gly Cys Asp Phe Asn Ser Tyr Arg Gln Gly
275 280 285
Asn Lys Thr Phe Tyr Gly Lys Gly Met Thr Val Asp Thr Thr Lys Lys
290 295 300
Ile Thr Val Val Thr Gln Phe Leu Lys Asp Ala Asn Gly Asp Leu Gly
305 310 315 320
Glu Ile Lys Arg Phe Tyr Val Gln Asp Gly Lys Ile Ile Pro Asn Ser
325 330 335
Glu Ser Thr Ile Pro Gly Val Glu Gly Asn Ser Ile Thr Gln Asp Trp
340 345 350
Cys Asp Arg Gln Lys Val Ala Phe Gly Asp Ile Asp Asp Phe Asn Arg
355 360 365
Lys Gly Gly Met Lys Gln Met Gly Lys Ala Leu Ala Gly Pro Met Val
370 375 380
Leu Val Met Ser Ile Trp Asp Asp His Ala Ser Asn Met Leu Trp Leu
385 390 395 400
Asp Ser Thr Phe Pro Val Asp Ala Ala Gly Lys Pro Gly Ala Glu Arg
405 410 415
Gly Ala Cys Pro Thr Thr Ser Gly Val Pro Ala Glu Val Glu Ala Glu
420 425 430
Ala Pro Asn Ser Asn Val Val Phe Ser Asn Ile Arg Phe Gly Pro Ile
435 440 445
Gly Ser Thr Val Ala Gly Leu Pro Gly Ala Gly Asn Gly Gly Asn Asn
450 455 460
Gly Gly Asn Pro Pro Pro Pro Thr Thr Thr Thr Ser Ser Ala Pro Ala
465 470 475 480
Thr Thr Thr Thr Ala Ser Ala Gly Pro Lys Ala Gly Arg Trp Gln Gln
485 490 495
Cys Gly Gly Ile Gly Phe Thr Gly Pro Thr Gln Cys Glu Glu Pro Tyr
500 505 510
Thr Cys Thr Lys Leu Asn Asp Trp Tyr Ser Gln Cys Leu
515 520 525

<210> 54
<211> 514
<212> PRT
<213> Fusarium oxysporum

<400> 54
Met Tyr Arg Ile Val Ala Thr Ala Ser Ala Leu Ile Ala Ala Ala Arg
1 5 10 15
Ala Gln Gln Val Cys Ser Leu Asn Thr Glu Thr Lys Pro Ala Leu Thr
20 25 30
Trp Ser Lys Cys Thr Ser Ser Gly Cys Ser Asp Val Lys Gly Ser Val
35 40 45
Val Ile Asp Ala Asn Trp Arg Trp Thr His Gln Thr Ser Gly Ser Thr
50 55 60
Asn Cys Tyr Thr Gly Asn Lys Trp Asp Thr Ser Ile Cys Thr Asp Gly
65 70 75 80
Lys Thr Cys Ala Glu Lys Cys Cys Leu Asp Gly Ala Asp Tyr Ser Gly
85 90 95
Thr Tyr Gly Ile Thr Ser Ser Gly Asn Gln Leu Ser Leu Gly Phe Val
100 105 110
Thr Asn Gly Pro Tyr Ser Lys Asn Ile Gly Ser Arg Thr Tyr Leu Met
115 120 125
Glu Asn Glu Asn Thr Tyr Gln Met Phe Gln Leu Leu Gly Asn Glu Phe

```
    130                 135                 140
Thr Phe Asp Val Asp Val Ser Gly Ile Gly Cys Gly Leu Asn Gly Ala
145                 150                 155                 160
Pro His Phe Val Ser Met Asp Glu Asp Gly Gly Lys Ala Lys Tyr Ser
                165                 170                 175
Gly Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ala Gln
            180                 185                 190
Cys Pro Arg Asp Val Lys Phe Ile Asn Gly Val Ala Asn Ser Glu Gly
        195                 200                 205
Trp Lys Pro Ser Asp Ser Asp Val Asn Ala Gly Val Gly Asn Leu Gly
    210                 215                 220
Thr Cys Cys Pro Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Thr
225                 230                 235                 240
Ala Phe Thr Pro His Pro Cys Thr Lys Leu Thr Gln His Ser Cys Thr
                245                 250                 255
Gly Asp Ser Cys Gly Gly Thr Tyr Ser Ser Asp Arg Tyr Gly Gly Thr
            260                 265                 270
Cys Asp Ala Asp Gly Cys Asp Phe Asn Ala Tyr Arg Gln Gly Asn Lys
        275                 280                 285
Thr Phe Tyr Gly Pro Gly Ser Asn Phe Asn Ile Asp Thr Thr Lys Lys
    290                 295                 300
Met Thr Val Val Thr Gln Phe His Lys Gly Ser Asn Gly Arg Leu Ser
305                 310                 315                 320
Glu Ile Thr Arg Leu Tyr Val Gln Asn Gly Lys Val Ile Ala Asn Ser
                325                 330                 335
Glu Ser Lys Ile Ala Gly Asn Pro Gly Ser Ser Leu Thr Ser Asp Phe
            340                 345                 350
Cys Ser Lys Gln Lys Ser Val Phe Gly Asp Ile Asp Asp Phe Ser Lys
        355                 360                 365
Lys Gly Gly Trp Asn Gly Met Ser Asp Ala Leu Ser Ala Pro Met Val
    370                 375                 380
Leu Val Met Ser Leu Trp His Asp His His Ser Asn Met Leu Trp Leu
385                 390                 395                 400
Asp Ser Thr Tyr Pro Thr Asp Ser Thr Lys Val Gly Ser Gln Arg Gly
                405                 410                 415
Ser Cys Ala Thr Thr Ser Gly Lys Pro Ser Asp Leu Glu Arg Asp Val
            420                 425                 430
Pro Asn Ser Lys Val Ser Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly
        435                 440                 445
Ser Thr Tyr Lys Ser Asp Gly Thr Thr Pro Asn Pro Pro Ala Ser Ser
    450                 455                 460
Ser Thr Thr Gly Ser Ser Thr Pro Thr Asn Pro Pro Ala Gly Ser Val
465                 470                 475                 480
Asp Gln Trp Gly Gln Cys Gly Gly Gln Asn Tyr Ser Gly Pro Thr Thr
                485                 490                 495
Cys Lys Ser Pro Phe Thr Cys Lys Lys Ile Asn Asp Phe Tyr Ser Gln
            500                 505                 510
Cys Gln
```

<210> 55
<211> 456
<212> PRT
<213> Claviceps purpurea

<400> 55

```
Met His Pro Ser Leu Gln Thr Ile Leu Leu Ser Ala Leu Phe Thr Thr
 1               5                  10                  15
Ala His Ala Gln Gln Ala Cys Ser Ser Lys Pro Glu Thr His Pro Pro
            20                  25                  30
Leu Ser Trp Ser Arg Cys Ser Arg Ser Gly Cys Arg Ser Val Gln Gly
```

```
         35                  40                  45
Ala Val Thr Val Asp Ala Asn Trp Leu Trp Thr Thr Val Asp Gly Ser
    50                  55                  60
Gln Asn Cys Tyr Thr Gly Asn Arg Trp Asp Thr Ser Ile Cys Ser Ser
65                  70                  75                  80
Glu Lys Thr Cys Ser Glu Ser Cys Cys Ile Asp Gly Ala Asp Tyr Ala
                85                  90                  95
Gly Thr Tyr Gly Val Thr Thr Thr Gly Asp Ala Leu Ser Leu Lys Phe
            100                 105                 110
Val Gln Gln Gly Pro Tyr Ser Lys Asn Val Gly Ser Arg Leu Tyr Leu
        115                 120                 125
Met Lys Asp Glu Ser Arg Tyr Glu Met Phe Thr Leu Leu Gly Asn Glu
    130                 135                 140
Phe Thr Phe Asp Val Asp Val Ser Lys Leu Gly Cys Gly Leu Asn Gly
145                 150                 155                 160
Ala Leu Tyr Phe Val Ser Met Asp Glu Asp Gly Gly Met Lys Arg Phe
                165                 170                 175
Pro Met Asn Lys Ala Gly Ala Lys Phe Gly Thr Gly Tyr Cys Asp Ser
            180                 185                 190
Gln Cys Pro Arg Asp Val Lys Phe Ile Asn Gly Met Ala Asn Ser Lys
        195                 200                 205
Asp Trp Ile Pro Ser Lys Ser Asp Ala Asn Ala Gly Ile Gly Ser Leu
    210                 215                 220
Gly Ala Cys Cys Arg Glu Met Asp Ile Trp Glu Ala Asn Asn Ile Ala
225                 230                 235                 240
Ser Ala Phe Thr Pro His Pro Cys Lys Asn Ser Ala Tyr His Ser Cys
                245                 250                 255
Thr Gly Asp Gly Cys Gly Gly Thr Tyr Ser Lys Asn Arg Tyr Ser Gly
            260                 265                 270
Asp Cys Asp Pro Asp Gly Cys Asp Phe Asn Ser Tyr Arg Leu Gly Asn
        275                 280                 285
Thr Thr Phe Tyr Gly Pro Gly Pro Lys Phe Thr Ile Asp Thr Thr Arg
    290                 295                 300
Lys Ile Ser Val Val Thr Gln Phe Leu Lys Gly Arg Asp Gly Ser Leu
305                 310                 315                 320
Arg Glu Ile Lys Arg Phe Tyr Val Gln Asn Gly Lys Val Ile Pro Asn
                325                 330                 335
Ser Val Ser Arg Val Arg Gly Val Pro Gly Asn Ser Ile Thr Gln Gly
            340                 345                 350
Phe Cys Asn Ala Gln Lys Lys Met Phe Gly Ala His Glu Ser Phe Asn
        355                 360                 365
Ala Lys Gly Gly Met Lys Gly Met Ser Ala Ala Val Ser Lys Pro Met
    370                 375                 380
Val Leu Val Met Ser Leu Trp Asp Asp His Asn Ser Asn Met Leu Trp
385                 390                 395                 400
Leu Asp Ser Thr Tyr Pro Thr Asn Ser Arg Gln Arg Gly Ser Lys Arg
                405                 410                 415
Gly Ser Cys Pro Ala Ser Ser Gly Arg Pro Thr Asp Val Glu Ser Ser
            420                 425                 430
Ala Pro Asp Ser Thr Val Val Phe Ser Asn Ile Lys Phe Gly Pro Ile
        435                 440                 445
Gly Ser Thr Phe Ser Arg Gly Lys
    450                 455
```

<210> 56
<211> 451
<212> PRT
<213> Humicola grisea var. thermoidea

<400> 56
Met Gln Ile Lys Ser Tyr Ile Gln Tyr Leu Ala Ala Ala Leu Pro Leu

```
 1               5                  10                  15
Leu Ser Ser Val Ala Ala Gln Gln Ala Gly Thr Ile Thr Ala Glu Asn
            20                  25                  30
His Pro Arg Met Thr Trp Lys Arg Cys Ser Gly Pro Gly Asn Cys Gln
        35                  40                  45
Thr Val Gln Gly Glu Val Val Ile Asp Ala Asn Trp Arg Trp Leu His
    50                  55                  60
Asn Asn Gly Gln Asn Cys Tyr Glu Gly Asn Lys Trp Thr Ser Gln Cys
65                  70                  75                  80
Ser Ser Ala Thr Asp Cys Ala Gln Arg Cys Ala Leu Asp Gly Ala Asn
                85                  90                  95
Tyr Gln Ser Thr Tyr Gly Ala Ser Thr Ser Gly Asp Ser Leu Thr Leu
            100                 105                 110
Lys Phe Val Thr Lys His Glu Tyr Gly Thr Asn Ile Gly Ser Arg Phe
        115                 120                 125
Tyr Leu Met Ala Asn Gln Asn Lys Tyr Gln Met Phe Thr Leu Met Asn
    130                 135                 140
Asn Glu Phe Ala Phe Asp Val Asp Leu Ser Lys Val Glu Cys Gly Ile
145                 150                 155                 160
Asn Ser Ala Leu Tyr Phe Val Ala Met Glu Glu Asp Gly Gly Met Ala
                165                 170                 175
Ser Tyr Pro Ser Asn Arg Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys
            180                 185                 190
Asp Ala Gln Cys Ala Arg Asp Leu Lys Phe Ile Gly Gly Lys Ala Asn
        195                 200                 205
Ile Glu Gly Trp Arg Pro Ser Thr Asn Asp Pro Asn Ala Gly Val Gly
    210                 215                 220
Pro Met Gly Ala Cys Cys Ala Glu Ile Asp Val Trp Glu Ser Asn Ala
225                 230                 235                 240
Tyr Ala Tyr Ala Phe Thr Pro His Ala Cys Gly Ser Lys Asn Arg Tyr
                245                 250                 255
His Ile Cys Glu Thr Asn Asn Cys Gly Gly Thr Tyr Ser Asp Asp Arg
            260                 265                 270
Phe Ala Gly Tyr Cys Asp Ala Asn Gly Cys Asp Tyr Asn Pro Tyr Arg
        275                 280                 285
Met Gly Asn Lys Asp Phe Tyr Gly Lys Gly Lys Thr Val Asp Thr Asn
    290                 295                 300
Arg Lys Phe Thr Val Val Ser Arg Phe Glu Arg Asn Arg Leu Ser Gln
305                 310                 315                 320
Phe Phe Val Gln Asp Gly Arg Lys Ile Glu Val Pro Pro Pro Thr Trp
                325                 330                 335
Pro Gly Leu Pro Asn Ser Ala Asp Ile Thr Pro Glu Leu Cys Asp Ala
            340                 345                 350
Gln Phe Arg Val Phe Asp Asp Arg Asn Arg Phe Ala Glu Thr Gly Gly
        355                 360                 365
Phe Asp Ala Leu Asn Glu Ala Leu Thr Ile Pro Met Val Leu Val Met
    370                 375                 380
Ser Ile Trp Asp Asp His His Ser Asn Met Leu Trp Leu Asp Ser Ser
385                 390                 395                 400
Tyr Pro Pro Glu Lys Ala Gly Leu Pro Gly Gly Asp Arg Gly Pro Cys
                405                 410                 415
Pro Thr Thr Ser Gly Val Pro Ala Glu Val Glu Ala Gln Tyr Pro Asn
            420                 425                 430
Ala Gln Val Val Trp Ser Asn Ile Arg Phe Gly Pro Ile Gly Ser Thr
        435                 440                 445
Val Asn Val
    450
```

<210> 57
<211> 451
<212> PRT

<213> Humicola grisea var. thermoidea

<400> 57

```
Met Gln Ile Lys Ser Tyr Ile Gln Tyr Leu Ala Ala Ala Leu Pro Leu
 1               5                  10                  15
Leu Ser Ser Val Ala Ala Gln Gln Ala Gly Thr Ile Thr Ala Glu Asn
            20                  25                  30
His Pro Arg Met Thr Trp Lys Arg Cys Ser Gly Pro Gly Asn Cys Gln
        35                  40                  45
Thr Val Gln Gly Glu Val Val Ile Asp Ala Asn Trp Arg Trp Leu His
    50                  55                  60
Asn Asn Gly Gln Asn Cys Tyr Glu Gly Asn Lys Trp Thr Ser Gln Cys
65                  70                  75                  80
Ser Ser Ala Thr Asp Cys Ala Gln Arg Cys Ala Leu Asp Gly Ala Asn
                85                  90                  95
Tyr Gln Ser Thr Tyr Gly Ala Ser Thr Ser Gly Asp Ser Leu Thr Leu
            100                 105                 110
Lys Phe Val Thr Lys His Glu Tyr Gly Thr Asn Ile Gly Ser Arg Phe
        115                 120                 125
Tyr Leu Met Ala Asn Gln Asn Lys Tyr Gln Met Phe Thr Leu Met Asn
    130                 135                 140
Asn Glu Phe Ala Phe Asp Val Asp Leu Ser Lys Val Glu Cys Gly Ile
145                 150                 155                 160
Asn Ser Ala Leu Tyr Phe Val Ala Met Glu Glu Asp Gly Gly Met Ala
                165                 170                 175
Ser Tyr Pro Ser Asn Arg Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys
            180                 185                 190
Asp Ala Gln Cys Ala Arg Asp Leu Lys Phe Ile Gly Gly Lys Ala Asn
        195                 200                 205
Ile Glu Gly Trp Arg Pro Ser Thr Asn Asp Pro Asn Ala Gly Val Gly
    210                 215                 220
Pro Met Gly Ala Cys Cys Ala Glu Ile Asp Val Trp Glu Ser Asn Ala
225                 230                 235                 240
Tyr Ala Tyr Ala Phe Thr Pro His Ala Cys Gly Ser Lys Asn Arg Tyr
                245                 250                 255
His Ile Cys Glu Thr Asn Asn Cys Gly Gly Thr Tyr Ser Asp Asp Arg
            260                 265                 270
Phe Ala Gly Tyr Cys Asp Ala Asn Gly Cys Asp Tyr Asn Pro Tyr Arg
        275                 280                 285
Met Gly Asn Lys Asp Phe Tyr Gly Lys Gly Lys Thr Val Asp Thr Asn
    290                 295                 300
Arg Lys Phe Thr Val Val Ser Arg Phe Glu Arg Asn Arg Leu Ser Gln
305                 310                 315                 320
Phe Phe Val Gln Asp Gly Arg Lys Ile Glu Val Pro Pro Pro Thr Trp
                325                 330                 335
Pro Gly Leu Pro Asn Ser Ala Asp Ile Thr Pro Glu Leu Cys Asp Ala
            340                 345                 350
Gln Phe Arg Val Phe Asp Asp Arg Asn Arg Phe Ala Glu Thr Gly Gly
        355                 360                 365
Phe Asp Ala Leu Asn Glu Ala Leu Thr Ile Pro Met Val Leu Val Met
    370                 375                 380
Ser Ile Trp Asp Asp His His Ser Asn Met Leu Trp Leu Asp Ser Ser
385                 390                 395                 400
Tyr Pro Pro Glu Lys Ala Gly Leu Pro Gly Gly Asp Arg Gly Pro Cys
                405                 410                 415
Pro Thr Thr Ser Gly Val Pro Ala Glu Val Glu Ala Gln Tyr Pro Asp
            420                 425                 430
Ala Gln Val Val Trp Ser Asn Ile Arg Phe Gly Pro Ile Gly Ser Thr
        435                 440                 445
Val Asn Val
    450
```

<210> 58
<211> 447
<212> PRT
<213> Leptosphaeria maculans

<400> 58
Met Leu Ser Ala Ser Lys Ala Ala Ala Ile Leu Ala Phe Cys Ala His
1 5 10 15
Thr Ala Ser Ala Trp Val Val Gly Asp Gln Gln Thr Glu Thr His Pro
20 25 30
Lys Leu Asn Trp Gln Arg Cys Thr Gly Lys Gly Arg Ser Ser Cys Thr
35 40 45
Asn Val Asn Gly Glu Val Val Ile Asp Ala Asn Trp Arg Trp Leu Ala
50 55 60
His Arg Ser Gly Tyr Thr Asn Cys Tyr Thr Gly Ser Glu Trp Asn Gln
65 70 75 80
Ser Ala Cys Pro Asn Asn Glu Ala Cys Thr Lys Asn Cys Ala Ile Glu
85 90 95
Gly Ser Asp Tyr Ala Gly Thr Tyr Gly Ile Thr Thr Ser Gly Asn Gln
100 105 110
Met Asn Ile Lys Phe Ile Thr Lys Arg Pro Tyr Ser Thr Asn Ile Gly
115 120 125
Ala Arg Thr Tyr Leu Met Lys Asp Glu Gln Asn Tyr Glu Met Phe Gln
130 135 140
Leu Ile Gly Asn Glu Phe Thr Phe Asp Val Asp Leu Ser Gln Arg Cys
145 150 155 160
Gly Met Asn Gly Ala Leu Tyr Phe Val Ser Met Pro Gln Lys Gly Gln
165 170 175
Gly Ala Pro Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ala Gln Cys
180 185 190
Ala Arg Asp Leu Lys Phe Val Arg Gly Ser Ala Asn Ala Glu Gly Trp
195 200 205
Thr Lys Ser Ala Ser Asp Pro Asn Ser Gly Val Gly Lys Lys Gly Ala
210 215 220
Cys Cys Ala Gln Met Asp Val Trp Glu Ala Asn Ser Ala Ala Thr Ala
225 230 235 240
Leu Thr Pro His Ser Cys Gln Pro Ala Gly Tyr Ser Val Cys Glu Asp
245 250 255
Thr Asn Cys Gly Gly Thr Tyr Ser Glu Asp Arg Tyr Ala Gly Thr Cys
260 265 270
Asp Ala Asn Gly Cys Asp Phe Asn Pro Phe Arg Val Gly Val Lys Asp
275 280 285
Phe Tyr Gly Lys Gly Lys Thr Val Asp Thr Thr Lys Lys Met Thr Val
290 295 300
Val Thr Gln Phe Val Gly Ser Gly Asn Gln Leu Ser Glu Ile Lys Arg
305 310 315 320
Phe Tyr Val Gln Asp Gly Lys Val Ile Ala Asn Pro Glu Pro Thr Ile
325 330 335
Pro Gly Met Glu Trp Cys Asn Thr Gln Lys Lys Val Phe Gln Glu Glu
340 345 350
Ala Tyr Pro Phe Asn Glu Phe Gly Gly Met Ala Ser Met Ser Glu Gly
355 360 365
Met Ser Gln Gly Met Val Leu Val Met Ser Leu Trp Asp Asp His Tyr
370 375 380
Ala Asn Met Leu Trp Leu Asp Ser Asn Trp Pro Arg Glu Ala Asp Pro
385 390 395 400
Ala Lys Pro Gly Val Ala Arg Arg Asp Cys Pro Thr Ser Gly Gly Lys
405 410 415
Pro Ser Glu Val Glu Ala Ala Asn Pro Asn Ala Gln Val Met Phe Ser

420 425 430
Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Phe Ala His Ala Ala
435 440 445

<210> 59
<211> 516
<212> PRT
<213> Neurospora crassa

<400> 59
Met Arg Ala Ser Leu Leu Ala Phe Ser Leu Ala Ala Ala Val Ala Gly
1 5 10 15
Gly Gln Gln Ala Gly Thr Leu Thr Ala Lys Arg His Pro Ser Leu Thr
20 25 30
Trp Gln Lys Cys Thr Arg Gly Gly Cys Pro Thr Leu Asn Thr Thr Met
35 40 45
Val Leu Asp Ala Asn Trp Arg Trp Thr His Ala Thr Ser Gly Ser Thr
50 55 60
Lys Cys Tyr Thr Gly Asn Lys Trp Gln Ala Thr Leu Cys Pro Asp Gly
65 70 75 80
Lys Ser Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr Thr Gly
85 90 95
Thr Tyr Gly Ile Thr Gly Ser Gly Trp Ser Leu Thr Leu Gln Phe Val
100 105 110
Thr Asp Asn Val Gly Ala Arg Ala Tyr Leu Met Ala Asp Asp Thr Gln
115 120 125
Tyr Gln Met Leu Glu Leu Leu Asn Gln Glu Leu Trp Phe Asp Val Asp
130 135 140
Met Ser Asn Ile Pro Cys Gly Leu Asn Gly Ala Leu Tyr Leu Ser Ala
145 150 155 160
Met Asp Ala Asp Gly Gly Met Arg Lys Tyr Pro Thr Asn Lys Ala Gly
165 170 175
Ala Lys Tyr Ala Thr Gly Tyr Cys Asp Ala Gln Cys Pro Arg Asp Leu
180 185 190
Lys Tyr Ile Asn Gly Ile Ala Asn Val Glu Gly Trp Thr Pro Ser Thr
195 200 205
Asn Asp Ala Asn Gly Ile Gly Asp His Gly Ser Cys Cys Ser Glu Met
210 215 220
Asp Ile Trp Glu Ala Asn Lys Val Ser Thr Ala Phe Thr Pro His Pro
225 230 235 240
Cys Thr Thr Ile Glu Gln His Met Cys Glu Gly Asp Ser Cys Gly Gly
245 250 255
Thr Tyr Ser Asp Asp Arg Tyr Gly Val Leu Cys Asp Ala Asp Gly Cys
260 265 270
Asp Phe Asn Ser Tyr Arg Met Gly Asn Thr Thr Phe Tyr Gly Glu Gly
275 280 285
Lys Thr Val Asp Thr Ser Ser Lys Phe Thr Val Val Thr Gln Phe Ile
290 295 300
Lys Asp Ser Ala Gly Asp Leu Ala Glu Ile Lys Ala Phe Tyr Val Gln
305 310 315 320
Asn Gly Lys Val Ile Glu Asn Ser Gln Ser Asn Val Asp Gly Val Ser
325 330 335
Gly Asn Ser Ile Thr Gln Ser Phe Cys Lys Ser Gln Lys Thr Ala Phe
340 345 350
Gly Asp Ile Asp Asp Phe Asn Lys Lys Gly Gly Leu Lys Gln Met Gly
355 360 365
Lys Ala Leu Ala Gln Ala Met Val Leu Val Met Ser Ile Trp Asp Asp
370 375 380
His Ala Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Val Pro Lys
385 390 395 400
Val Pro Gly Ala Tyr Arg Gly Ser Gly Pro Thr Thr Ser Gly Val Pro

405 410 415
Ala Glu Val Asp Ala Asn Ala Pro Asn Ser Lys Val Ala Phe Ser Asn
420 425 430
Ile Lys Phe Gly His Leu Gly Ile Ser Pro Phe Ser Gly Gly Ser Ser
435 440 445
Gly Thr Pro Pro Ser Asn Pro Ser Ser Ser Ala Ser Pro Thr Ser Ser
450 455 460
Thr Ala Lys Pro Ser Ser Thr Ser Thr Ala Ser Asn Pro Ser Gly Thr
465 470 475 480
Gly Ala Ala His Trp Ala Gln Cys Gly Gly Ile Gly Phe Ser Gly Pro
485 490 495
Thr Thr Cys Pro Glu Pro Tyr Thr Cys Ala Lys Asp His Asp Ile Tyr
500 505 510
Ser Gln Cys Val
515

<210> 60
<211> 540
<212> PRT
<213> Aspergillus aculeatus

<400> 60
Met Val Asp Ser Phe Ser Ile Tyr Lys Thr Ala Leu Leu Leu Ser Met
1 5 10 15
Leu Ala Thr Ser Asn Ala Gln Gln Val Gly Thr Tyr Thr Ala Glu Thr
20 25 30
His Pro Ser Leu Thr Trp Gln Thr Cys Ser Gly Ser Gly Ser Cys Thr
35 40 45
Thr Thr Ser Gly Ser Val Val Ile Asp Ala Asn Trp Arg Trp Val His
50 55 60
Glu Val Gly Gly Tyr Thr Asn Cys Tyr Ser Gly Asn Thr Trp Asp Ser
65 70 75 80
Ser Ile Cys Ser Thr Asp Thr Thr Cys Ala Ser Glu Cys Ala Leu Glu
85 90 95
Gly Ala Thr Tyr Glu Ser Thr Tyr Gly Val Thr Thr Ser Gly Ser Ser
100 105 110
Leu Arg Leu Asn Phe Val Thr Thr Ala Ser Gln Lys Asn Ile Gly Ser
115 120 125
Arg Leu Tyr Leu Leu Ala Asp Asp Ser Thr Tyr Glu Thr Phe Lys Leu
130 135 140
Phe Asn Arg Glu Phe Thr Phe Asp Val Asp Val Ser Asn Leu Pro Cys
145 150 155 160
Gly Leu Asn Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly
165 170 175
Val Ser Arg Phe Pro Thr Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly
180 185 190
Tyr Cys Asp Ser Gln Cys Pro Arg Asp Leu Lys Phe Ile Asp Gly Gln
195 200 205
Ala Asn Ile Glu Gly Trp Glu Pro Ser Ser Thr Asp Val Asn Ala Gly
210 215 220
Thr Gly Asn His Gly Ser Cys Cys Pro Glu Met Asp Ile Trp Glu Ala
225 230 235 240
Asn Ser Ile Ser Ser Ala Phe Thr Ala His Pro Cys Asp Ser Val Gln
245 250 255
Gln Thr Met Cys Thr Gly Asp Thr Cys Gly Gly Thr Tyr Ser Asp Thr
260 265 270
Thr Asp Arg Tyr Ser Gly Thr Cys Asp Pro Asp Gly Cys Asp Phe Asn
275 280 285
Pro Tyr Arg Phe Gly Asn Thr Asn Phe Tyr Gly Pro Gly Lys Thr Val
290 295 300
Asp Asn Ser Lys Pro Phe Thr Val Val Thr Gln Phe Ile Thr His Asp

305 310 315 320
Gly Thr Asp Thr Gly Thr Leu Thr Glu Ile Arg Arg Leu Tyr Val Gln
325 330 335
Asn Gly Val Val Ile Gly Asn Gly Pro Ser Thr Tyr Thr Ala Ala Ser
340 345 350
Gly Asn Ser Ile Thr Glu Ser Phe Cys Lys Ala Glu Lys Thr Leu Phe
355 360 365
Gly Asp Thr Asn Val Phe Glu Thr His Gly Gly Leu Ser Ala Met Gly
370 375 380
Asp Ala Leu Gly Asp Gly Met Val Leu Val Leu Ser Leu Trp Asp Asp
385 390 395 400
His Ala Ala Asp Met Leu Trp Leu Asp Ser Asp Tyr Pro Thr Thr Ser
405 410 415
Cys Ala Ser Ser Pro Gly Val Ala Arg Gly Thr Cys Pro Thr Thr Thr
420 425 430
Gly Asn Ala Thr Tyr Val Glu Ala Asn Tyr Pro Asn Ser Tyr Val Thr
435 440 445
Tyr Ser Asn Ile Lys Phe Gly Thr Leu Asn Ser Thr Tyr Ser Gly Thr
450 455 460
Ser Ser Gly Gly Ser Ser Ser Ser Ser Thr Thr Leu Thr Thr Lys Ala
465 470 475 480
Ser Thr Ser Thr Thr Ser Ser Lys Thr Thr Thr Thr Thr Ser Lys Thr
485 490 495
Ser Thr Thr Ser Ser Ser Ser Thr Asn Val Ala Gln Leu Tyr Gly Gln
500 505 510
Cys Gly Gly Gln Gly Trp Thr Gly Pro Thr Thr Cys Ala Ser Gly Thr
515 520 525
Cys Thr Lys Gln Asn Asp Tyr Tyr Ser Gln Cys Leu
530 535 540

<210> 61
<211> 536
<212> PRT
<213> Aspergillus niger

<400> 61
Met Ser Ser Phe Gln Ile Tyr Arg Ala Ala Leu Leu Leu Ser Ile Leu
1 5 10 15
Ala Thr Ala Asn Ala Gln Gln Val Gly Thr Tyr Thr Thr Glu Thr His
20 25 30
Pro Ser Leu Thr Trp Gln Thr Cys Thr Ser Asp Gly Ser Cys Thr Thr
35 40 45
Asn Asp Gly Glu Val Val Ile Asp Ala Asn Trp Arg Trp Val His Ser
50 55 60
Thr Ser Ser Ala Thr Asn Cys Tyr Thr Gly Asn Glu Trp Asp Thr Ser
65 70 75 80
Ile Cys Thr Asp Asp Val Thr Cys Ala Ala Asn Cys Ala Leu Asp Gly
85 90 95
Ala Thr Tyr Glu Ala Thr Tyr Gly Val Thr Thr Ser Gly Ser Glu Leu
100 105 110
Arg Leu Asn Phe Val Thr Gln Gly Ser Ser Lys Asn Ile Gly Ser Arg
115 120 125
Leu Tyr Leu Met Ser Asp Asp Ser Asn Tyr Glu Leu Phe Lys Leu Leu
130 135 140
Gly Gln Glu Phe Thr Phe Asp Val Asp Val Ser Asn Leu Pro Cys Gly
145 150 155 160
Leu Asn Gly Ala Leu Tyr Phe Val Ala Met Asp Ala Asp Gly Gly Thr
165 170 175
Ser Glu Tyr Ser Gly Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr
180 185 190
Cys Asp Ser Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Glu Ala

```
            195                 200                 205
Asn Cys Asp Gly Trp Glu Pro Ser Ser Asn Asn Val Asn Thr Gly Val
    210                 215                 220
Gly Asp His Gly Ser Cys Cys Ala Glu Met Asp Val Trp Glu Ala Asn
225                 230                 235                 240
Ser Ile Ser Asn Ala Phe Thr Ala His Pro Cys Asp Ser Val Ser Gln
                245                 250                 255
Thr Met Cys Asp Gly Asp Ser Cys Gly Gly Thr Tyr Ser Ala Ser Gly
            260                 265                 270
Asp Arg Tyr Ser Gly Thr Cys Asp Pro Asp Gly Cys Asp Tyr Asn Pro
        275                 280                 285
Tyr Arg Leu Gly Asn Thr Asp Phe Tyr Gly Pro Gly Leu Thr Val Asp
    290                 295                 300
Thr Asn Ser Pro Phe Thr Val Val Thr Gln Phe Ile Thr Asp Asp Gly
305                 310                 315                 320
Thr Ser Ser Gly Thr Leu Thr Glu Ile Lys Arg Leu Tyr Val Gln Asn
                325                 330                 335
Gly Glu Val Ile Ala Asn Gly Ala Ser Thr Tyr Ser Ser Val Asn Gly
            340                 345                 350
Ser Ser Ile Thr Ser Ala Phe Cys Glu Ser Glu Lys Thr Leu Phe Gly
        355                 360                 365
Asp Glu Asn Val Phe Asp Lys His Gly Gly Leu Glu Gly Met Gly Glu
    370                 375                 380
Ala Met Ala Lys Gly Met Val Leu Val Leu Ser Leu Trp Asp Asp Tyr
385                 390                 395                 400
Ala Ala Asp Met Leu Trp Leu Asp Ser Asp Tyr Pro Val Asn Ser Ser
                405                 410                 415
Ala Ser Thr Pro Gly Val Ala Arg Gly Thr Cys Ser Thr Asp Ser Gly
            420                 425                 430
Val Pro Ala Thr Val Glu Ala Glu Ser Pro Asn Ala Tyr Val Thr Tyr
        435                 440                 445
Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Tyr Ser Ser Gly Ser
    450                 455                 460
Ser Ser Gly Ser Gly Ser Ser Ser Ser Ser Ser Ser Thr Thr Thr Lys
465                 470                 475                 480
Ala Thr Ser Thr Thr Leu Lys Thr Thr Ser Thr Thr Ser Ser Gly Ser
                485                 490                 495
Ser Ser Thr Ser Ala Ala Gln Ala Tyr Gly Gln Cys Gly Gly Gln Gly
            500                 505                 510
Trp Thr Gly Pro Thr Thr Cys Val Ser Gly Tyr Thr Cys Thr Tyr Glu
        515                 520                 525
Asn Ala Tyr Tyr Ser Gln Cys Leu
    530                 535
```

<210> 62
<211> 537
<212> PRT
<213> Penicillum janthinellum

<220>
<221> VARIANT
<222> 48, 64
<223> Xaa = Any Amino Acid

<400> 62

```
Met Lys Gly Ser Ile Ser Tyr Gln Ile Tyr Lys Gly Ala Leu Leu Leu
 1               5                  10                  15
Ser Ala Leu Leu Asn Ser Val Ser Ala Gln Gln Val Gly Thr Leu Thr
            20                  25                  30
Ala Glu Thr His Pro Ala Leu Thr Trp Ser Lys Cys Thr Ala Gly Xaa
        35                  40                  45
```

```
Cys Ser Gln Val Ser Gly Ser Val Val Ile Asp Ala Asn Trp Pro Xaa
    50                  55                  60
Val His Ser Thr Ser Gly Ser Thr Asn Cys Tyr Thr Gly Asn Thr Trp
65                  70                  75                  80
Asp Ala Thr Leu Cys Pro Asp Asp Val Thr Cys Ala Ala Asn Cys Ala
                85                  90                  95
Val Asp Gly Ala Arg Arg Gln His Leu Arg Val Thr Thr Ser Gly Asn
            100                 105                 110
Ser Leu Arg Ile Asn Phe Val Thr Thr Ala Ser Gln Lys Asn Ile Gly
        115                 120                 125
Ser Arg Leu Tyr Leu Leu Glu Asn Asp Thr Thr Tyr Gln Lys Phe Asn
    130                 135                 140
Leu Leu Asn Gln Glu Phe Thr Phe Asp Val Asp Val Ser Asn Leu Pro
145                 150                 155                 160
Cys Gly Leu Asn Gly Ala Leu Tyr Phe Val Asp Met Asp Ala Asp Gly
                165                 170                 175
Gly Met Ala Lys Tyr Pro Thr Asn Lys Ala Gly Ala Lys Tyr Gly Thr
            180                 185                 190
Gly Tyr Cys Asp Ser Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly
        195                 200                 205
Gln Ala Asn Val Asp Gly Trp Thr Pro Ser Lys Asn Asp Val Asn Ser
    210                 215                 220
Gly Ile Gly Asn His Gly Ser Cys Cys Ala Glu Met Asp Ile Trp Glu
225                 230                 235                 240
Ala Asn Ser Ile Ser Asn Ala Val Thr Pro His Pro Cys Asp Thr Pro
                245                 250                 255
Ser Gln Thr Met Cys Thr Gly Gln Arg Cys Gly Gly Thr Tyr Ser Thr
            260                 265                 270
Asp Arg Tyr Gly Gly Thr Cys Asp Pro Asp Gly Cys Asp Phe Asn Pro
        275                 280                 285
Tyr Arg Met Gly Val Thr Asn Phe Tyr Gly Pro Gly Glu Thr Ile Asp
    290                 295                 300
Thr Lys Ser Pro Phe Thr Val Val Thr Gln Phe Leu Thr Asn Asp Gly
305                 310                 315                 320
Thr Ser Thr Gly Thr Leu Ser Glu Ile Lys Arg Phe Tyr Val Gln Gly
                325                 330                 335
Gly Lys Val Ile Gly Asn Pro Gln Ser Thr Ile Val Gly Val Ser Gly
            340                 345                 350
Asn Ser Ile Thr Asp Ser Trp Cys Asn Ala Gln Lys Ser Ala Phe Gly
        355                 360                 365
Asp Thr Asn Glu Phe Ser Lys His Gly Gly Met Ala Gly Met Gly Ala
    370                 375                 380
Gly Leu Ala Asp Gly Met Val Leu Val Met Ser Leu Trp Asp Asp His
385                 390                 395                 400
Ala Ser Asp Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn Ala Thr
                405                 410                 415
Ser Thr Thr Pro Gly Ala Lys Arg Gly Thr Cys Asp Ile Ser Arg Arg
            420                 425                 430
Pro Asn Thr Val Glu Ser Thr Tyr Pro Asn Ala Tyr Val Ile Tyr Ser
        435                 440                 445
Asn Ile Lys Thr Gly Pro Leu Asn Ser Thr Phe Thr Gly Gly Thr Thr
    450                 455                 460
Ser Ser Ser Ser Thr Thr Thr Thr Thr Ser Lys Ser Thr Ser Thr Ser
465                 470                 475                 480
Ser Ser Ser Lys Thr Thr Thr Thr Val Thr Thr Thr Thr Thr Ser Ser
                485                 490                 495
Gly Ser Ser Gly Thr Gly Ala Arg Asp Trp Ala Gln Cys Gly Gly Asn
            500                 505                 510
Gly Trp Thr Gly Pro Thr Thr Cys Val Ser Pro Tyr Thr Cys Thr Lys
        515                 520                 525
Gln Asn Asp Trp Tyr Ser Gln Cys Leu
```

530 535

<210> 63
<211> 452
<212> PRT
<213> Aspergillus niger

<400> 63

Met	His	Gln	Arg	Ala	Leu	Leu	Phe	Ser	Ala	Leu	Leu	Thr	Ala	Val	Arg
1				5				10					15		
Ala	Gln	Gln	Ala	Gly	Thr	Leu	Thr	Glu	Glu	Val	His	Pro	Ser	Leu	Thr
			20					25					30		
Trp	Gln	Lys	Cys	Thr	Ser	Glu	Gly	Ser	Cys	Thr	Glu	Gln	Ser	Gly	Ser
		35					40					45			
Val	Val	Ile	Asp	Ser	Asn	Trp	Arg	Trp	Thr	His	Ser	Val	Asn	Asp	Ser
	50					55					60				
Thr	Asn	Cys	Tyr	Thr	Gly	Asn	Thr	Trp	Asp	Ala	Thr	Leu	Cys	Pro	Asp
65					70					75					80
Asp	Glu	Thr	Cys	Ala	Ala	Asn	Cys	Ala	Leu	Asp	Gly	Ala	Asp	Tyr	Glu
				85					90					95	
Ser	Thr	Tyr	Gly	Val	Thr	Thr	Asp	Gly	Asp	Ser	Leu	Thr	Leu	Lys	Phe
			100					105					110		
Val	Thr	Gly	Ser	Asn	Val	Gly	Ser	Arg	Leu	Tyr	Leu	Met	Asp	Thr	Ser
		115					120					125			
Asp	Glu	Gly	Tyr	Gln	Thr	Phe	Asn	Leu	Leu	Asp	Ala	Glu	Phe	Thr	Phe
	130					135					140				
Asp	Val	Asp	Val	Ser	Asn	Leu	Pro	Cys	Gly	Leu	Asn	Gly	Ala	Leu	Tyr
145					150					155					160
Phe	Thr	Ala	Met	Asp	Ala	Asp	Gly	Gly	Val	Ser	Lys	Tyr	Pro	Ala	Asn
				165					170					175	
Lys	Ala	Gly	Ala	Lys	Tyr	Gly	Thr	Gly	Tyr	Cys	Asp	Ser	Gln	Cys	Pro
			180					185					190		
Arg	Asp	Leu	Lys	Phe	Ile	Asp	Gly	Gln	Ala	Asn	Val	Asp	Gly	Trp	Glu
		195					200					205			
Pro	Ser	Ser	Asn	Asn	Asp	Asn	Thr	Gly	Ile	Gly	Asn	His	Gly	Ser	Cys
	210					215					220				
Cys	Pro	Glu	Met	Asp	Ile	Trp	Glu	Ala	Asn	Lys	Ile	Ser	Thr	Ala	Leu
225					230					235					240
Thr	Pro	His	Pro	Cys	Asp	Ser	Ser	Glu	Gln	Thr	Met	Cys	Glu	Gly	Asn
				245					250					255	
Asp	Cys	Gly	Gly	Thr	Tyr	Ser	Asp	Asp	Arg	Tyr	Gly	Gly	Thr	Cys	Asp
			260					265					270		
Pro	Asp	Gly	Cys	Asp	Phe	Asn	Pro	Tyr	Arg	Met	Gly	Asn	Asp	Ser	Phe
		275					280					285			
Tyr	Gly	Pro	Gly	Lys	Thr	Ile	Asp	Thr	Gly	Ser	Lys	Met	Thr	Val	Val
	290					295					300				
Thr	Gln	Phe	Ile	Thr	Asp	Gly	Ser	Gly	Ser	Leu	Ser	Glu	Ile	Lys	Arg
305					310					315					320
Tyr	Tyr	Val	Gln	Asn	Gly	Asn	Val	Ile	Ala	Asn	Ala	Asp	Ser	Asn	Ile
				325					330					335	
Ser	Gly	Val	Thr	Gly	Asn	Ser	Ile	Thr	Thr	Asp	Phe	Cys	Thr	Ala	Gln
			340					345					350		
Lys	Lys	Ala	Phe	Gly	Asp	Glu	Asp	Ile	Phe	Ala	Glu	His	Asn	Gly	Leu
		355					360					365			
Ala	Gly	Ile	Ser	Asp	Ala	Met	Ser	Ser	Met	Val	Leu	Ile	Leu	Ser	Leu
	370					375					380				
Trp	Asp	Asp	Tyr	Tyr	Ala	Ser	Met	Glu	Trp	Leu	Asp	Ser	Asp	Tyr	Pro
385					390					395					400
Glu	Asn	Ala	Thr	Ala	Thr	Asp	Pro	Gly	Val	Ala	Arg	Gly	Thr	Cys	Asp
				405					410					415	
Ser	Glu	Ser	Gly	Val	Pro	Ala	Thr	Val	Glu	Gly	Ala	His	Pro	Asp	Ser

420 425 430
Ser Val Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Asn Ser Thr Phe
435 440 445
Ser Ala Ser Ala
450

<210> 64
<211> 513
<212> PRT
<213> Hypocrea ceramica

<400> 64
Met Tyr Arg Lys Leu Ala Val Ile Ser Ala Phe Leu Ala Thr Ala Arg
1 5 10 15
Ala Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr
20 25 30
Trp Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser
35 40 45
Val Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser
50 55 60
Thr Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp
65 70 75 80
Asn Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala
85 90 95
Ser Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe
100 105 110
Val Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met
115 120 125
Ala Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe
130 135 140
Ser Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala
145 150 155 160
Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro
165 170 175
Thr Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln
180 185 190
Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly
195 200 205
Trp Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly
210 215 220
Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu
225 230 235 240
Ala Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu
245 250 255
Gly Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr
260 265 270
Cys Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr
275 280 285
Ser Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys
290 295 300
Leu Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr
305 310 315 320
Tyr Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly
325 330 335
Ser Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu
340 345 350
Ala Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln
355 360 365
Phe Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp
370 375 380
Asp Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr

385 390 395 400
Asn Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr
405 410 415
Ser Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys
420 425 430
Val Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn
435 440 445
Pro Ser Gly Gly Asn Pro Pro Gly Gly Asn Arg Gly Thr Thr Thr Thr
450 455 460
Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser
465 470 475 480
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys
485 490 495
Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
500 505 510
Leu

<210> 65
<211> 491
<212> PRT
<213> Hypocrea jecorina

<220>
<221> VARIANT
<222> (348)...(349)
<223> Xaa = Any Amino Acid

<400> 65
Glu Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
1 5 10 15
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
20 25 30
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
35 40 45
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
50 55 60
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Thr Ser
65 70 75 80
Ala Tyr Ser Ser Glx Pro Gly Gly Gly Gly Gly Val Val Ile Phe Phe
85 90 95
Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala Ser Asp Thr Thr Tyr
100 105 110
Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser Phe Asp Val Asp Val
115 120 125
Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr Phe Val Ser Met
130 135 140
Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr Asn Thr Ala Gly Ala
145 150 155 160
Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro Arg Asp Leu Lys
165 170 175
Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp Glu Pro Ser Ser Asn
180 185 190
Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser Cys Cys Ser Glu Met
195 200 205
Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala Leu Thr Pro His Pro
210 215 220
Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly Asp Gly Cys Gly Gly
225 230 235 240
Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys Asp Pro Asp Gly Cys
245 250 255

```
Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser Phe Tyr Gly Pro Gly
            260                 265                 270
Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu Thr Val Val Thr Gln
        275                 280                 285
Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr Val Gln Asp Gly Val
    290                 295                 300
Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser Tyr Ser Gly Asn Glu
305                 310                 315                 320
Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala Glu Phe Gly Gly Ser
                325                 330                 335
Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe Xaa Xaa Ala Thr Ser
            340                 345                 350
Gly Gly Met Val Leu Val Met Ser Leu Trp Asp Asp Tyr Tyr Ala Asn
        355                 360                 365
Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn Glu Thr Ser Ser Thr
    370                 375                 380
Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser Ser Gly Val Pro Ala
385                 390                 395                 400
Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val Thr Phe Ser Asn Ile
                405                 410                 415
Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro Ser Gly Gly Asn Pro
            420                 425                 430
Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr Thr Thr Thr Thr Ser Ser
        435                 440                 445
Ser Glx Pro Pro Pro Gly Ala His Arg Arg Tyr Gly Gln Cys Gly Gly
    450                 455                 460
Ile Gly Tyr Ser Gly Pro Thr Val Cys Ala Ser Gly Thr Thr Cys Gln
465                 470                 475                 480
Val Leu Asn Pro Tyr Tyr Ser Gln Cys Leu Val
                485                 490
```

<210> 66
<211> 513
<212> PRT
<213> Trichoderma viride

<400> 66

```
Met Tyr Gln Lys Leu Ala Leu Ile Ser Ala Phe Leu Ala Thr Ala Arg
 1               5                  10                  15
Ala Gln Ser Ala Cys Thr Leu Gln Ala Glu Thr His Pro Pro Leu Thr
            20                  25                  30
Trp Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser
        35                  40                  45
Val Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser
    50                  55                  60
Thr Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp
65                  70                  75                  80
Asn Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala
                85                  90                  95
Ser Thr Tyr Gly Val Thr Thr Ser Ala Asp Ser Leu Ser Ile Gly Phe
            100                 105                 110
Val Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met
        115                 120                 125
Ala Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe
    130                 135                 140
Ser Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala
145                 150                 155                 160
Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Thr Lys Tyr Pro
                165                 170                 175
Thr Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln
            180                 185                 190
```

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Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly
        195                 200                 205
Trp Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly
    210                 215                 220
Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu
225                 230                 235                 240
Ala Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu
                245                 250                 255
Gly Asp Ser Cys Gly Gly Thr Tyr Ser Gly Asp Arg Tyr Gly Gly Thr
            260                 265                 270
Cys Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr
        275                 280                 285
Ser Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys
    290                 295                 300
Leu Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr
305                 310                 315                 320
Tyr Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly
                325                 330                 335
Asp Tyr Ser Gly Asn Ser Leu Asp Asp Asp Tyr Cys Ala Ala Glu Glu
            340                 345                 350
Ala Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln
        355                 360                 365
Phe Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp
    370                 375                 380
Asp Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr
385                 390                 395                 400
Asp Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Ser Ser Thr
                405                 410                 415
Ser Ser Gly Val Pro Ala Gln Leu Glu Ser Asn Ser Pro Asn Ala Lys
            420                 425                 430
Val Val Tyr Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn
        435                 440                 445
Pro Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr
    450                 455                 460
Pro Arg Pro Ala Thr Ser Thr Gly Ser Ser Pro Gly Pro Thr Gln Thr
465                 470                 475                 480
His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ile Gly Pro Thr Val Cys
                485                 490                 495
Ala Ser Gly Ser Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys
            500                 505                 510
Leu
```

<210> 67
<211> 514
<212> PRT
<213> Trichoderma viride

<400> 67

```
Met Tyr Gln Lys Leu Ala Leu Ile Ser Ala Phe Leu Ala Thr Ala Arg
 1               5                  10                  15
Ala Gln Ser Ala Cys Thr Leu Gln Ala Glu Thr His Pro Pro Leu Thr
            20                  25                  30
Trp Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser
        35                  40                  45
Val Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser
    50                  55                  60
Thr Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp
65                  70                  75                  80
Asn Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala
                85                  90                  95
```

Ser Thr Tyr Gly Val Thr Thr Ser Ala Asp Ser Leu Ser Ile Gly Phe
100 105 110
Val Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met
115 120 125
Ala Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe
130 135 140
Ser Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala
145 150 155 160
Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro
165 170 175
Thr Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln
180 185 190
Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly
195 200 205
Trp Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly
210 215 220
Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu
225 230 235 240
Ala Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Asp
245 250 255
Gly Asp Ser Cys Gly Gly Thr Tyr Ser Gly Asp Arg Tyr Gly Gly Thr
260 265 270
Cys Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr
275 280 285
Ser Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys
290 295 300
Leu Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr
305 310 315 320
Tyr Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly
325 330 335
Asp Tyr Ser Gly Asn Ser Leu Asp Asp Asp Tyr Cys Ala Ala Glu Glu
340 345 350
Ala Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln
355 360 365
Phe Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp
370 375 380
Asp Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr
385 390 395 400
Asn Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr
405 410 415
Ser Ser Gly Val Pro Ala Gln Leu Glu Ser Asn Ser Pro Asn Ala Lys
420 425 430
Val Val Tyr Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn
435 440 445
Ser Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr
450 455 460
Thr Arg Arg Pro Ala Thr Ser Thr Gly Ser Ser Pro Gly Pro Thr Gln
465 470 475 480
Thr His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val
485 490 495
Cys Ala Ser Gly Ser Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln
500 505 510
Cys Leu

<210> 68
<211> 505
<212> PRT
<213> Trichoderma harzianum

<400> 68

Met Tyr Arg Lys Leu Ala Val Ile Ser Ala Phe Leu Ala Ala Ala Arg
1 5 10 15
Ala Gln Gln Val Cys Thr Gln Gln Ala Glu Thr His Pro Pro Leu Thr
20 25 30
Trp Gln Lys Cys Thr Ala Ser Gly Cys Thr Pro Gln Gln Gly Ser Val
35 40 45
Val Leu Asp Ala Asn Trp Arg Trp Thr His Asp Thr Lys Ser Thr Thr
50 55 60
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asp
65 70 75 80
Ala Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Asn Tyr Ser Gly
85 90 95
Thr Tyr Gly Val Thr Thr Ser Gly Asp Ala Leu Thr Leu Gln Phe Val
100 105 110
Thr Ala Ser Asn Val Gly Ser Arg Leu Tyr Leu Met Ala Asn Asp Ser
115 120 125
Thr Tyr Gln Glu Phe Thr Leu Ser Gly Asn Glu Phe Ser Phe Asp Val
130 135 140
Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr Phe Val
145 150 155 160
Ser Met Asp Ala Asp Gly Gly Gln Ser Lys Tyr Pro Gly Asn Ala Ala
165 170 175
Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro Arg Asp
180 185 190
Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp Glu Pro Ser
195 200 205
Ser Asn Asn Ala Asn Thr Gly Val Gly Gly His Gly Ser Cys Cys Ser
210 215 220
Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala Leu Thr Pro
225 230 235 240
His Pro Cys Glu Thr Val Gly Gln Thr Met Cys Ser Gly Asp Ser Cys
245 250 255
Gly Gly Thr Tyr Ser Asn Asp Arg Tyr Gly Gly Thr Cys Asp Pro Asp
260 265 270
Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser Phe Tyr Gly
275 280 285
Pro Gly Ser Ser Phe Ala Leu Asp Thr Thr Lys Lys Leu Thr Val Val
290 295 300
Thr Gln Phe Ala Thr Asp Gly Ser Ile Ser Arg Tyr Tyr Val Gln Asn
305 310 315 320
Gly Val Lys Phe Gln Gln Pro Asn Ala Gln Val Gly Ser Tyr Ser Gly
325 330 335
Asn Thr Ile Asn Thr Asp Tyr Cys Ala Ala Glu Gln Thr Ala Phe Gly
340 345 350
Gly Thr Ser Phe Thr Asp Lys Gly Gly Leu Ala Gln Ile Asn Lys Ala
355 360 365
Phe Gln Gly Gly Met Val Leu Val Met Ser Leu Trp Asp Asp Tyr Ala
370 375 380
Val Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn Ala Thr Ala
385 390 395 400
Ser Thr Pro Gly Ala Lys Arg Gly Ser Cys Ser Thr Ser Ser Gly Val
405 410 415
Pro Ala Gln Val Glu Ala Gln Ser Pro Asn Ser Lys Val Ile Tyr Ser
420 425 430
Asn Ile Arg Phe Gly Pro Ile Gly Ser Thr Gly Gly Asn Thr Gly Ser
435 440 445
Asn Pro Pro Gly Thr Ser Thr Thr Arg Ala Pro Pro Ser Ser Thr Gly
450 455 460
Ser Ser Pro Thr Ala Thr Gln Thr His Tyr Gly Gln Cys Gly Gly Thr
465 470 475 480
Gly Trp Thr Gly Pro Thr Arg Cys Ala Ser Gly Tyr Thr Cys Gln Val

485 490 495
Leu Asn Pro Phe Tyr Ser Gln Cys Leu
500 505

<210> 69
<211> 506
<212> PRT
<213> Aspergillus bisporus

<400> 69
Met Phe Pro Arg Ser Ile Leu Leu Ala Leu Ser Leu Thr Ala Val Ala
1 5 10 15
Leu Gly Gln Gln Val Gly Thr Asn Met Ala Glu Asn His Pro Ser Leu
20 25 30
Thr Trp Gln Arg Cys Thr Ser Ser Gly Cys Gln Asn Val Asn Gly Lys
35 40 45
Val Thr Leu Asp Ala Asn Trp Arg Trp Thr His Arg Ile Asn Asp Phe
50 55 60
Thr Asn Cys Tyr Thr Gly Asn Glu Trp Asp Thr Ser Ile Cys Pro Asp
65 70 75 80
Gly Val Thr Cys Ala Glu Asn Cys Ala Leu Asp Gly Ala Asp Tyr Ala
85 90 95
Gly Thr Tyr Gly Val Thr Ser Ser Gly Thr Ala Leu Thr Leu Lys Phe
100 105 110
Val Thr Glu Ser Gln Gln Lys Asn Ile Gly Ser Arg Leu Tyr Leu Met
115 120 125
Ala Asp Asp Ser Asn Tyr Glu Ile Phe Asn Leu Leu Asn Lys Glu Phe
130 135 140
Thr Phe Asp Val Asp Val Ser Lys Leu Pro Cys Gly Leu Asn Gly Ala
145 150 155 160
Leu Tyr Phe Ser Glu Met Ala Ala Asp Gly Gly Met Ser Ser Thr Asn
165 170 175
Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
180 185 190
Arg Asp Ile Lys Phe Ile Asp Gly Glu Ala Asn Ser Glu Gly Trp Glu
195 200 205
Gly Ser Pro Asn Asp Val Asn Ala Gly Thr Gly Asn Phe Gly Ala Cys
210 215 220
Cys Gly Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Ser Ala Tyr
225 230 235 240
Thr Pro His Pro Cys Arg Glu Pro Gly Leu Gln Arg Cys Glu Gly Asn
245 250 255
Thr Cys Ser Val Asn Asp Arg Tyr Ala Thr Glu Cys Asp Pro Asp Gly
260 265 270
Cys Asp Phe Asn Ser Phe Arg Met Gly Asp Lys Ser Phe Tyr Gly Pro
275 280 285
Gly Met Thr Val Asp Thr Asn Gln Pro Ile Thr Val Val Thr Gln Phe
290 295 300
Ile Thr Asp Asn Gly Ser Asp Asn Gly Asn Leu Gln Glu Ile Arg Arg
305 310 315 320
Ile Tyr Val Gln Asn Gly Gln Val Ile Gln Asn Ser Asn Val Asn Ile
325 330 335
Pro Gly Ile Asp Ser Gly Asn Ser Ile Ser Ala Glu Phe Cys Asp Gln
340 345 350
Ala Lys Glu Ala Phe Gly Asp Glu Arg Ser Phe Gln Asp Arg Gly Gly
355 360 365
Leu Ser Gly Met Gly Ser Ala Leu Asp Arg Gly Met Val Leu Val Leu
370 375 380
Ser Ile Trp Asp Asp His Ala Val Asn Met Leu Trp Leu Asp Ser Asp
385 390 395 400
Tyr Pro Leu Asp Ala Ser Pro Ser Gln Pro Gly Ile Ser Arg Gly Thr

405 410 415
Cys Ser Arg Asp Ser Gly Lys Pro Glu Asp Val Glu Ala Asn Ala Gly
420 425 430
Gly Val Gln Val Val Tyr Ser Asn Ile Lys Phe Gly Asp Ile Asn Ser
435 440 445
Thr Phe Asn Asn Asn Gly Gly Gly Gly Gly Asn Pro Ser Pro Thr Thr
450 455 460
Thr Arg Pro Asn Ser Pro Ala Gln Thr Met Trp Gly Gln Cys Gly Gly
465 470 475 480
Gln Gly Trp Thr Gly Pro Thr Ala Cys Gln Ser Pro Ser Thr Cys His
485 490 495
Val Ile Asn Asp Phe Tyr Ser Gln Cys Phe
500 505

<210> 70
<211> 536
<212> PRT
<213> Volvariella volvacea

<400> 70
Met Arg Ala Ser Leu Leu Ala Phe Ser Leu Asn Ser Ala Ala Gly Gln
1 5 10 15
Gln Ala Gly Thr Leu Gln Thr Lys Asn His Pro Ser Leu Thr Ser Gln
20 25 30
Lys Cys Arg Gln Gly Gly Cys Pro Gln Val Asn Thr Thr Ile Val Leu
35 40 45
Asp Ala Asn Trp Arg Trp Thr His Ser Thr Ser Gly Ser Thr Asn Cys
50 55 60
Tyr Thr Gly Asn Thr Trp Gln Ala Thr Leu Cys Pro Asp Gly Lys Thr
65 70 75 80
Cys Ala Ala Asn Cys Ala Leu Asp Gly Ala Asp Tyr Thr Gly Thr Tyr
85 90 95
Gly Val Thr Thr Ser Gly Asn Ser Leu Thr Leu Gln Phe Val Thr Gln
100 105 110
Ser Asn Val Gly Ala Arg Leu Gly Tyr Leu Met Ala Asp Asp Thr Thr
115 120 125
Tyr Gln Met Phe Asn Leu Leu Asn Gln Glu Phe Trp Phe Asp Val Asp
130 135 140
Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala Leu Tyr Phe Ser Ala
145 150 155 160
Met Ala Arg Thr Ala Ala Trp Met Pro Met Val Val Cys Ala Ser Thr
165 170 175
Pro Leu Ile Ser Thr Arg Arg Ser Thr Ala Arg Leu Leu Arg Leu Pro
180 185 190
Val Pro Pro Arg Ser Arg Tyr Gly Arg Gly Ile Cys Asp Ser Gln Cys
195 200 205
Pro Arg Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Val Gln Gly Trp
210 215 220
Gln Pro Ser Pro Asn Asp Thr Asn Ala Gly Thr Gly Asn Tyr Gly Ala
225 230 235 240
Cys Cys Asn Lys Met Asp Val Trp Glu Ala Asn Ser Ile Ser Thr Ala
245 250 255
Tyr Thr Pro His Pro Cys Thr Gln Arg Gly Leu Val Arg Cys Ser Gly
260 265 270
Thr Ala Cys Gly Gly Gly Ser Asn Arg Tyr Gly Ser Ile Cys Asp His
275 280 285
Asp Gly Leu Gly Phe Gln Asn Leu Phe Gly Met Gly Arg Thr Arg Val
290 295 300
Arg Ala Arg Val Gly Arg Val Lys Gln Phe Asn Arg Ser Ser Arg Val
305 310 315 320
Val Glu Pro Ile Ser Trp Thr Lys Gln Thr Thr Leu His Leu Gly Asn

```
                325                 330                 335
Leu Pro Trp Lys Ser Ala Asp Cys Asn Val Gln Asn Gly Arg Val Ile
            340                 345                 350
Gln Asn Ser Lys Val Asn Ile Pro Gly Met Pro Ser Thr Met Asp Ser
        355                 360                 365
Val Thr Thr Glu Phe Cys Asn Ala Gln Lys Thr Ala Phe Asn Asp Thr
    370                 375                 380
Phe Ser Phe Gln Gln Lys Gly Gly Met Ala Asn Met Ser Glu Ala Leu
385                 390                 395                 400
Arg Arg Gly Met Val Leu Val Leu Ser Ile Trp Asp Asp His Ala Ala
                405                 410                 415
Asn Met Leu Trp Leu Asp Ser Ile Thr Ser Ala Ala Ala Cys Arg Ser
            420                 425                 430
Thr Pro Ser Glu Val His Ala Thr Pro Leu Arg Glu Ser Gln Ile Arg
        435                 440                 445
Ser Ser His Ser Arg Gln Thr Arg Tyr Val Thr Phe Thr Asn Ile Lys
    450                 455                 460
Phe Gly Pro Phe Asn Ser Thr Gly Thr Thr Tyr Thr Thr Gly Ser Val
465                 470                 475                 480
Pro Thr Thr Ser Thr Ser Thr Gly Thr Thr Gly Ser Ser Thr Pro Pro
                485                 490                 495
Gln Pro Thr Gly Val Thr Val Pro Gln Gly Gln Cys Gly Gly Ile Gly
            500                 505                 510
Tyr Thr Gly Pro Thr Thr Cys Ala Ser Pro Thr Thr Cys His Val Leu
        515                 520                 525
Asn Pro Tyr Tyr Ser Gln Cys Tyr
    530                 535
```

<210> 71
<211> 463
<212> PRT
<213> Trichoderma longibrachiatum

<400> 71

```
Met Ala Pro Ser Ala Thr Leu Pro Leu Thr Thr Ala Ile Leu Ala Ile
 1               5                  10                  15
Gly Arg Leu Val Ala Ala Gln Gln Pro Gly Thr Ser Thr Pro Glu Val
            20                  25                  30
His Pro Lys Leu Thr Thr Tyr Lys Cys Thr Thr Ser Gly Gly Cys Val
        35                  40                  45
Ala Gln Asp Thr Ser Val Val Leu Asp Trp Asn Tyr Arg Trp Met His
    50                  55                  60
Asp Ala Asn Tyr Asn Ser Cys Thr Val Asn Gly Gly Val Asn Thr Thr
65                  70                  75                  80
Leu Cys Pro Asp Glu Ala Thr Cys Gly Lys Asn Cys Tyr Ile Glu Gly
                85                  90                  95
Val Asp Tyr Ala Ala Ser Gly Val Thr Ala Ser Gly Ser Thr Leu Thr
            100                 105                 110
Leu Asn Gln Tyr Met Pro Ser Ser Ser Gly Gly Tyr Ser Ser Val Ser
        115                 120                 125
Pro Arg Leu Tyr Leu Leu Gly Pro Asp Gly Glu Tyr Val Met Leu Lys
    130                 135                 140
Leu Asn Gly Gln Glu Leu Ser Phe Asp Val Asp Leu Ser Ala Leu Pro
145                 150                 155                 160
Cys Gly Glu Asn Gly Ser Leu Tyr Leu Ser Gln Met Asp Glu Asn Gly
                165                 170                 175
Gly Ala Asn Gln Tyr Asn Thr Ala Gly Ala Asn Tyr Gly Ser Gly Tyr
            180                 185                 190
Cys Asp Ala Gln Cys Pro Val Gln Thr Trp Arg Asn Gly Thr Leu Asn
        195                 200                 205
Thr Ser Gly Gln Gly Phe Cys Cys Asn Glu Met Asp Ile Leu Glu Gly
```

```
    210                 215                 220
Asn Ser Arg Ala Asn Ala Leu Thr Pro His Ser Cys Thr Ala Thr Ala
225                 230                 235                 240
Cys Asp Ser Ala Gly Cys Gly Phe Asn Pro Tyr Gly Ser Gly Tyr Pro
                245                 250                 255
Asn Tyr Phe Gly Pro Gly Asp Thr Val Asp Thr Ser Lys Thr Phe Thr
            260                 265                 270
Ile Ile Thr Gln Phe Asn Thr Asp Asn Gly Ser Pro Ser Gly Asn Leu
        275                 280                 285
Val Ser Ile Thr Arg Lys Tyr Arg Gln Asn Gly Val Asp Ile Pro Ser
    290                 295                 300
Ala Lys Pro Gly Gly Asp Thr Ile Ser Ser Cys Pro Ser Ala Ser Ala
305                 310                 315                 320
Tyr Gly Gly Leu Ala Thr Met Gly Lys Ala Leu Ser Ser Gly Met Val
                325                 330                 335
Leu Val Phe Ser Ile Trp Asn Asp Asn Ser Gln Tyr Met Asn Trp Leu
            340                 345                 350
Asp Ser Gly Arg Ala Gly Pro Cys Ser Ser Thr Glu Gly Asn Pro Ser
        355                 360                 365
Asn Ile Leu Ala Asn Asn Pro Gly Thr His Val Val Tyr Ser Asn Ile
    370                 375                 380
Arg Trp Gly Asp Ile Gly Ser Thr Thr Asn Ser Thr Gly Gly Asn Pro
385                 390                 395                 400
Pro Pro Pro Pro Pro Pro Ala Ser Ser Thr Thr Phe Ser Thr Thr Arg
                405                 410                 415
Arg Ser Ser Thr Thr Ser Ser Ser Pro Ser Cys Thr Gln Thr His Trp
            420                 425                 430
Gly Gln Cys Gly Gly Ile Gly Tyr Thr Gly Cys Lys Thr Cys Thr Ser
        435                 440                 445
Gly Thr Thr Cys Gln Tyr Gly Asn Asp Tyr Tyr Ser Gln Cys Leu
    450                 455                 460
```

<210> 72
<211> 459
<212> PRT
<213> Hypocrea jecorina

<400> 72

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Met Ala Pro Ser Val Thr Leu Pro Leu Thr Thr Ala Ile Leu Ala Ile
 1               5                  10                  15
Ala Arg Leu Val Ala Ala Gln Gln Pro Gly Thr Ser Thr Pro Glu Val
            20                  25                  30
His Pro Lys Leu Thr Thr Tyr Lys Cys Thr Lys Ser Gly Gly Cys Val
        35                  40                  45
Ala Gln Asp Thr Ser Val Val Leu Asp Trp Asn Tyr Arg Trp Met His
    50                  55                  60
Asp Ala Asn Tyr Asn Ser Cys Thr Val Asn Gly Gly Val Asn Thr Thr
65                  70                  75                  80
Leu Cys Pro Asp Glu Ala Thr Cys Gly Lys Asn Cys Phe Ile Glu Gly
                85                  90                  95
Val Asp Tyr Ala Ala Ser Gly Val Thr Thr Ser Gly Ser Ser Leu Thr
            100                 105                 110
Met Asn Gln Tyr Met Pro Ser Ser Ser Gly Gly Tyr Ser Ser Val Ser
        115                 120                 125
Pro Arg Leu Tyr Leu Leu Asp Ser Asp Gly Glu Tyr Val Met Leu Lys
    130                 135                 140
Leu Asn Gly Gln Glu Leu Ser Phe Asp Val Asp Leu Ser Ala Leu Pro
145                 150                 155                 160
Cys Gly Glu Asn Gly Ser Leu Tyr Leu Ser Gln Met Asp Glu Asn Gly
                165                 170                 175
Gly Ala Asn Gln Tyr Asn Thr Ala Gly Ala Asn Tyr Gly Ser Gly Tyr
```

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

<210> 73
<211> 416
<212> PRT
<213> Aspergillus oryzae

<400> 73

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Met Ile Trp Thr Leu Ala Pro Phe Val Ala Leu Leu Pro Leu Val Thr
 1               5                  10                  15
Ala Gln Gln Val Gly Thr Thr Ala Asp Ala His Pro Arg Leu Thr Thr
            20                  25                  30
Tyr Lys Cys Thr Ser Gln Asn Gly Cys Thr Arg Gln Asn Thr Ser Leu
        35                  40                  45
Val Leu Asp Ala Ala Thr His Phe Ile His Lys Lys Gly Thr Gln Thr
    50                  55                  60
Ser Cys Thr Asn Ser Asn Gly Leu Asp Thr Ala Ile Cys Pro Asp Lys
65                  70                  75                  80
Gln Thr Cys Ala Asp Asn Cys Val Val Asp Gly Ile Thr Asp Tyr Ala
                85                  90                  95
Ser Tyr Gly Val Gln Thr Lys Asn Asp Thr Leu Thr Leu Gln Gln Tyr
            100                 105                 110
Leu Gln Thr Gly Asn Ala Thr Lys Ser Leu Ser Pro Arg Val Tyr Leu
        115                 120                 125
Leu Ala Glu Asp Gly Glu Asn Tyr Ser Met Leu Lys Leu Leu Asn Gln
    130                 135                 140
Glu Phe Thr Phe Asp Val Asp Ala Ser Thr Leu Val Cys Gly Met Asn
```

145 150 155 160
Gly Ala Leu Tyr Leu Ser Glu Met Glu Ala Ser Gly Gly Lys Ser Ser
165 170 175
Leu Asn Gln Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ala Gln
180 185 190
Cys Tyr Thr Thr Pro Trp Ile Asn Gly Glu Gly Asn Thr Glu Ser Val
195 200 205
Gly Ser Cys Cys Gln Glu Met Asp Ile Trp Glu Ala Asn Ala Arg Ala
210 215 220
Thr Gly Leu Thr Pro His Pro Cys Asn Thr Thr Gly Leu Tyr Glu Cys
225 230 235 240
Ser Gly Ser Gly Cys Gly Asp Ser Gly Val Cys Asp Lys Ala Gly Cys
245 250 255
Gly Phe Asn Pro Tyr Gly Leu Gly Ala Lys Asp Tyr Tyr Gly Tyr Gly
260 265 270
Leu Lys Val Asn Thr Asn Glu Thr Phe Thr Val Val Thr Gln Phe Leu
275 280 285
Thr Asn Asp Asn Thr Thr Ser Gly Gln Leu Ser Glu Ile Arg Arg Leu
290 295 300
Tyr Ile Gln Asn Gly Gln Val Ile Gln Asn Ala Ala Val Thr Ser Gly
305 310 315 320
Gly Lys Thr Val Asp Ser Ile Thr Lys Asp Phe Cys Ser Gly Glu Gly
325 330 335
Ser Ala Phe Asn Arg Leu Gly Gly Leu Glu Glu Met Gly His Ala Leu
340 345 350
Gly Arg Gly Met Val Leu Ala Leu Ser Ile Trp Asn Asp Ala Gly Ser
355 360 365
Phe Met Gln Trp Leu Asp Gly Gly Ser Ala Gly Pro Cys Asn Ala Thr
370 375 380
Glu Gly Asn Pro Ala Leu Ile Glu Lys Leu Tyr Pro Asp Thr His Val
385 390 395 400
Lys Phe Ser Lys Ile Arg Trp Gly Asp Ile Gly Ser Thr Tyr Arg His
405 410 415

<210> 74
<211> 381
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus sequence

<400> 74
Met Phe Ile Leu Met Val Ala Ala Gln Gln Gly Thr Thr Ala Glu His
1 5 10 15
Pro Leu Thr Trp Gln Lys Cys Thr Gly Cys Thr Gly Ser Val Val Leu
20 25 30
Asp Ala Asn Trp Arg Trp Ile His Thr Gly Tyr Thr Asn Cys Tyr Thr
35 40 45
Gly Asn Trp Asp Ser Thr Leu Cys Pro Asp Thr Cys Ala Asn Cys Ala
50 55 60
Leu Asp Gly Ala Asp Tyr Ser Gly Thr Tyr Gly Ile Thr Thr Ser Gly
65 70 75 80
Ser Leu Ser Leu Phe Val Thr Gly Ser Asn Val Gly Ser Arg Val Tyr
85 90 95
Leu Met Ala Asp Asp Thr Tyr Gln Met Phe Leu Leu Asn Asn Glu Phe
100 105 110
Thr Phe Asp Val Asp Met Ser Asn Leu Pro Cys Gly Leu Asn Gly Ala
115 120 125
Leu Tyr Phe Val Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Asn
130 135 140

```
Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys Pro
145                 150                 155                 160
Arg Asp Leu Lys Phe Ile Asn Gly Ala Asn Val Glu Gly Trp Ser Ser
                165                 170                 175
Asn Gly Gly Gly Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn
            180                 185                 190
Ser Ile Ala Ala Phe Thr Pro His Pro Cys Thr Thr Gly Gln Thr Cys
        195                 200                 205
Gly Asp Cys Gly Gly Thr Tyr Ser Asp Arg Tyr Gly Cys Asp Asp Gly
    210                 215                 220
Cys Asp Phe Asn Tyr Arg Met Gly Asn Ser Phe Tyr Gly Gly Thr Val
225                 230                 235                 240
Asp Thr Thr Lys Lys Phe Thr Val Val Thr Gln Phe Val Thr Ser Gly
                245                 250                 255
Leu Glu Ile Arg Arg Phe Tyr Val Gln Asn Gly Lys Val Ile Asn Ile
            260                 265                 270
Pro Gly Val Gly Asn Ser Ile Thr Asp Glu Phe Cys Gln Lys Phe Gly
        275                 280                 285
Asp Ser Phe Gly Gly Leu Gln Met Gly Ala Leu Gly Met Val Leu Val
    290                 295                 300
Met Ser Ile Trp Asp Asp His Ala Ala Asn Met Leu Trp Leu Asp Ser
305                 310                 315                 320
Tyr Pro Thr Ser Pro Gly Arg Gly Ser Cys Thr Thr Ser Gly Val Pro
                325                 330                 335
Ala Val Glu Gln Pro Asn Val Val Phe Ser Asn Ile Lys Phe Gly Pro
            340                 345                 350
Ile Gly Ser Thr Tyr Gly Ser Ser Phe Gly Gln Cys Gly Gly Gly Tyr
        355                 360                 365
Thr Gly Thr Cys Ser Thr Cys Asn Tyr Tyr Ser Gln Cys
    370                 375                 380
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<210> 75
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide

<400> 75
ggtttggatc cggtcaccag g 21

<210> 76
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide

<400> 76
cgcgcctggt gaccggatcc aaaccgc 27

<210> 77
<211> 353
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus sequence

<400> 77
Thr Pro Gly Thr Thr Lys Glu Val His Pro Lys Leu Thr Thr Tyr Arg
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Cys Thr Lys Ala Gly Gly Cys Lys Gln Thr Asn Ser Ile Val Leu Asp
20 25 30
Ala Asn Trp Trp Ile His Asn Cys Gly Cys Gly Asp Trp Gly Gln Pro
35 40 45
Asn Ser Thr Leu Cys Pro Asp Glu Ser Cys Ala Lys Asn Cys Ile Leu
50 55 60
Glu Gly Met Ala Tyr Ala Asn Tyr Gly Val Thr Thr Ser Gly Asn Ser
65 70 75 80
Leu Arg Leu Gln Gln Leu Ile Pro Ser Asn Arg Leu Val Ser Pro Arg
85 90 95
Val Tyr Leu Leu Asp Thr Lys Lys Tyr Glu Met Leu His Leu Thr Gly
100 105 110
Asn Glu Phe Ser Phe Asp Val Asp Met Ser Lys Leu Pro Cys Gly Met
115 120 125
Asn Gly Ala Leu Tyr Leu Ser Glu Met Asp Asp Gly Gly Lys Ser Arg
130 135 140
Tyr Asn Thr Ala Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys Asp Ala Gln
145 150 155 160
Cys Pro Val Thr Pro Phe Ile Asn Gly Val Gly Asn Ile Glu Gly Gln
165 170 175
Gly Ser Cys Cys Asn Glu Met Asp Ile Trp Asx Ala Asn Ser Arg Ala
180 185 190
Thr Leu Pro His Pro Cys Thr Lys Gly Leu Tyr Leu Cys Glu Gly Asp
195 200 205
Glu Cys Gly Phe Gly Ile Cys Asp Lys Ala Gly Cys Gly Trp Asn Pro
210 215 220
Tyr Arg Ile Val Thr Phe Tyr Gly Gly Phe Val Asp Thr Thr Lys Lys
225 230 235 240
Phe Thr Val Val Thr Gln Phe Val Asn Lys Gly Leu Ile Ile His Arg
245 250 255
Phe Tyr Val Gln Gly Val Ile Glu Ser Ala Asn Asn Gly Pro Gly Asn
260 265 270
Ile Asn Asp Glu Tyr Cys Ala Thr Gly Ala Ser Tyr Glu Leu Gly Gly
275 280 285
Gln Met Gly Lys Ala Leu Ser Arg Gly Asn Val Leu Met Ser Ile Trp
290 295 300
Trp Asp Gln Gly Gly Asn Met Trp Leu Asp Ser Gly Val Ala Gly Pro
305 310 315 320
Cys Ser Thr Thr Glu Gly Pro Ser Asn Ile Val Val Gln Pro Asn Pro
325 330 335
Glu Val Thr Phe Ser Asn Ile Arg Trp Gly Glu Ile Gly Ser Thr Ser
340 345 350
Gln

<210> 78
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> SstII site of pUC219M vector

<400> 78
tgagccgagg cctcc 15

<210> 79
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic BgIII site at HindIII site

<400> 79
actctagact tcga 14

<210> 80
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic cloning linker

<400> 80
ccccaaattt gggcgccctt aa 22

CLAIMS

1. A variant CBH I cellulase, wherein said variant comprises an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 2 and an amino acid substitution at position P227(L/T/A) and wherein said variant has cellobiohydrolase activity and an increased melting temperature or thermal stability.

2. The variant CBH I cellulase according to claim 1, wherein said variant further comprises a substitution of one or more residues selected from the group consisting of S8P, Q17L, G22D, T41I, N49S, S57N, N64D, A68T, A77D, N89D, S92T, N103I, A112E, S113(T/N/D), E193V, S196T, M213I, L225F, T226A, R251A, Y252(A/Q), D257E, D259W, S278P, S279N, K286M, L288F, E295K, T296P, S297T, A299E, N301(R/K), E325K, T332(K/Y/H), F338Y, S342Y, F352L, T356L, Y371C, T380G, Y381D, V393G, R394A, S398T, V403D, S411F, G430F, G440R, T462I, T484S, Q487L and P491L in CBH I from Hypocrea jecorina (SEQ ID NO: 2).

3. The variant CBH I cellulose according to claim 1 or 2, further comprising a deletion at position T445 in CBH I from Hypocrea jecorina (SEQ ID NO: 2).

4. A variant CBH I cellulase, wherein said variant CBH I has cellobiohydrolase activity and an increased melting temperature or thermal stability and consists of the mutations selected from the group consisting of

i. P227L/E325K/Q487L;

ii. P227T/T484S/F352L;

iii. S8P/N49S/A68T/S113N/P227L;

iv. T41I/A112E/P227L/S278P/T296P;

v. S8P/N49S/A68T/A112E/P227L;

vi. S8P/T411/N49S/A68T/A112E/P227L;

vii. G22D/N49S/A68T/P227L/S278P/T296P;

viii. S8P/G22D/T411/N49S/A68T/N103I/S113N/P227L/S278P /T296P;

ix. G22D/N49S/A68T/N103I/S113N/P227L/S278P/T296P;

x. G22D/N49S/A68T/N103I/A112E/P227L/S278P/T296P;

xi. S8P/G22D/T41I/N49S/A68T/N103I/S113N/P227L/S278P/T296P /N301R;

in CBH I from Hypocrea jecorina (SEQ ID NO: 2).

5. A nucleic acid encoding a CBH I variant according to any one of claims 1 to 4.

6. A vector comprising a nucleic acid encoding a CBH I variant of claim 5.

7. A host cell transformed with the vector of claim 6.

8. A method of producing a CBH I variant according to any one of claims 1 to 4 comprising the steps of:

(a) culturing the host cell according to claim 7 in a suitable culture medium under suitable conditions to produce CBH I variant;

(b) obtaining said produced CBH I variant.

9. A detergent composition comprising a surfactant and a CBH I variant, wherein said CBH I variant comprises a CBH I variant according to any one of claims 1 to 4.

10. The detergent according to claim 9, wherein said detergent is a laundry detergent.

11. The detergent according to claim 9, wherein said detergent is a dish detergent.

12. A feed additive comprising a CBH I variant according to any one of claims 1 to 4.

13. A method of treating wood pulp comprising contacting said wood pulp with a CBH I variant according to any one of claims 1 to 4.

14. A method of converting biomass to sugars comprising contacting said biomass with a CBH I variant according to any one of claims 1 to 4.

CA 02495664 2005-02-15

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A

```
      GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
    1 CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
      ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
  101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
      ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
  201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
      GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
  301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
      ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
  401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC·TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
      ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
  501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
      ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
  601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
      ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
  701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
      ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
  801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
      AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
  901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
      ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
 1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
      ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
 1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC
      SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
 1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
      ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
 1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
      ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
 1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
```

FIG._1

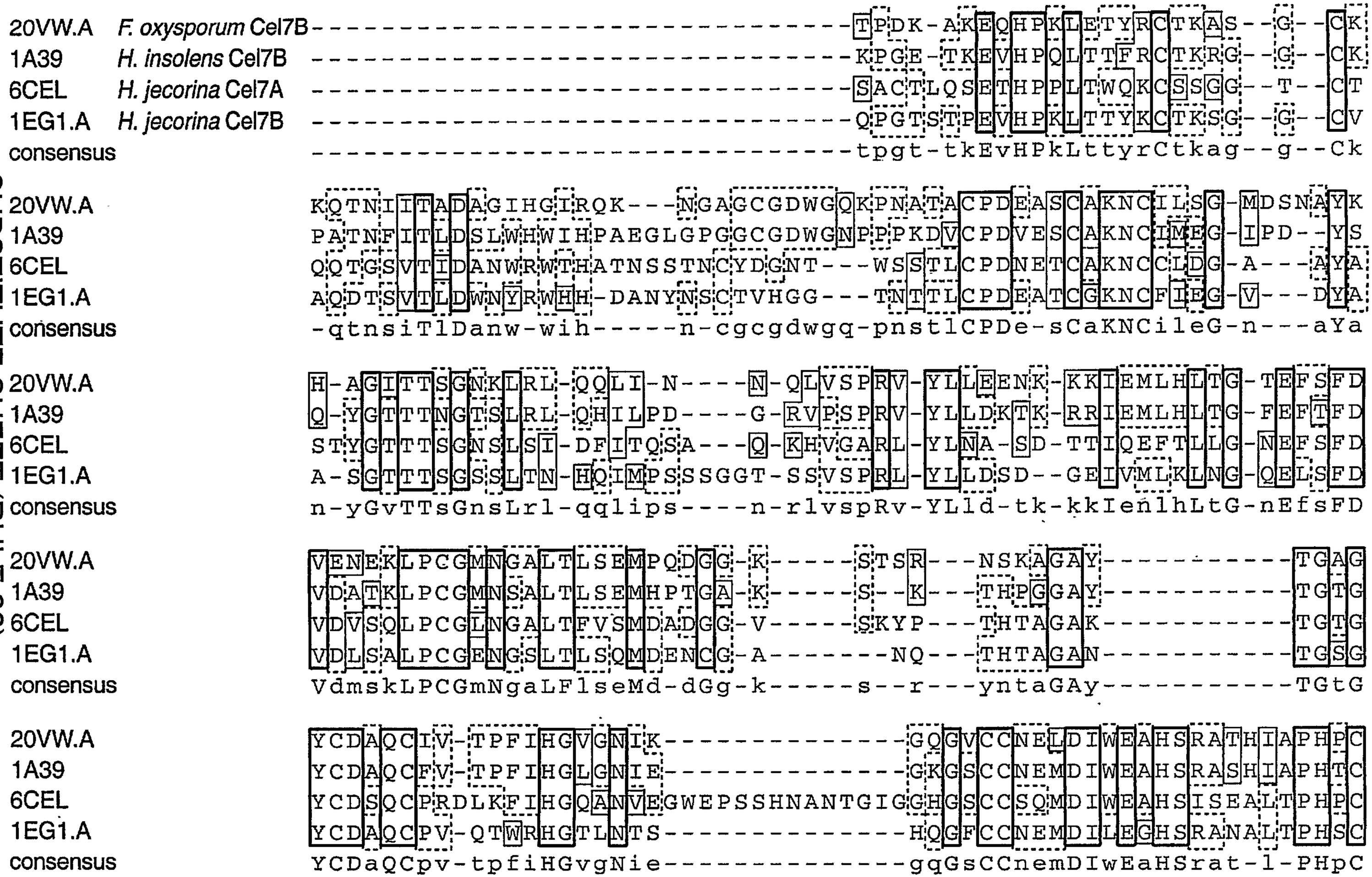
Cel7A Catalytic Module
(PDB Reference 8Cel)

***Hypocrea jecornia* Cel7A**
497 Amino Acids
1-431 in the Catalytic Module
432-461 in the Linker Region
462-497 in the Cellulose Binding Module
12 Disulfide Bonds--10 in the Catalytic Module
E212 and E217 are the Active Site Residues

FIG._2

FIG._3A

SUBSTITUTE SHEET (RULE 26)



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20VW.A    SKPGL-TGC-TGDECG---------SSGICDK-AGCGW-HHNRINTTDFIGR-GKQIKTD
1A39      HKKGL-TLC-EGEECA---------FEGVCDK-NGCGW-HNYRTNTTDYIGR-GEEFKTN
6CEL      TTVGQ-EIC-EGDGCGGTYSD--NRIGGTCDP-DGCDW-HPYRLGNTSPIGP-GSSFTLD
1EG1.A    TA-------------------------TACDS-AGCGP-HPIGSGIKSYIGP-G--DTTD
consensus tk-gl-ylc-egdecg---------f-giCDk-aGCgw-Hpyri-vt-fIG--G--f-vd

20VW.A    STRKFTTTSQFVAHK---QGD-LI-ELHRHIIQDNKVIESATVNISGPPK-IHFINDKIC
1A39      TLKPFTTVTQFLAHR---RGK-LE-KIHRPIVQDGKVIESFITNKEGVPI-THMIDDEFC
6CEL      TTKKLTTVTQFETS---------G-AINRYIVQNGVTFQQPNAE-LGSYS-GHELNDDIC
1EG1.A    TSKTFTIITQFHTDNGSPSGN-LV-SITRKIQQNGVDIPSAQP------G-GDTISSC--
consensus ttkkfTvvtQFv-nk----g--li--ihRfIvQ-g-viesan-n--g-p--gn-indeyc

20VW.A    AATG----ANE--YMRLGGTKQNGDANSRGNVLANSVWWSEGDFMAWLDQG---------
1A39      EATG----SRK--YMELGATQGNGEALTRGNVLANSIWWDQGGNMEWLDHG---------
6CEL      TAEEAEFGGSS--FSDKGGLTQFKKATSGGNVLVNSLWDDIIANMLWLDSTFPTNETS-S
1EG1.A    ---------PS--ASAYGGLATNGKALSSGNVLVFSIWNDNSQIMNWLDSG---------
consensus -atg----a-s--y-elGg--qmgkAlsrGNVL-mSiWwdqggnM-WLDsg---------

20VW.A    ----TAGPCDATEGDPKNITKTQPHPEVTFSNIRIGEIG-ST------------------
1A39      ----EAGPCAKGEGAPSNITQTEPFPEVTYTNLRWGEIG-ST------------------
6CEL      TPGATRGSCSTSSGVPAQVESQSPHAKVTFSNIKFGPIG-ST------------------
1EG1.A    ----NAGPCSSTEGNPSNILANHPHTHVVFSNIRWGDIG-ST------------------
consensus ----vaGpCstteG-Psniv-vqPnpeVtfsNirwGeIG-ST------------------

20VW.A    ----------------------------------------------------------S
1A39      ----------------------------------------------------------YQEL-Q
6CEL      ----------------------------------------------------------GNPS-G
1EG1.A    ----------------------------------------------------------T
consensus ----------------------------------------------------------sq
```

FIG._3B

C-α Trace of the Crystal Structures from the Catalytic Domains of Four Cel7 Homologues Aligned and Overlaid as Described.

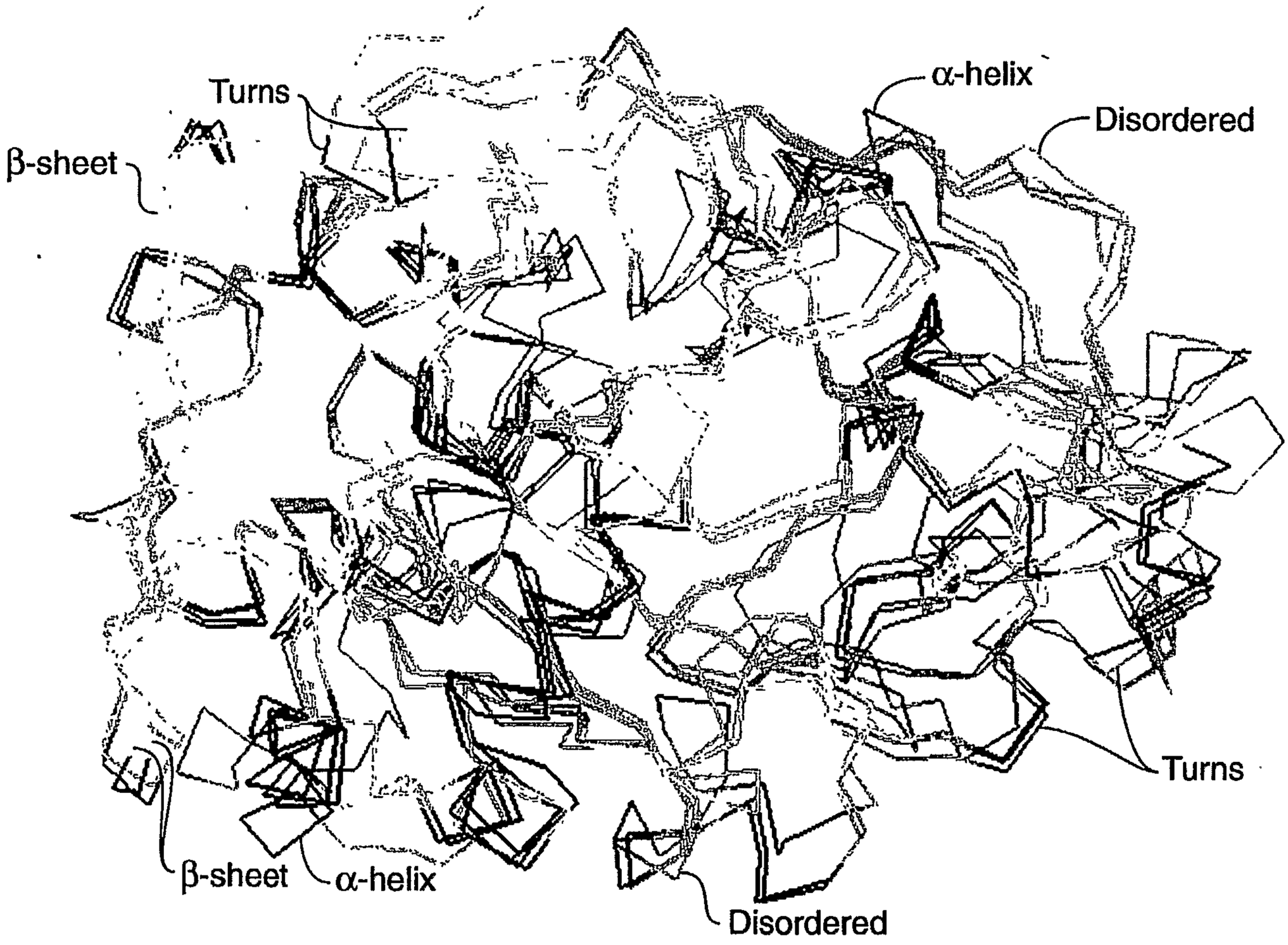

FIG._4

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant L225F

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
1   CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn·GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla PheThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTTTTACCCC CCACCCTTGC ACGACTGTCG
    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC
    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5A

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant S113D

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
1   CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA

    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG

    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG

    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaAspAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGGACG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT

    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA

    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC

    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG

    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG

    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC

    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG

    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG

    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC

    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG

    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG

    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5B

WO 2004/016760
PCT/US2003/025625
CA 02495666 2005-02-15

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant A77D

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
  1 CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
    ·AlaLysAsn CysCysLeuAsp GlyAlaAsp TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGA CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC
    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5C

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant L288F

```
      GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
   1  CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
      ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
 101  TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
      ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
 201  CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
      GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
 301  CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
      ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
 401  CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
      ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
 501  CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
      ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
 601  ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
      ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
 701  GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
      ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys PheThrVal ValThrGlnPhe GluThrSer GlyAlaIle
 801  CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTTACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
      AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
 901  AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
      ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001  AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
      ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101  GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC
      SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201  TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
      ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301  GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
      ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401  CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5D

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant L299F

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    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
1   CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA

    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG

    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG

    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT

    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA

    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC

    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG

    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG

    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyGluIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGAGATC

    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG

    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG

    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC

    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG

    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG

    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5E

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant N301K

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
  1 CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
    LysArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AAGCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC
    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5F

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant T356L

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
  1 CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaLeuSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTCTCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC
    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5G

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant G430F

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
1   CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA

    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG

    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG

    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT

    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA

    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC

    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG

    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG

    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC

    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG

    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG

    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC

    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrPhe AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCTTC AACCCTAGCG

    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG

    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5H

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant T246/Y371C

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
1   CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA

    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG

    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG

    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT

    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA

    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC

    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG

    ·GGlnGluIle CysGluGly AspGlyCysGly GlyCysTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGATGTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG

    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC

    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG

    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG

    ·AspAspTyr CysAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TGCGCCAACA TGCTGTGGCT·GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC

    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG

    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG

    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5I

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant T246A/R251A/Y252A

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
1   CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
    ·GGlnGluIle CysGluGly AspGlyCysGly GlyAlaTyr SerAspAsn AlaAlaGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAGCTTA CTCCGATAAC GCAGCTGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC
    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5J

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant T380G/Y381D/R394A

```
    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
  1 CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerGly AspProThrAsn GluThrSer SerThrPro GlyAlaValAla GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCGGC GACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGG CCGGAAGCTG CTCCACCAGC
    SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG
    ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG
    ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5K

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant T380G/Y381D/R394A with Residues 381 Through 393 Deleted

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    GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
  1 CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA
    ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG
    ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG
    GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT
    ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA
    ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC
    ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG
    ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgTyrGlyGly ThrCysAsp ProAspGly CysAspTrpAsn ProTyrArg·
701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGATATGGCG GCACTTGCGA TCCCGATGGC TGCGACTGGA ACCCATACCG
    ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC
    AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG
    ·GGluAlaGlu PheGlyGly SerSerPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTCTTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG
    ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerGly AspAlaGlySer CysSerThr SerSerGly ValProAlaGln ValGluSer GlnSerPro
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCGGC GACGCCGGAA GCTGCTCCAC CAGCTCCGGT GTCCCTGCTC AGGTCGAATC TCAGTCTCCC
    AsnAlaLysVal ThrPheSer AsnIleLys PheGlyProIle GlySerThr GlyAsnPro SerGlyGlyAsn ProProGly GlyAsnPro ProGlyThrThr·
1201 AACGCCAAGG TCACCTTCTC CAACATCAAG TTCGGACCCA TTGGCAGCAC CGGCAACCCT AGCGGCGGCA ACCCTCCCGG CGGAAACCCG CCTGGCACCA
    ·TThrThrArg ArgProAla ThrThrThrGly SerSerPro GlyProThr GlnSerHisTyr GlyGlnCys GlyGlyIle GlyTyrSerGly ProThrVal·
1301 CCACCACCCG CCGCCCAGCC ACTACCACTG GAAGCTCTCC CGGACCTACC CAGTCTCACT ACGGCCAGTG CGGCGGTATT GGCTACAGCG GCCCCACGGT
    ·CysAlaSer GlyThrThrCys GlnValLeu AsnProTyr TyrSerGlnCys Leu
1401 CTGCGCCAGC GGCACAACTT GCCAGGTCCT GAACCCTTAC TACTCTCAGT GCCTG
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FIG._5L

Amino Acid and Nucleic Acid Sequence of *Hypocrea jecorina* Cel7A Mutant Y252Q/D259W/S342Y

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     GlnSerAlaCys ThrLeuGln SerGluThr HisProProLeu ThrTrpGln LysCysSer SerGlyGlyThr CysThrGln GlnThrGly SerValValIle·
   1 CAGTCGGCCT GCACTCTCCA ATCGGAGACT CACCCGCCTC TGACATGGCA GAAATGCTCG TCTGGTGGCA CTTGCACTCA ACAGACAGGC TCCGTGGTCA

     ·IAspAlaAsn TrpArgTrp ThrHisAlaThr AsnSerSer ThrAsnCys TyrAspGlyAsn ThrTrpSer SerThrLeu CysProAspAsn GluThrCys·
 101 TCGACGCCAA CTGGCGCTGG ACTCACGCTA CGAACAGCAG CACGAACTGC TACGATGGCA ACACTTGGAG CTCGACCCTA TGTCCTGACA ACGAGACCTG

     ·AlaLysAsn CysCysLeuAsp GlyAlaAla TyrAlaSer ThrTyrGlyVal ThrThrSer GlyAsnSer LeuSerIleGly PheValThr GlnSerAla
 201 CGCGAAGAAC TGCTGTCTGG ACGGTGCCGC CTACGCGTCC ACGTACGGAG TTACCACGAG CGGTAACAGC CTCTCCATTG GCTTTGTCAC CCAGTCTGCG

     GlnLysAsnVal GlyAlaArg LeuTyrLeu MetAlaSerAsp ThrThrTyr GlnGluPhe ThrLeuLeuGly AsnGluPhe SerPheAsp ValAspValSer·
 301 CAGAAGAACG TTGGCGCTCG CCTTTACCTT ATGGCGAGCG ACACGACCTA CCAGGAATTC ACCCTGCTTG GCAACGAGTT CTCTTTCGAT GTTGATGTTT

     ·SGlnLeuPro CysGlyLeu AsnGlyAlaLeu TyrPheVal SerMetAsp AlaAspGlyGly ValSerLys TyrProThr AsnThrAlaGly AlaLysTyr·
 401 CGCAGCTGCC GTGCGGCTTG AACGGAGCTC TCTACTTCGT GTCCATGGAC GCGGATGGTG GCGTGAGCAA GTATCCCACC AACACCGCTG GCGCCAAGTA

     ·GlyThrGly TyrCysAspSer GlnCysPro ArgAspLeu LysPheIleAsn GlyGlnAla AsnValGlu GlyTrpGluPro SerSerAsn AsnAlaAsn
 501 CGGCACGGGG TACTGTGACA GCCAGTGTCC CCGCGATCTG AAGTTCATCA ATGGCCAGGC CAACGTTGAG GGCTGGGAGC CGTCATCCAA CAACGCGAAC

     ThrGlyIleGly GlyHisGly SerCysCys SerGluMetAsp IleTrpGlu AlaAsnSer IleSerGluAla LeuThrPro HisProCys ThrThrValGly·
 601 ACGGGCATTG GAGGACACGG AAGCTGCTGC TCTGAGATGG ATATCTGGGA GGCCAACTCC ATCTCCGAGG CTCTTACCCC CCACCCTTGC ACGACTGTCG

     ·GGlnGluIle CysGluGly AspGlyCysGly GlyThrTyr SerAspAsn ArgGlnGlyGly ThrCysAsp ProTrpGly CysAspTrpAsn ProTyrArg·
 701 GCCAGGAGAT CTGCGAGGGT GATGGGTGCG GCGGAACTTA CTCCGATAAC AGACAGGGCG GCACTTGCGA TCCCTGGGGC TGCGACTGGA ACCCATACCG

     ·LeuGlyAsn ThrSerPheTyr GlyProGly SerSerPhe ThrLeuAspThr ThrLysLys LeuThrVal ValThrGlnPhe GluThrSer GlyAlaIle
 801 CCTGGGCAAC ACCAGCTTCT ACGGCCCTGG CTCAAGCTTT ACCCTCGATA CCACCAAGAA ATTGACCGTT GTCACCCAGT TCGAGACGTC GGGTGCCATC

     AsnArgTyrTyr ValGlnAsn GlyValThr PheGlnGlnPro AsnAlaGlu LeuGlySer TyrSerGlyAsn GluLeuAsn AspAspTyr CysThrAlaGlu·
 901 AACCGATACT ATGTCCAGAA TGGCGTCACT TTCCAGCAGC CCAACGCCGA GCTTGGTAGT TACTCTGGCA ACGAGCTCAA CGATGATTAC TGCACAGCTG

     ·GGluAlaGlu PheGlyGly SerTyrPheSer AspLysGly GlyLeuThr GlnPheLysLys AlaThrSer GlyGlyMet ValLeuValMet SerLeuTrp·
1001 AGGAGGCAGA ATTCGGCGGA TCCTATTTCT CAGACAAGGG CGGCCTGACT CAGTTCAAGA AGGCTACCTC TGGCGGCATG GTTCTGGTCA TGAGTCTGTG

     ·AspAspTyr TyrAlaAsnMet LeuTrpLeu AspSerThr TyrProThrAsn GluThrSer SerThrPro GlyAlaValArg GlySerCys SerThrSer
1101 GGATGATTAC TACGCCAACA TGCTGTGGCT GGACTCCACC TACCCGACAA ACGAGACCTC CTCCACACCC GGTGCCGTGC GCGGAAGCTG CTCCACCAGC

     SerGlyValPro AlaGlnVal GluSerGln SerProAsnAla LysValThr PheSerAsn IleLysPheGly ProIleGly SerThrGly AsnProSerGly·
1201 TCCGGTGTCC CTGCTCAGGT CGAATCTCAG TCTCCCAACG CCAAGGTCAC CTTCTCCAAC ATCAAGTTCG GACCCATTGG CAGCACCGGC AACCCTAGCG

     ·GGlyAsnPro ProGlyGly AsnProProGly ThrThrThr ThrArgArg ProAlaThrThr ThrGlySer SerProGly ProThrGlnSer HisTyrGly·
1301 GCGGCAACCC TCCCGGCGGA AACCCGCCTG GCACCACCAC CACCCGCCGC CCAGCCACTA CCACTGGAAG CTCTCCCGGA CCTACCCAGT CTCACTACGG

     ·GlnCysGly GlyIleGlyTyr SerGlyPro ThrValCys AlaSerGlyThr ThrCysGln ValLeuAsn ProTyrTyrSer GlnCysLeu
1401 CCAGTGCGGC GGTATTGGCT ACAGCGGCCC CACGGTCTGC GCCAGCGGCA CAACTTGCCA GGTCCTGAAC CCTTACTACT CTCAGTGCCT G
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FIG._5M

pTEX2

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AAGCTTAAGG TGCACGGCCC ACGTGGCCAC TAGTACTTCT CGAGCTCTGT      50
ACATGTCCGG TCGCGACGTA CGCGTATCGA TGGCGCCAGC TGCAGGCGGC     100
CGCCTGCAGC CACTTGCAGT CCCGTGGAAT TCTCACGGTG AATGTAGGCC     150
TTTTGTAGGG TAGGAATTGT CACTCAAGCA CCCCCAACCT CCATTACGCC     200
TCCCCCATAG AGTTCCCAAT CAGTGAGTCA TGGCACTGTT CTCAAATAGA     250
TTGGGGAGAA GTTGACTTCC GCCCAGAGCT GAAGGTCGCA CAACCGCATG     300
ATATAGGGTC GGCAACGGCA AAAAAGCACG TGGCTCACCG AAAAGCAAGA     350
TGTTTGCGAT CTAACATCCA GGAACCTGGA TACATCCATC ATCACGCACG     400
ACCACTTTGA TCTGCTGGTA AACTCGTATT CGCCCTAAAC CGAAGTGCGT     450
GGTAAATCTA CACGTGGGCC CCTTTCGGTA TACTGCGTGT GTCTTCTCTA     500
GGTGCCATTC TTTTCCCTTC CTCTAGTGTT GAATTGTTTG TGTTGGAGTC     550
CGAGCTGTAA CTACCTCTGA ATCTCTGGAG AATGGTGGAC TAACGACTAC     600
CGTGCACCTG CATCATGTAT ATAATAGTGA TCCTGAGAAG GGGGGTTTGG.    650
AGCAATGTGG GACTTTGATG GTCATCAAAC AAAGAACGAA GACGCCTCTT     700
TTGCAAAGTT TTGTTTCGGC TACGGTGAAG AACTGGATAC TTGTTGTGTC     750
TTCTGTGTAT TTTTGTGGCA ACAAGAGGCC AGAGACAATC TATTCAAACA     800
CCAAGCTTGC TCTTTTGAGC TACAAGAACC TGTGGGGTAT ATATCTAGAG     850
TTGTGAAGTC GGTAATCCCG CTGTATAGTA ATACGAGTCG CATCTAAATA     900
CTCCGAAGCT GCTGCGAACC CGGAGAATCG AGATGTGCTG GAAAGCTTCT     950
AGCGAGCGGC TAAATTAGCA TGAAAGGCTA TGAGAAATTC TGGAGACGGC    1000
TTGTTGAATC ATGGCGTTCC ATTCTTCGAC AAGCAAAGCG TTCCGTCGCA    1050
GTAGCAGGCA CTCATTCCCG AAAAAACTCG GAGATTCCTA AGTAGCGATG    1100
GAACCGGAAT AATATAATAG GCAATACATT GAGTTGCCTC GACGGTTGCA    1150
ATGCAGGGGT ACTGAGCTTG GACATAACTG TTCCGTACCC CACCTCTTCT    1200
CAACCTTTGG CGTTTCCCTG ATTCAGCGTA CCCGTACAAG TCGTAATCAC    1250
TATTAACCCA GACTGACCGG ACGTGTTTTG CCCTTCATTT GGAGAAATAA    1300
TGTCATTGCG ATGTGTAATT TGCCTGCTTG ACCGACTGGG GCTGTTCGAA    1350
GCCCGAATGT AGGATTGTTA TCCGAACTCT GCTCGTAGAG GCATGTTGTG    1400
AATCTGTGTC GGGCAGGACA CGCCTCGAAG GTTCACGGCA AGGGAAACCA    1450
CCGATAGCAG TGTCTAGTAG CAACCTGTAA AGCCGCAATG CAGCATCACT    1500
GGAAAATACA AACCAATGGC TAAAAGTACA TAAGTTAATG CCTAAAGAAG    1550
TCATATACCA GCGGCTAATA ATTGTACAAT CAAGTGGCTA AACGTACCGT    1600
AATTTGCCAA CGGCTTGTGG GGTTGCAGAA GCAACGGCAA AGCCCCACTT    1650
CCCCACGTTT GTTTCTTCAC TCAGTCCAAT CTCAGCTGGT GATCCCCCAA    1700
TTGGGTCGCT TGTTTGTTCC GGTGAAGTGA AAGAAGACAG AGGTAAGAAT    1750
GTCTGACTCG GAGCGTTTTG CATACAACCA AGGGCAGTGA TGGAAGACAG    1800
TGAAATGTTG ACATTCAAGG AGTATTTAGC CAGGGATGCT TGAGTGTATC    1850
GTGTAAGGAG GTTTGTCTGC CGATACGACG AATACTGTAT AGTCACTTCT    1900
GATGAAGTGG TCCATATTGA AATGTAAGTC GGCACTGAAC AGGCAAAAGA    1950
TTGAGTTGAA ACTGCCTAAG ATCTCGGGCC CTCGGGCCTT CGGCCTTTGG    2000
GTGTACATGT TTGTGCTCCG GGCAAATGCA AAGTGTGGTA GGATCGAACA    2050
CACTGCTGCC TTTACCAAGC AGCTGAGGGT ATGTGATAGG CAAATGTTCA.   2100
GGGGCCACTG CATGGTTTCG AATAGAAAGA GAAGCTTAGC CAAGAACAAT    2150
AGCCGATAAA GATAGCCTCA TTAAACGGAA TGAGCTAGTA GGCAAAGTCA    2200
GCGAATGTGT ATATATAAAG GTTCGAGGTC CGTGCCTCCC TCATGCTCTC    2250
CCCATCTACT CATCAACTCA GATCCTCCAG GAGACTTGTA CACCATCTTT    2300
TGAGGCACAG AAACCCAATA GTCAACCGCG GTTTAGGCGC GCCAGCTCCG    2350
TGCGAAAGCC TGACGCACCG GTAGATTCTT GGTGAGCCCG TATCATGACG    2400
GCGGCGGGAG CTACATGGCC CCGGGTGATT TATTTTTTTT GTATCTACTT    2450
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FIG. 6A

pTEX2

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CTGACCCTTT TCAAATATAC GGTCAACTCA TCTTTCACTG GAGATGCGGC      2500
CTGCTTGGTA TTGCGATGTT GTCAGCTTGG CAAATTGTGG CTTTCGAAAA      2550
CACAAAACGA TTCCTTAGTA GCCATGCATT TTAAGATAAC GGAATAGAAG      2600
AAAGAGGAAA TTAAAAAAAA AAAAAAAACA AACATCCCGT TCATAACCCG      2650
TAGAATCGCC GCTCTTCGTG TATCCCAGTA CCAGTTTAAA CGGATCTCAA      2700
GCTTGCATGC AAAGATACAC ATCAATCGCA GCTGGGGTAC AATCATCCAT      2750
CATCCCAACT GGTACGTCAT AACAAAAATC GACAAGATGG AAAAAGAGGT      2800
CGCCTAAATA CAGCTGCATT CTATGATGCC GGGCTTTGGA CAAGAGCTCT      2850
TTCTCAGCTC CGTTTGTCCT CCCTCCCTTT TCCCCCTTCT TGCTAAATGC      2900
CTTTCTTTAC TTCTTTCTTC CCTTCCCTCC CCTATCGCAG CAGCCTCTCG      2950
GTGTAGGCTT TCCACGCTGC TGATCGGTAC CGCTCTGCCT CCTCTACGGG      3000
GTCTGAGGCC TTGAGGATGC CCCGGCCCAC AATGGCAATG TCGCTGCCGG      3050
CGATGCCAAT CAGCTTGTGC GGCGTGTTGT ACTGCTGGCC CTGGCCGTCT      3100
CCACCGACCG ATCCGTTGGT CTGCTGGTCC TCGTCTTCGG GGGGCAGCTG      3150
GCAGCCGGGC GTCATGTGGA TAAAGGCATC GTCGGGCTCG GTGTTGAGCG      3200
TCTCCTGCGA GATGAAGCCC ATGACAAAGT CCTTGTGCTC CCGGGCGGCC      3250
TCGACGCAGG CCTGCGTGTA CTCCTTGTTC ATGAAGTTGC CCTGGCTGGA      3300
CATTTGGGCG AGGATCAGGA GGCCTCGGCT CAGCGGCGCC TCCTCGATGC      3350
CCGGGAAGAG CGACTCGTCG CCCTCGGCGA TGGCCTTTGT TAACCGGGGC      3400
GAGGAGACGG ACTCGTACTG CTGGGTGACG GTGGTGATGG AGACGATGCT      3450
GCCCTTGCGG CCGTCGCCGG ACCGGTTCGA GTAGATGGGC TTGTCCAGGA      3500
CGCCAATGGA GCCCATGCCG TTGACGGCGC CGGCGGGCTC GGCGTCCCTG      3550
GAGTCGGCGT CGTCGTCAAA CGAGTCCATG GTGGGCGTGC CGACGGTGAC      3600
GGACGTCTTG ACCTCGCAGG GGTAGCGCTC GAGCCAGCGC TTGGCGCCCT      3650
GGGCCAGCGA GGCCACCGAC GCCTTGCCGG GCACCATGTT GACGTTGACA      3700
ATGTGCGCCC AGTCGATGAT GCGCGCCGAC CCGCCCGTGT ACTGCAGCTC      3750
GACGGTGTGG CCAATGTCGC CAAACTTGCG GTCCTCGAAG ATGAGGAAGC      3800
CGTGCTTGCG CGCCAGCGAC GCCAGCTGGG CTCCCGTGCC CGTCTCCGGG      3850
TGGAAGTCCC AGCCCGAGAC CATGTCGTAG TGCGTCTTGA GCACGACAAT      3900
CGACGGGCCA ATCTTGTCGG CCAGGTACAG CAGCTCGCGC GCTGTCGGCA      3950
CGTCGGCGCT CAGGCACAGG TTGGACGCCT TGAGGTCCAT GAGCTTGAAC      4000
AGGTAAGCCG TCAGCGGGTG CGTCGCCGTC TCGCTCCTGG CCGCGAAGGT      4050
GGCCTTGAGC GTCGGGTGTG GTGCCATGGC TGATGAGGCT GAGAGAGGCT      4100
GAGGCTGCGG CTGGTTGGAT AGTTTAACCC TTAGGGTGCC GTTGTGGCGG      4150
TTTAGAGGGG GGGAAAAAAA AGAGAGAGAT GGCACAATTC TGCTGTGCGA      4200
ATGACGTTGG AAGCGCGACA GCCGTGCGGG AGGAAGAGGA GTAGGAACTG      4250
TCGGCGATTG GGAGAATTTC GTGCGATCCG AGTCGTCTCG AGGCGAGGGA      4300
GTTGCTTTAA TGTCGGGCTC GTCCCCTGGT CAAAATTCTA GGGAGCAGCG      4350
CTGGCAACGA GAGCAGAGCA GCAGTAGTCG ATGCTAGAAA TCGATAGATC      4400
CACGATGCCA AAAAGCTTGT TCATTTCGGC TAGCCCGTGA TCCTGGCGCT      4450
TCTAGGGCTG AAACTGTGTT GTTAATGTAT TATTGGCTGT GTAACTGACT      4500
TGAATGGGGA ATGAGGAGCG CGATGGATTC GCTTGCATGT CCCCTGGCCA      4550
AGACGAGCCG CTTTGGCGGT TTGTGATTCG AAGGTGTGTC AGCGGAGGCG      4600
CCAGGGCAAC ACGCACTGAG CCAGCCAACA TGCATTGCTG CCGACATGAA      4650
TAGACACGCG CCGAGCAGAC ATAGGAGACG TGTTGACTGT AAAAATTCTA      4700
CTGAATATTA GCACGCATGG TCTCAATAAG AGCAATAGGA ATGCTTGCCA      4750
ATCATAAGTA CGTATGTGCT TTTTCCTGCA AATGGTACGT ACGGACAGTT      4800
CATGTTGTCT GTCATCCCCC ACTCAGGCTC TCATGATCAT TTTATGGGAC      4850
TGGGGTTTTG CTGACTGAAT GGATTCAGCC GCACGAAACA AATTGGGGGC      4900
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FIG. 6B

pTEX2

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CATGCAGAAG GGAAGCCCCC CCAGCCCCCT GTTCATAATT TGTTAAGAGT     4950
CGGAGAGCTG CCTAGTATGA AGCAGCAATT GATAACGTTG ACTTTGCGCA     5000
TGAGCTCTGA AGCCGGGCAT ATGTATCACG TTTCTGCCTA GAGCCGCACG     5050
GGACCCAAGA AGCTCTTGTC ATAAGGTATT TATGAGTGTT CAGCTGCCAA     5100
CGCTGGTTCT ACTTTGGCTC AACCGCATCC CATAAGCTGA ACTTTGGGAG     5150
CTGCCAGAAT GTCTCTTGAT GTACAGCGAT CAACAACCGT GCGCCGGTCG     5200
ACAACTGTTC ACCGATCAGG GACGCGAAGA GGACCCAATC CCGGTTAACG     5250
CACCTGCTCC GAAGAAGCAA AAGGGCTATG AGGTGGTGCA GCAAGGAATC     5300
AAAGAGCTCT ATCCACTTGA CAAGGCCAAT GTCGCTCCCG ATCTGGAGTA     5350
AGTCAACCCT GAAGTGGAAG TTTGCTTCTC TGATTAGTAT GTAGCATCGT     5400
GTTTGTCCCA GGACTGGGTG CAAATCCCGA AGACAGCTGG AAGTCCAGCA     5450
AGACCGACTT CAATTGGACC ACGCATACAG ATGGCCTCCA GAGAGACTTC     5500
CCAAGAGCTC GGTTGCTTCT GTATATGTAC GACTCAGCAT GGACTGGCCA     5550
GCTCAAAGTA AAACAATTCA TGGGCAATAT CGCGATGGGG CTCTTGGTTG     5600
GGCTGAGGAG CAAGAGAGAG GTAGGCCAAA CGCCAGACTC GAACCGCCAG     5650
CCAAGTCTCA AACTGACTGC AGGCGGCCGC CATATGCATC CTAGGCCTAT     5700
TAATATTCCG GAGTATACGT AGCCGGCTAA CGTTAACAAC CGGTACCTCT     5750
AGAACTATAG CTAGCATGCG CAAATTTAAA GCGCTGATAT CGATCGCGCG     5800
CAGATCCATA TATAGGGCCC GGGTTATAAT TACCTCAGGT CGACGTCCCA     5850
TGGCCATTCG AATTCGTAAT CATGGTCATA GCTGTTTCCT GTGTGAAATT     5900
GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT     5950
AAAGCCTGGG GTGCCTAATG AGTGAGCTAA CTCACATTAA TTGCGTTGCG     6000
CTCACTGCCC GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGCATTAAT     6050
GAATCGGCCA ACGCGCGGGG AGAGGCGGTT TGCGTATTGG GCGCTCTTCC     6100
GCTTCCTCGC TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC     6150
GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG     6200
ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC     6250
CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA     6300
CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG     6350
GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT     6400
CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC     6450
GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG     6500
TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG     6550
CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT     6600
AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA     6650
GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC     6700
TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC     6750
AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA     6800
CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA     6850
AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC     6900
TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA     6950
AAAGGATCTT CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA     7000
ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT     7050
CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG     7100
CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT     7150
GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA     7200
TTTATCAGCA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC     7250
CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT     7300
AGAGTAAGTA GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC     7350
```

FIG. 6C

pTEX2

```
TACAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT      7400
CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA      7450
AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC      7500
CGCAGTGTTA TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG      7550
TCATGCCATC CGTAAGATGC TTTTCTGTGA CTGGTGAGTA CTCAACCAAG      7600
TCATTCTGAG AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC      7650
AATACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA GTGCTCATCA      7700
TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG      7750
AGATCCAGTT CGATGTAACC CACTCGTGCA CCCAACTGAT CTTCAGCATC      7800
TTTTACTTTC ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG      7850
CCGCAAAAAA GGGAATAAGG GCGACACGGA AATGTTGAAT ACTCATACTC      7900
TTCCTTTTTC AATATTATTG AAGCATTTAT CAGGGTTATT GTCTCATGAG      7950
CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA GGGGTTCCGC      8000
GCACATTTCC CCGAAAAGTG CCACCTGACG TCTAAGAAAC CATTATTATC      8050
ATGACATTAA CCTATAAAAA TAGGCGTATC ACGAGGCCCT TTCGTCTCGC      8100
GCGTTTCGGT GATGACGGTG AAAACCTCTG ACACATGCAG CTCCCGGAGA      8150
CGGTCACAGC TTGTCTGTAA GCGGATGCCG GGAGCAGACA AGCCCGTCAG      8200
GGCGCGTCAG CGGGTGTTGG CGGGTGTCGG GGCTGGCTTA ACTATGCGGC      8250
ATCAGAGCAG ATTGTACTGA GAGTGCACCA TAAAATTGTA AACGTTAATA      8300
TTTTGTTAAA ATTCGCGTTA AATTTTTGTT AAATCAGCTC ATTTTTTAAC      8350
CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGCCCGA      8400
GATAGGGTTG AGTGTTGTTC CAGTTTGGAA CAAGAGTCCA CTATTAAAGA      8450
ACGTGGACTC CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC      8500
CCACTACGTG AACCATCACC CAAATCAAGT TTTTTGGGGT CGAGGTGCCG      8550
TAAAGCACTA AATCGGAACC CTAAAGGGAG CCCCCGATTT AGAGCTTGAC      8600
GGGGAAAGCC GGCGAACGTG GCGAGAAAGG AAGGGAAGAA AGCGAAAGGA      8650
GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC GCGTAACCAC      8700
CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTAC TATGGTTGCT      8750
TTGACGTATG CGGTGTGAAA TACCGCACAG ATGCGTAAGG AGAAAATACC      8800
GCATCAGGCG CCATTCGCCA TTCAGGCTGC GCAACTGTTG GGAAGGGCGA      8850
TCGGTGCGGG CCTCTTCGCT ATTACGCCAG CTGGCGAAAG GGGGATGTGC      8900
TGCAAGGCGA TTAAGTTGGG TAACGCCAGG GTTTTCCCAG TCACGACGTT      8950
GTAAAACGAC GGCCAGTGCC                                       8970
```

FIG._6D

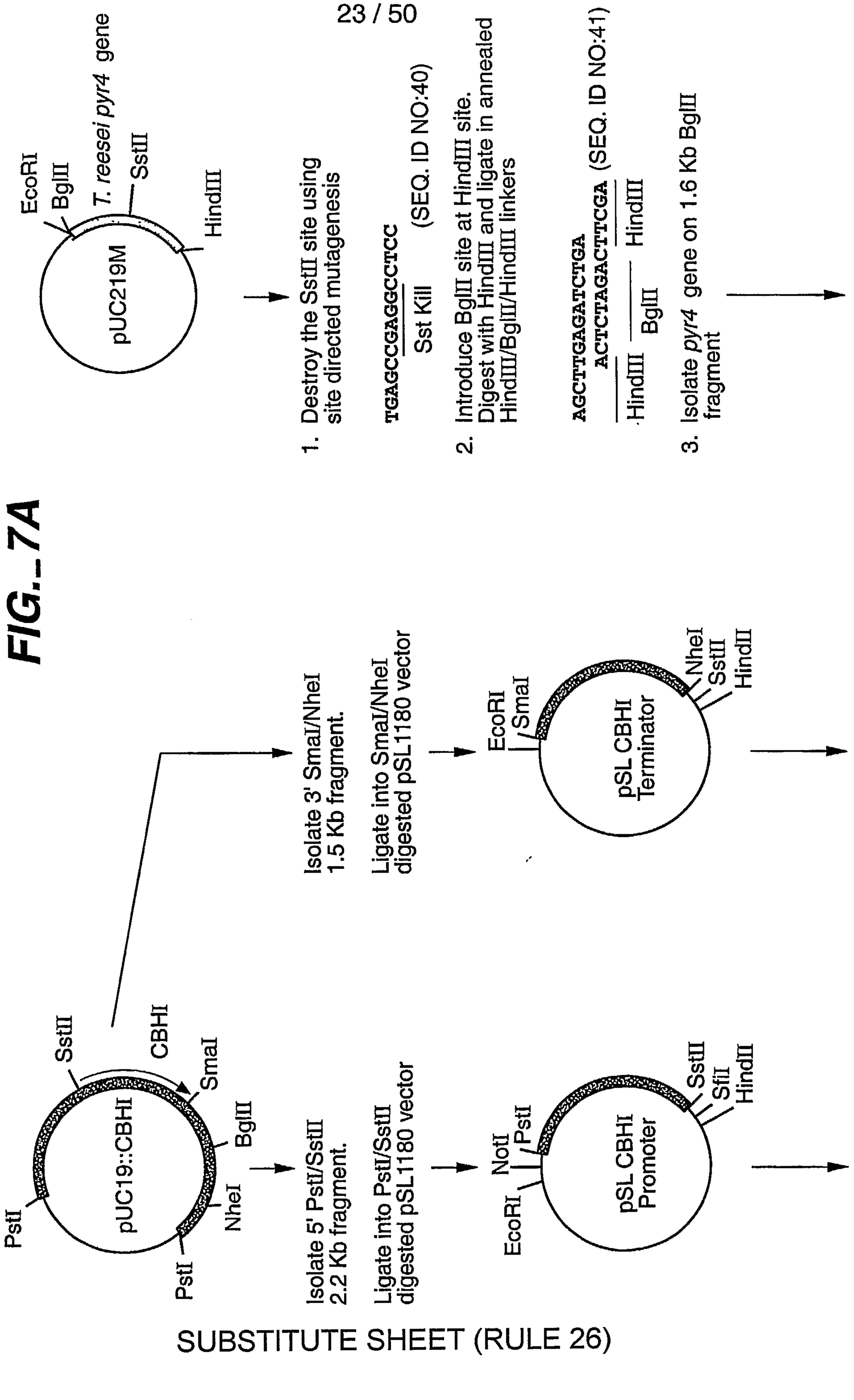

pUC219
EcoRI
BglII
T. reesei pyr4 gene
SstII
HindIII
1. Destroy the SstII site using site directed mutagenesis
TGAGCCGAGGCCTCC
Sst KIII
(SEQ. ID NO:40)
2. Introduce BglII site at HindIII site.
Digest with HindIII and ligate in annealed HindIII/BglII/HindIII linkers
AGCTTGAGATCTCGA
ACTCTAGAGCTTCGA
(SEQ. ID NO:41)
HindIII
BglII
HindIII
3. Isolate pyr4 gene on 1.6 Kb BglII fragment
Isolate 3' SmaI/NheI 1.5 Kb fragment.
Ligate into SmaI/NheI digested pSL1180 vector
EcoRI
SmaI
pSL CBHI Terminator
NheI
SstII
HindII
Isolate 5' PstI/SstII 2.2 Kb fragment.
Ligate into PstI/SstII digested pSL1180 vector
NotI
PstI
EcoRI
pSL CBHI Promoter
SstII
SfiI
HindII
SstII
CBHI
SmaI
BglII
NheI
PstI
PstI
pUC19::CBHI

FIG._7A

FIG._7A FIG._7B

FIG._7

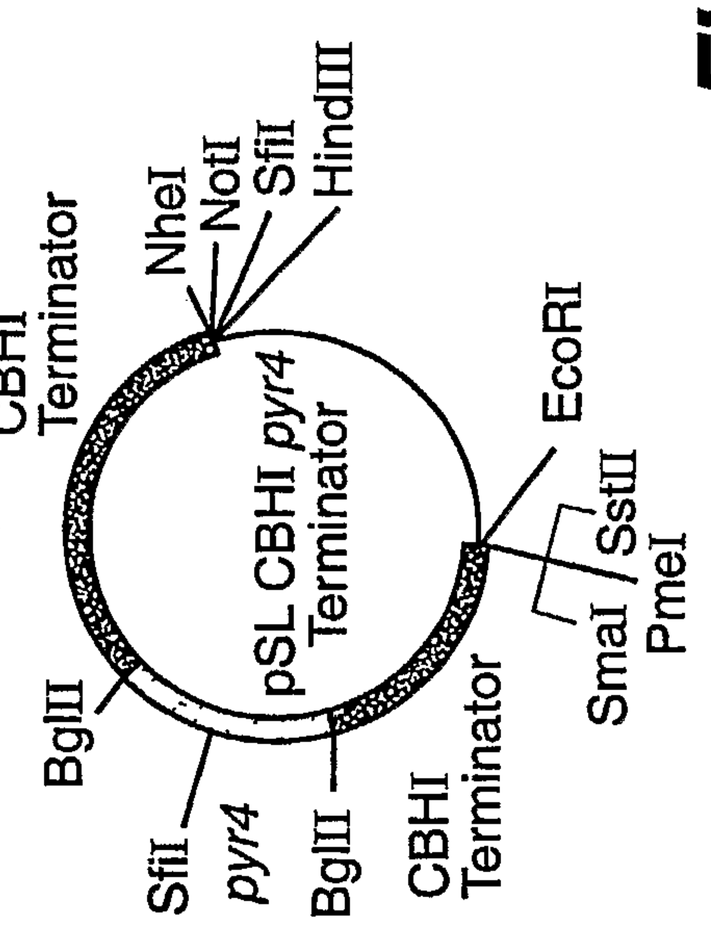
1. Destroy SstII site by:
   a) digestion with SstII
   b) Fill in the overhangs with T4 DNA polymerase
   c) Ligate back together.
2. Insert synthetic "cloning" linker upstream of terminator.
   a) Digest with EcoRI and SmaI
   b) Anneal synthetic linker and ligate into vector

GGGGTTTAAACCCGCGGG
CCCCAAATTTGGGCGCCCTTAA (SEQ. ID NO:39)

SmaI EcoRI
PmeI SstII

3. Digest with BglII

Ligate together



1. Digest with SstI to completion
   Then partial digest with SfiI
2. Isolate 3.2 Kb SstII/SfiI fragment

Ligate

Isolate vector fragment.

Digest with SstII and SfiI.

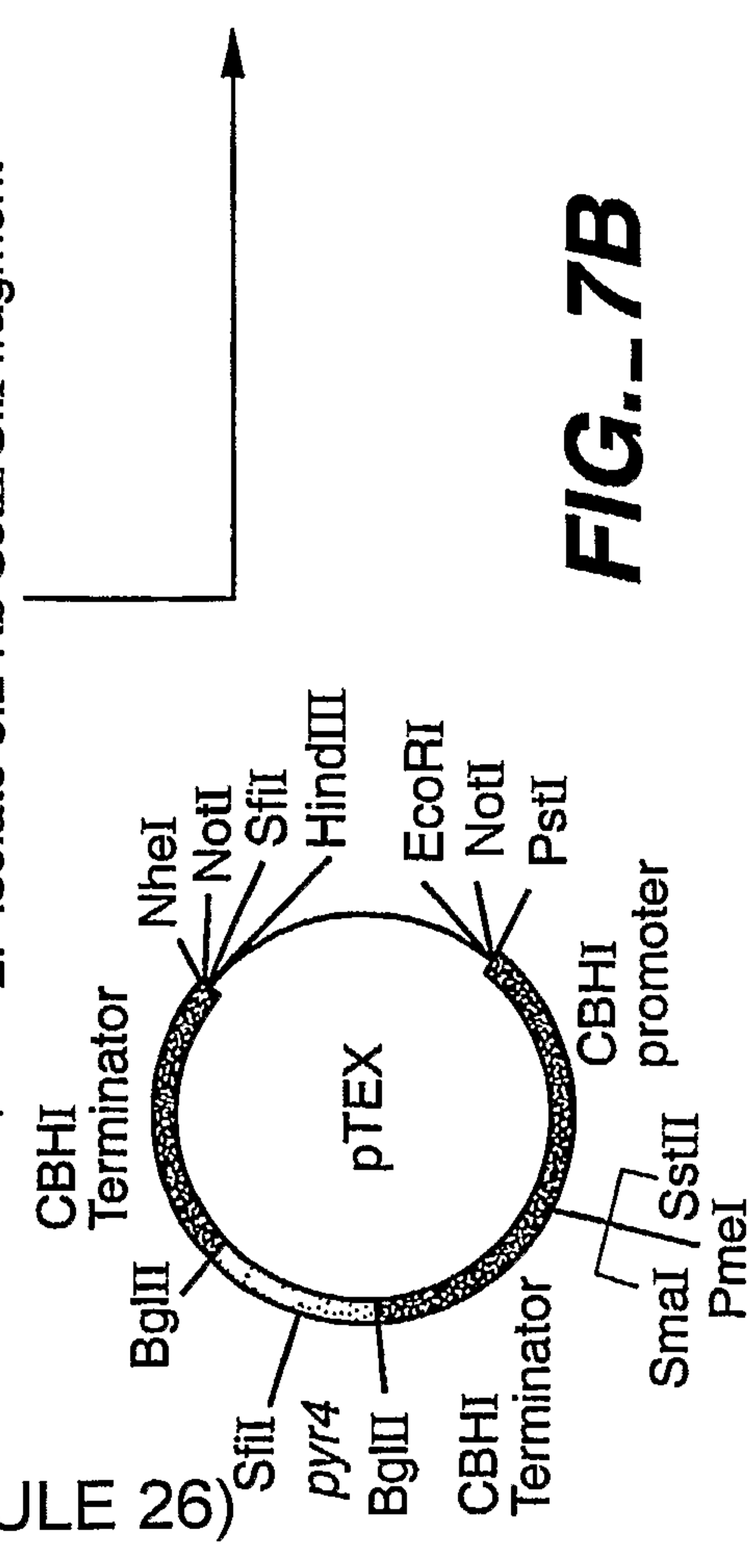


FIG._7B

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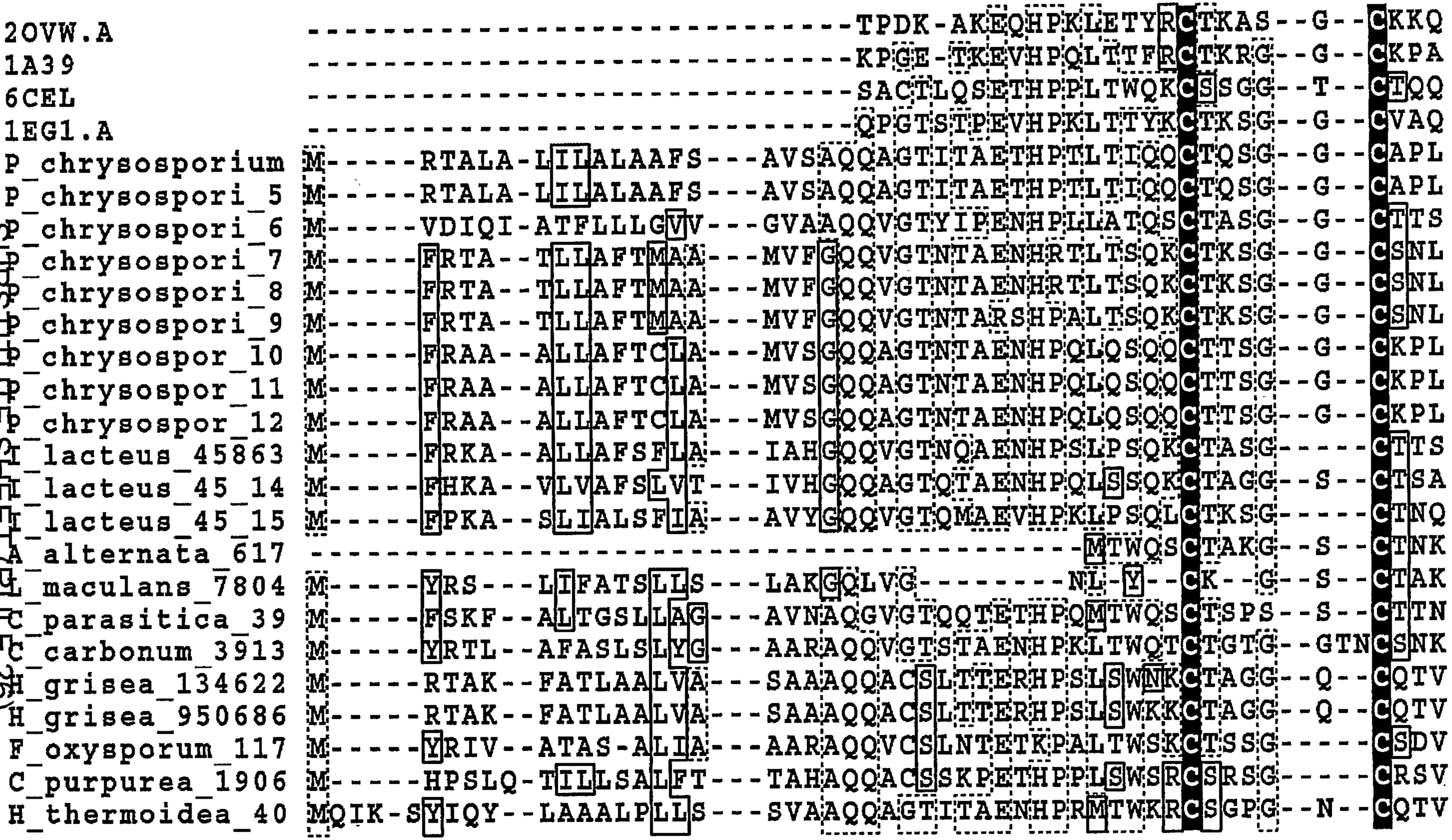

FIG._8A-1

```
H_thermoidea_74      MQIK-SYIQY--LAAALPLLS---SVAAQQAGTITAENHPRMTWKRCSGPG--N--CQTV
L_maculans_7_26      M-----LSASK-AAAILAFCA--HTASAWVVGDQQTETHPKLNWQRCTGKGRSS--CTNV
N_crassa_729649      M-----RASL--LAFSLA-AA---VAGGQQAGTLTAKRHPSLTWQKCTRGG-----CPTL
A_aculeatus_391      M-VD-SFSIYK-TALLLSMLA---TSNAQQVGTYTAETHPSLTWQTCSGSG--S--CTTT
A_niger_6164684      M--S-SFQIYR-AALLLSILA---TANAQQVGTYTTETHPSLTWQTCTSDG--S--CTTN
P_janthinellum_      MKGSISYQIYKGALLLSALLN---SVSAQQVGTLTAETHPALTWSKCTA-G--X--CSQV
A_niger_6164682      M-----HQRA--LLFS-ALLT---AVRAQQAGTLTEEVHPSLTWQKCTSEG--S--CTEQ
H_ceramica_1218      M-----YRKL--AVIS-AFLA---TARAQSACTLQSETHPPLTWQKCSSGG--T--CTQQ
H_jecorina_2238      ------------------------------ESACTLQSETHPPLTWQKCSSGG--T--CTQQ
T_viride_121854      M-----YQKL--ALIS-AFLA---TARAQSACTLQAETHPPLTWQKCSSGG--T--CTQQ
T_viride_406299      M-----YQKL--ALIS-AFLA---TARAQSACTLQAETHPPLTWQKCSSGG--T--CTQQ
T_harzianum_710      M-----YRKL--AVIS-AFLA---AARAQQVCTQQAETHPPLTWQKCTASG-----CTPQ
A_bisporus_3913      M-----FPRS--ILLALSLTA---VALGQQVGTNMAENHPSLTWQRCTSSG-----CQNV
V_volvacea_5231      M------RA---SLLAFS-LN---SAAGQQAGTLQTKNHPSLTSQKCRQGG-----CPQV
T_longibrachiat      M-----APSAT-LPLTTAILAIGRLVAAQQPGTSTPEVHPKLTTYKCTTSG--G--CVAQ
H_jecorina_1217      M-----APSVT-LPLTTAILAIARLVAAQQPGTSTPEVHPKLTTYKCTKSG--G--CVAQ
A_oryzae_246737      M-----IWTL--APFV-ALLP---LVTAQQVGT-TADAHPRLTTYKCTSQN--G--CTRQ
Consensus            m-----f------il---mva------aqq-gt-tae-hp-ltwqkCt--g-----Ct--
```

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FIG._8A-2

SUBSTITUTE SHEET (RULE 26)

```
2OVW.A                TNYIVADAGIHGIRQK---NGAGCGDWGQKPNATACPDEASCAKNCILSG-MDSNAYK-N
1A39                  TNFIVLDSLWHWIHRAEGLGPGGCGDWGNPPPKDVCPDVESCAKNCIMEG-IPD--YS-Q
6CEL                  TGSVVIDANWRWTHATNSSTNCYDGNT---WSSTLCPDNETCAKNCCLDG-A---AYAST
1EG1.A                DTSVVLDWNYRWMH-DANYNSCTVNGG---VNTTLCPDEATCGKNCFIEG-V---DYA-A
P_chrysosporium       TTKVVLDVNWRWIHSTTGYTNCYSGNT---WDAILCPDPVTCAANCALDG-A---DYTGT
P_chrysospori_5       TTKVVLDVNWRWIHSTTGYTNCYSGNT---WDAILCPDPVTCAANCALDG-A---DYTGT
P_chrysospori_6       SSKIVLDANRRWIHSTLGTTSCLTANG---WDPTLCPDGITCANYCALDG-V---SYSST
P_chrysospori_7       NTKIVLDANWRWLHSTSGYTNCYTGNQ---WDATLCPDGKTCAANCALDG-A---DYTGT
P_chrysospori_8       NTKIVLDANWRWLHSTSGYTNCYTGNQ---WDATLCPDGKTCAANCALDG-A---DYTGT
P_chrysospori_9       NTKIVLDANWRWLHSTSGYTNCYTGNQ---WDATLCPDGKTCAANCALDG-A---DYTGT
P_chrysospor_10       STKVVLDSNWRWVHSTSGYTNCYTGNE---WNTSLCPDGKTCAANCALDG-A---DYSGT
P_chrysospor_11       STKVVLDSNWRWVHSTSGYTNCYTGNE---WDTSLCPDGKTCAANCALDG-A---DYSGT
P_chrysospor_12       STKVVLDSNWRWVHSTSGYTNCYTGNE---WDTSLCPDGKTCAANCALDG-A---DYSGT
I_lacteus_45863       STSVVLDANWRWVHTTTGYTNCYTGQT---WDASICPDGVTCAKACALDG-A---DYSGT
I_lacteus_45_14       STSVVLDSNWRWVHTTSGYTNCYTGNT---WDASICSDPVSCAQNCALDG-A---DYAGT
I_lacteus_45_15       NTAVVLDANWRWLHTTSGYTNCYTGNS---WDATLCPDATTCAQNCAVDG-A---DYSGT
A_alternata_617       NGKIVIDANWRWLHKKEGYDNCYTGNE---WDATACPDNKACAANCAVDG-A---DYSGT
L_maculans_7804       NGKVVIDANWRWLHVKGGYTNCYTGNE---WNATACPDNKSCATNCAIDG-A---DYR-R
C_parasitica_39       QGEVVIDSNWRWVHDKDGYVNCYTGNT---WNTTLCPDDKTCAANCVLDG-A---DYSST
C_carbonum_3913       SGSVVLDSNWRWAHNVGGYTNCYTGNS---WSTQYCPDGDSCTKNCAIDG-A---DYSGT
H_grisea_134622       QASITLDSNWRWTHQVSGSTNCYTGNK---WDTSICTDAKSCAQNCCVDG-A---DYTST
H_grisea_950686       QASITLDSNWRWTHQVSGSTNCYTGNK---WDTSICTDAKSCAQNCCVDG-A---DYTST
F_oxysporum_117       KGSVVIDANWRWTHQTSGSTNCYTGNK---WDTSICTDGKTCAEKCCLDG-A---DYSGT
C_purpurea_1906       QGAVTVDANWLWT-TVDGSQNCYTGNR---WDTSICSSEKTCSESCCIDG-A---DYAGT
H_thermoidea_40       QGEVVIDANWRWLHN-NG-QNCYEGNK---WTSQ-CSSATDCAQRCALDG-A---NYQST
```

FIG._8B-1

```
H_thermoidea_74     QGEVVIDANWRWLHN-NG-QNCYEGNK---WTSQ-CSSATDCAQRCALDG-A---NYQST
L_maculans_7_26     NGEVVIDANWRWLAHRSGYTNCYTGSE---WNQSACPNNEACTKNCAIEG-S---DYAGT
N_crassa_729649     NTTMVLDANWRWTHATSGSTKCYTGNK---WQATLCPDGKSCAANCALDG-A---DYTGT
A_aculeatus_391     SGSVVIDANWRWVHEVGGYTNCYSGNT---WDSSICSTDTTCASECALEG-A---TYEST
A_niger_6164684     DGEVVIDANWRWVHSTSSATNCYTGNE---WDTSICTDDVTCAANCALDG-A---TYEAT
P_janthinellum_     SGSVVIDANWPXVHSTSGSTNCYTGNT---WDATLCPDDVTCAANCAVDG-A---RRQ-H
A_niger_6164682     SGSVVIDSNWRWTHSVNDSTNCYTGNT---WDATLCPDDETCAANCALDG-A---DYEST
H_ceramica_1218     TGSVVIDANWRWTHATNSSTNCYDGNT---WSSTLCPDNETCAKNCCLDG-A---AYAST
H_jecorina_2238     TGSVVIDANWRWTHATNSSTNCYDGNT---WSSTLCPDNETCAKNCCLDG-A---AYTSA
T_viride_121854     TGSVVIDANWRWTHATNSSTNCYDGNT---WSSTLCPDNETCAKNCCLDG-A---AYAST
T_viride_406299     TGSVVIDANWRWTHATNSSTNCYDGNT---WSSTLCPDNETCAKNCCLDG-A---AYAST
T_harzianum_710     QGSVVLDANWRWTHDTKSTTNCYDGNT---WSSTLCPDDATCAKNCCLDG-A---NYSGT
A_bisporus_3913     NGKVTLDANWRWTHRINDFTNCYTGNE---WDTSICPDGVTCAENCALDG-A---DYAGT
V_volvacea_5231     NTTIVLDANWRWTHSTSGSTNCYTGNT---WQATLCPDGKTCAANCALDG-A---DYTGT
T_longibrachiat     DTSVVLDWNYRWMHDAN-YNSCTVNGG---VNTTLCPDEATCGKNCYIEG-V---DYAAS
H_jecorina_1217     DTSVVLDWNYRWMHDAN-YNSCTVNGG---VNTTLCPDEATCGKNCFIEG-V---DYAAS
A_oryzae_246737     NTSLVLDAATHFIHKKGTQTSCTNSNG---LDTAICPDKQTCADNCVVDGIT---DYA-S
Consensus           -gsvvlDanwrwih-t-gytncytgn----wdstlCpd--tCa-nCaldG-a---dysgt
```

SUBSTITUTE SHEET (RULE 26)

FIG._8B-2

SUBSTITUTE SHEET (RULE 26)

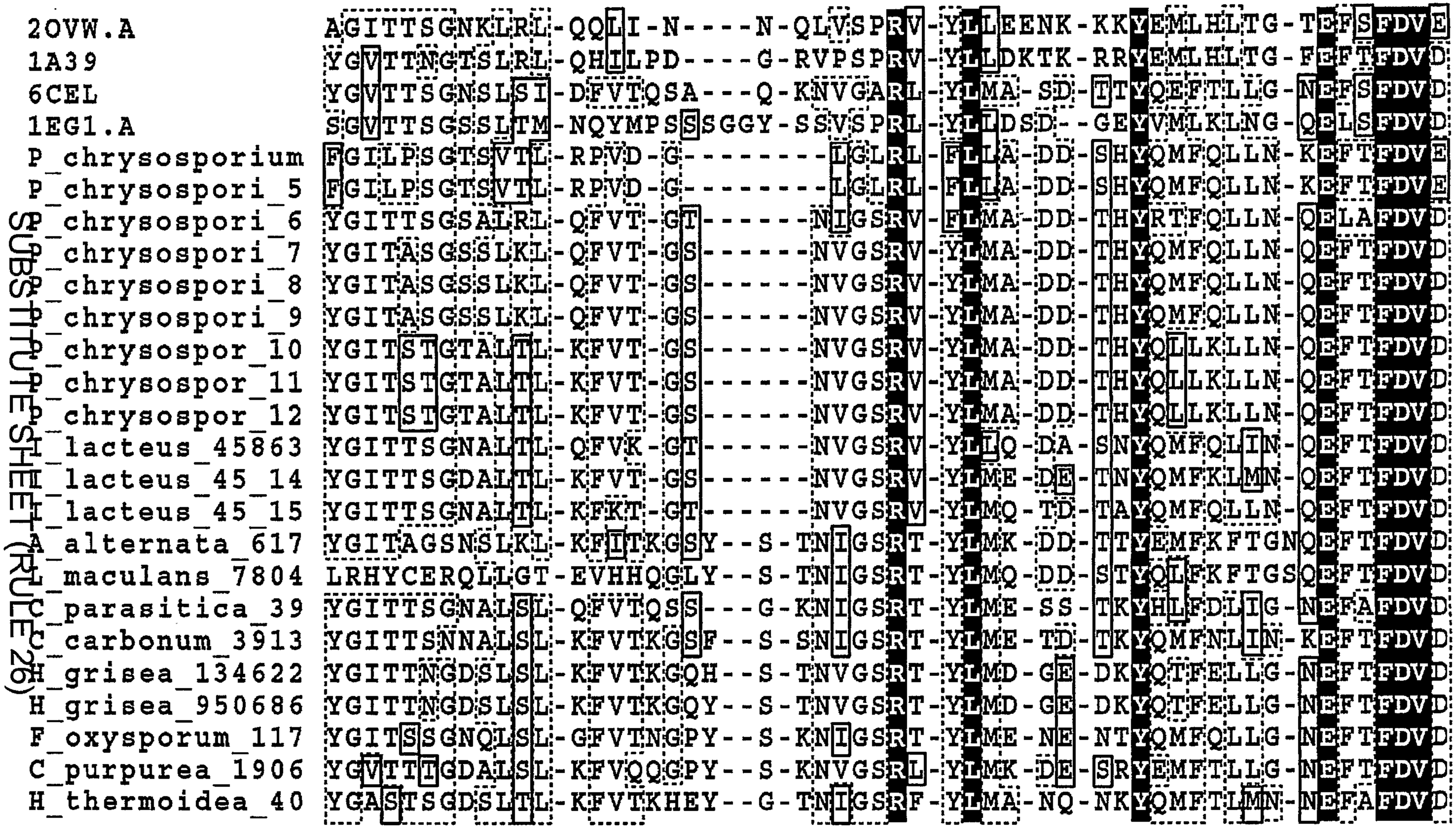

FIG._8C-1

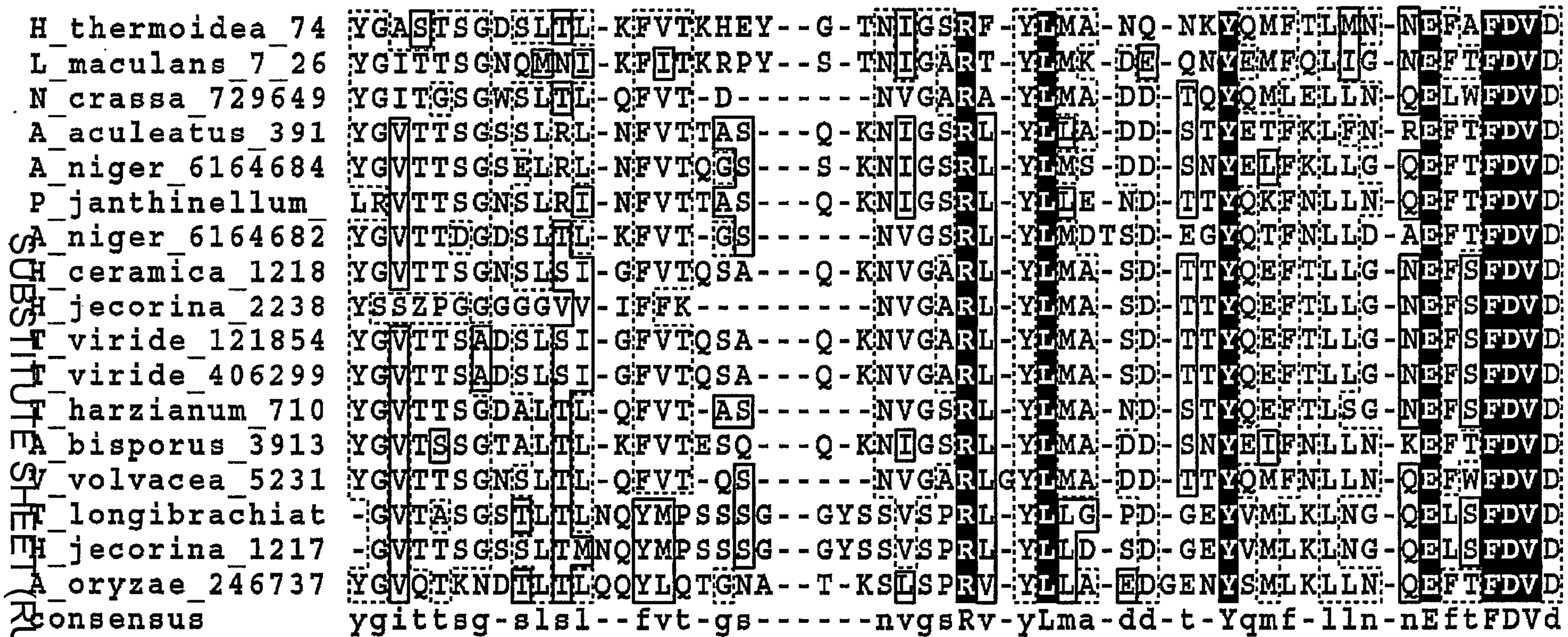

FIG._8C-2

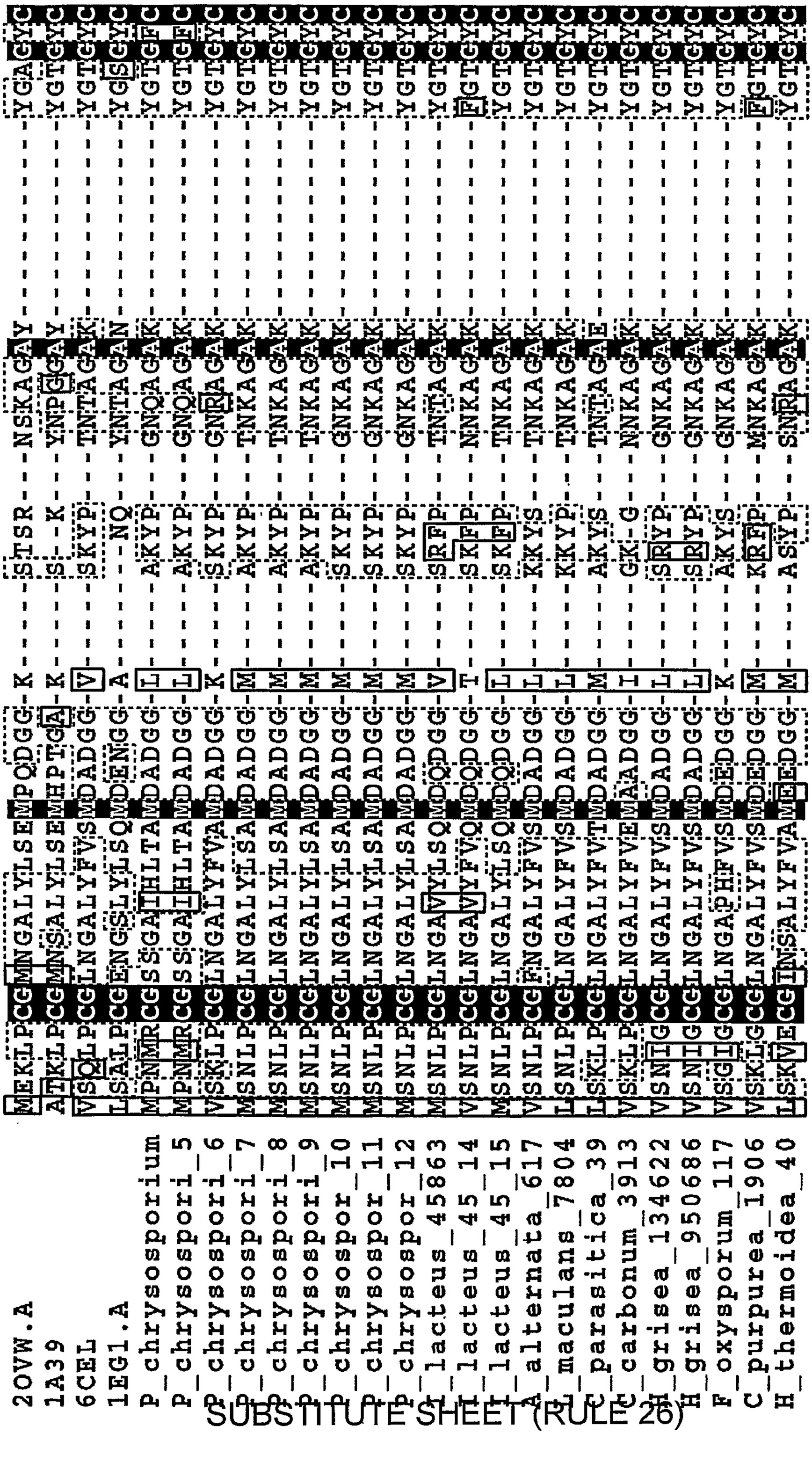

FIG._8D-1

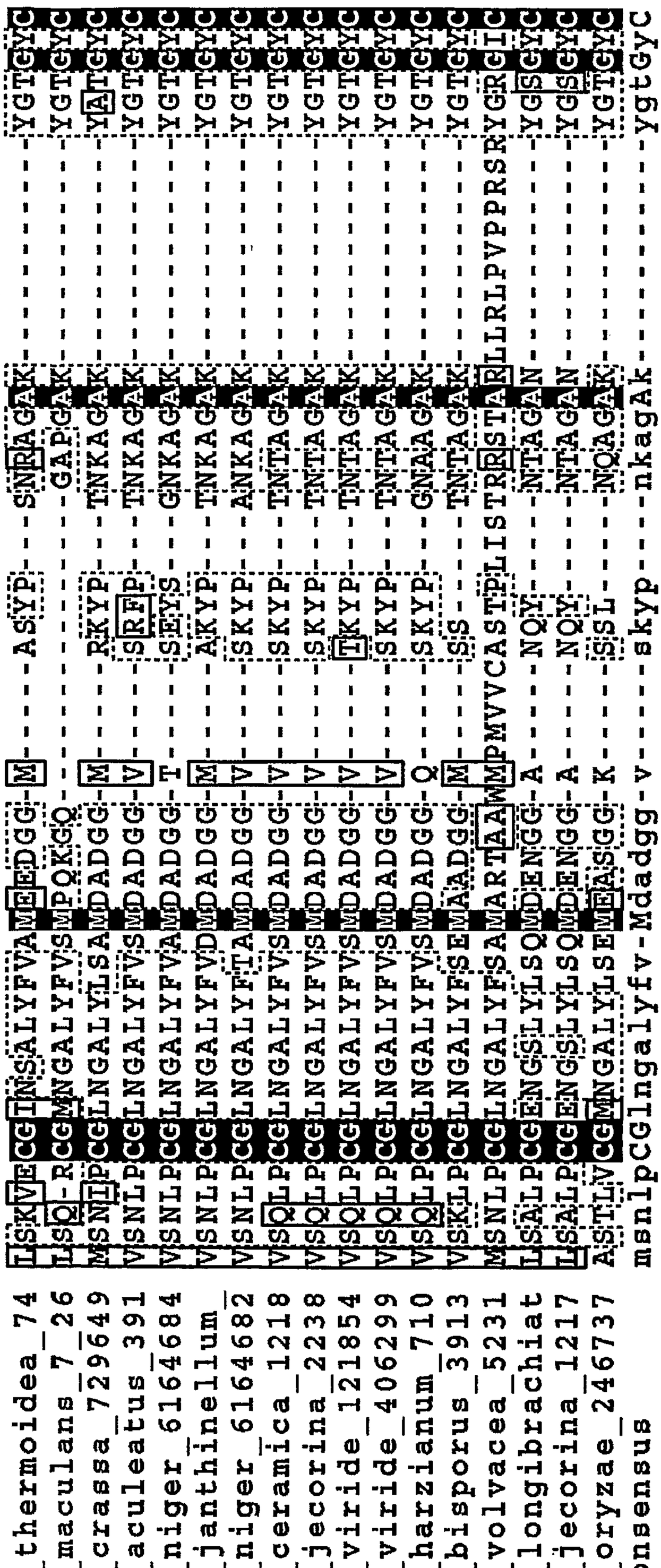

FIG._8D-2

SUBSTITUTE SHEET (RULE 26)

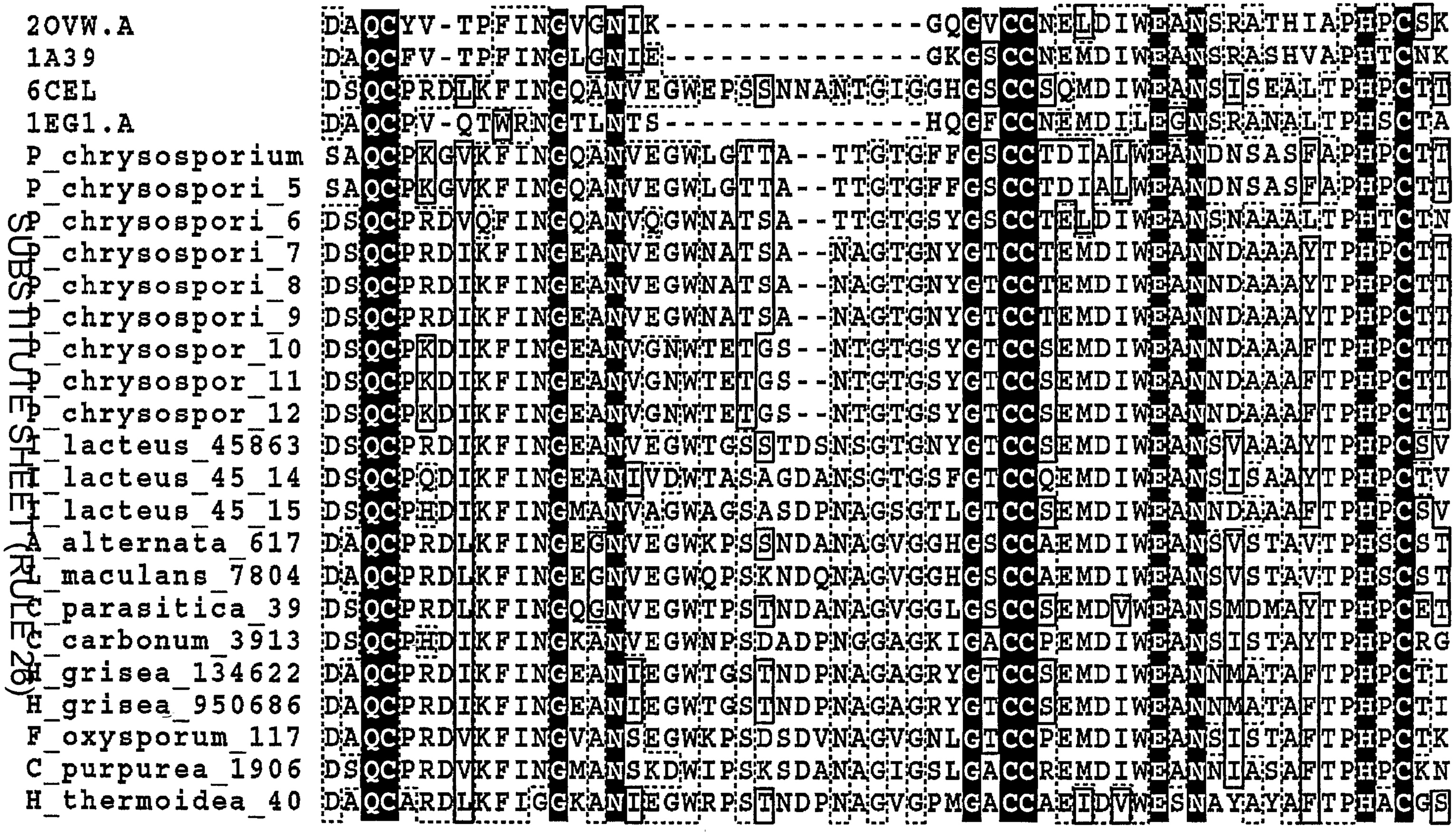

FIG._8E-1

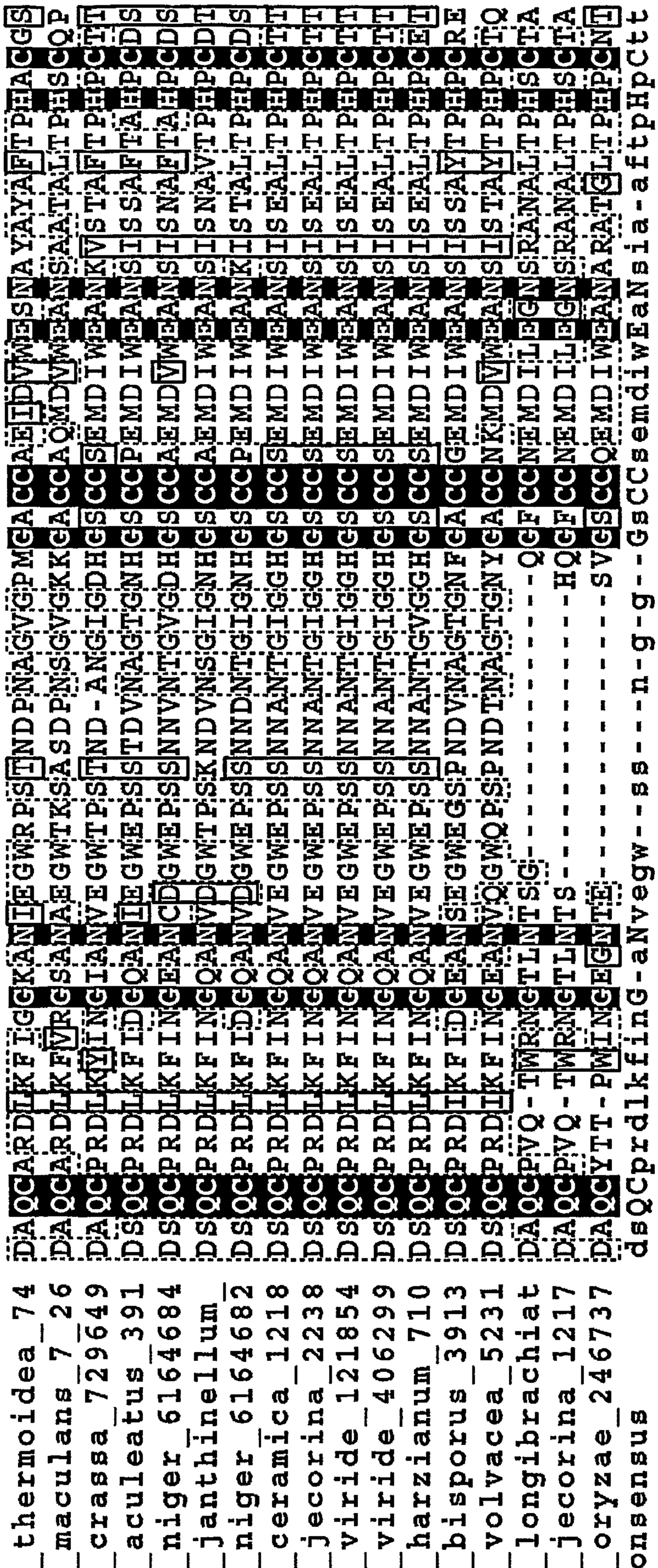

FIG._8E-2

SUBSTITUTE SHEET (RULE 26)

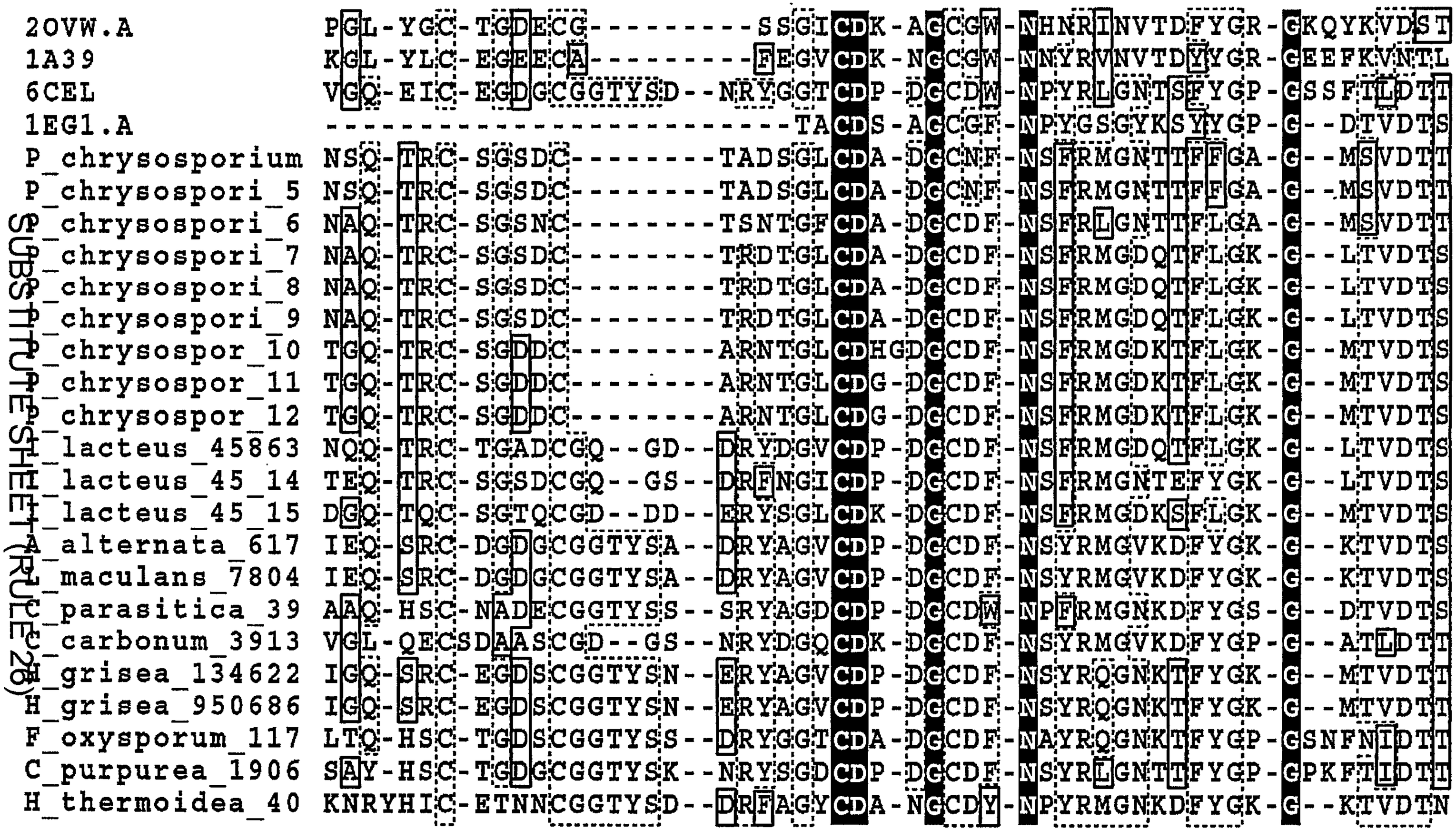

FIG._8F-1

```
H_thermoidea_74     KNRYHIC-ETNNCGGTYSD--DRFAGYCDA-NGCDY-NPYRMGNKDFYGK-G--KTVDTN
L_maculans_7_26     AGY-SVC-EDTNCGGTYSE--DRYAGTCDA-NGCDF-NPFRVGVKDFYGK-G--KTVDTT
N_crassa_729649     IEQ-HMC-EGDSCGGTYSD--DRYGVLCDA-DGCDF-NSYRMGNTTFYGE-G--KTVDTS
A_aculeatus_391     VQQ-TMC-TGDTCGGTYSDTTDRYSGTCDP-DGCDF-NPYRFGNTNFYGP-G--KTVDNS
A_niger_6164684     VSQ-TMC-DGDSCGGTYSASGDRYSGTCDP-DGCDY-NPYRLGNTDFYGP-G--LTVDTN
P_janthinellum_     PSQ-TMC-TGQRCGGTYST--DRYGGTCDP-DGCDF-NPYRMGVTNFYGP-G--ETIDTK
A_niger_6164682     SEQ-TMC-EGNDCGGTYSD--DRYGGTCDP-DGCDF-NPYRMGNDSFYGP-G--KTIDTG
H_ceramica_1218     VGQ-EIC-EGDGCGGTYSD--NRYGGTCDP-DGCDW-NPYRLGNTSFYGP-GSSFTLDTT
H_jecorina_2238     VGQ-EIC-EGDGCGGTYSD--NRYGGTCDP-DGCDW-NPYRLGNTSFYGP-GSSFTLDTT
T_viride_121854     VGQ-EIC-EGDSCGGTYSG--DRYGGTCDP-DGCDW-NPYRLGNTSFYGP-GSSFTLDTT
T_viride_406299     VGQ-EIC-DGDSCGGTYSG--DRYGGTCDP-DGCDW-NPYRLGNTSFYGP-GSSFTLDTT
T_harzianum_710     VGQ-TMC-SGDSCGGTYSN--DRYGGTCDP-DGCDW-NPYRLGNTSFYGP-GSSFALDTT
A_bisporus_3913     PGL-QRC-EGNTCS---VN--DRYATECDP-DGCDF-NSFRMGDKSFYGP-G--MTVDTN
V_volvacea_5231     RGL-VRC-SGTACGG--GS--NRYGSICDH-DGLGFQNLFGMGRTRVRARVGRVKQFNRS
T_longibrachiat     TA-------------------------CDS-AGCGF-NPYGSGYPNYFGP-G--DTVDTS
H_jecorina_1217     TA-------------------------CDS-AGCGF-NPYGSGYKSYYGP-G--DTVDTS
A_oryzae_246737     TGL-YEC-SGSGCG-------D--SGVCDK-AGCGF-NPYGLGAKDYYGY-G--LKVNIN
Consensus           -gq-t-c--gd-cggtys---dry-g-CD--dGcdf-N-yrmgn-sfyg--G---tvdtt
```

FIG._8F-2

SUBSTITUTE SHEET (RULE 26)

FIG._8G-1

```
H_thermoidea_74      RKFTVVSRF------ERNR----LSQFFVQDGRKIEVPPPTWPGLPN-SADITPELCDAQ
L_maculans_7_26      KKMTVVTQFV-G---SGNQLS-EIKRFYVQDGKVIANPEPTIPGM---------EWCNTQ
N_crassa_729649      SKFTVVTQFIKD---SAGDLA-EIKAFYVQNGKVIENSQSNVDGVSG--NSITQSFCKSQ
A_aculeatus_391      KPFTVVTQFITHDGTDTGTLT-EIRRLYVQNGVVIGNGPSTYTAASG--NSITESFCKAE
A_niger_6164684      SPFTVVTQFITDDGTSSGTLT-EIKRLYVQNGEVIANGASTYSSVNG--SSITSAFCESE
P_janthinellum_      SPFTVVTQFLTNDGTSTGTLS-EIKRFYVQGGKVIGNPQSTIVGVSG--NSITDSWCNAQ
A_niger_6164682      SKMTVVTQFITD---GSGSLS-EIKRYYVQNGNVIANADSNISGVTG--NSITTDFCTAQ
H_ceramica_1218      KKLTVVTQF--E---TSGA----INRYYVQNGVTFQQPNAELGSYSG--NELNDDYCTAE
H_jecorina_2238      KKLTVVTQF--E---TSGA----INRYYVQDGVTFQQPNAELGSYSG--NELNDDYCTAE
T_viride_121854      KKLTVVTQF--E---TSGA----INRYYVQNGVTFQQPNAELGDYSG--NSLDDDYCAAE
T_viride_406299      KKLTVVTQF--E---TSGA----INRYYVQNGVTFQQPNAELGDYSG--NSLDDDYCAAE
T_harzianum_710      KKLTVVTQFA-----TDGS----ISRYYVQNGVKFQQPNAQVGSYSG--NTINTDYCAAE
A_bisporus_3913      QPITVVTQFITDNGSDNGNLQ-EIRRIYVQNGQVIQNSNVNIPGIDS-GNSISAEFCDQA
V_volvacea_5231      SRVVEPISWTKQTTLHLGNLPWKSADCNVQNGRVIQNSKVNIPGMPSTMDSVTTEFCNAQ
T_longibrachiat      KTFTIITQFNTDNGSPSGNLV-SITRKYRQNGVDIPSAK---PG--G--DTISS--CPSA
H_jecorina_1217      KTFTIITQFNTDNGSPSGNLV-SITRKYQQNGVDIPSAQ---PG--G--DTISS--CPSA
A_oryzae_246737      ETFTVVTQFLTNDNTTSGQLS-EIRRLYIQNGQVIQNAAVTSGGKTV--DSITKDFCSGE
consensus            kkftvvtqfvt----s-g-l--eirrfyvQngkvi-n----ipgv-g--nsitdefc--q
```

FIG._8G-2

SUBSTITUTE SHEET (RULE 26)

```
2OVW.A                G----ANE--YMRLGGTKQMGDAMSRGMVLAMSVWWSEGDFMAWLDQG-----------
1A39                  G-----SRK--YMELGATQGMGEALTRGMVLAMSIWWDQGGNMEWLDHG-----------
6CEL                  EAEFGGSS--FSDKGGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNETS-STPG
1EG1.A                -------PS--ASAYGGLATMGKALSSGMVLVFSIWNDNSQYMNWLDSG-----------
P_chrysosporium       NAAFGGTSS-FAQHGGLAQVGEALRSGMVLALSIVNS-AADTLWLDSNYPADADP-SAPG
P_chrysospori_5       NAAFGGTSS-FAQHGGLAQVGEALRSGMVLALSIVNS-AADTLWLDSNYPADADP-SAPG
P_chrysospori_6       KKAFIETNY-FAQHGGLAQLGQALRTGMVLAFSISDDPANHMLWLDSNFPPSANP-AVPG
P_chrysospori_7       KTAFGDTNY-FAQHGGLKQVGEALRTGMVLALSIWDDYAANMLWLDSNYPTNKDP-STPG
P_chrysospori_8       KTAFGDTNY-FAQHGGLKQVGEALRTGMVLALSIWDDYAANMLWLDSNYPTNKDP-STPG
P_chrysospori_9       KTAFGDTNY-FAQHGGLKQVGEALRTGMVLALSIWDDYAANMLWLDSNYPTNKDP-STPG
P_chrysospor_10       KTAFGDTNW-FAQKGGLKQMGEALGNGMVLALSIWDDHAANMLWLDSDYPTDKDP-SAPG
P_chrysospor_11       KTAFGDTNW-FAQKGGLKQMGEALGNGMVLALSIWDDHAANMLWLDSDYPTDKDP-SAPG
P_chrysospor_12       KTAFGDTNW-FAQKGGLKQMGEALGNGMVLALSIWDDHAANMLWLDSDYPTDKDP-SAPG
L_lacteus_45863       KTAFGDTNR-FAAQGGLKQMGAALKSGMVLALSLWDDHAANMLWLDSDYPTTADA-SNPG
L_lacteus_45_14       KTVFGDTNN-FAAKGGLKQMGEAVKNGMVLALSLWDDYAAQMLWLDSDYPTTADP-SQPG
L_lacteus_45_15       KSVFGDTNY-FATLGGLKKMGAALKSGMVLAMSVWDDHAASMQWLDSNYPADGDA-TKPG
A_alternata_617       KAVFGDTYT-FKDKGGMANMAKALANGMVLVMSLWDDHYSNMLWLDSTYPTDKNPDTDLG
L_maculans_7804       KKAFGDKYT-FKDKGGMANM-PSTCNGMVLVMSLWDDHYSNMLWLDSTYPTDKNPDTDAG
C_parasitica_39       KVEFNDTDV-FSEKGGLAQMGAGMADGMVLVMSLWDDHYANMLWLDSTYPVDADA-SSPG
C_carbonum_3913       KKAFGDTSS-FAALGGLNEMGASLARGHVLIMSLWGDHAVNMLWLDSTYPTDADP-SKPG
H_grisea_134622       KVAFGDIDD-FNRKGGMKQMGKALAGPMVLVMSIWDDHASNMLWLDSTFPVDAAG--KPG
H_grisea_950686       KVAFGDIDD-FNRKGGMKQMGKALAGPMVLVMSIWDDHASNMLWLDSTFPVDAAG--KPG
F_oxysporum_117       KSVFGDIDD-FSKKGGWNGMSDALSAPMVLVMSLWHDHHSNMLWLDSTYPTDST---KVG
C_purpurea_1906       KKMFGAHES-FNAKGGMKGMSAAVSKPMVLVMSLWDDHNSNMLWLDSTYPTNSR---QRG
H_thermoidea_40       FRVFDDRNR-FAETGGFDALNEALTIPMVLVMSIWDDHHSNMLWLDSSYPPEKAG--LPG
```

FIG._8H-1

```
H_thermoidea_74      FRVFDDRNR-FAETGGFDALNEALTIPMVLVMSIWDDHHSNMLWLDSSYPPEKAG--LPG
L_maculans_7_26      KKVFQEEAYPFNEFGGMASMSEGMSQGMVLVMSLWDDHYANMLWLDSNWPREADP-AKPG
N_crassa_729649      KTAFGDIDD-FNKKGGLKQMGKALAQAMVLVMSIWDDHAANMLWLDSTYPV---P-KVPG
A_aculeatus_391      KTLFGDTNV-FETHGGLSAMGDALGDGMVLVLSLWDDHAADMLWLDSDYPTTSCA-SSPG
A_niger_6164684      KTLFGDENV-FDKHGGLEGMGEAMAKGMVLVLSLWDDYAADMLWLDSDYPVNSSA-STPG
P_janthinellum_      KSAFGDTNE-FSKHGGMAGMGAGLADGMVLVMSLWDDHASDMLWLDSTYPTNATS-TTPG
A_niger_6164682      KKAFGDEDI-FAEHNGLAGISDAMSS-MVLILSLWDDYYASMEWLDSDYPENATA-TDPG
H_ceramica_1218      EAEFGGSS--FSDKGGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNETS-STPG
H_jecorina_2238      EAEFGGSS--FSDKGGLTQFXXATSGGMVLVMSLWDDYYANMLWLDSTYPTNETS-STPG
T_viride_121854      EAEFGGSS--FSDKGGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTDETS-STPG
T_viride_406299      EAEFGGSS--FSDKGGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNETS-STPG
T_harzianum_710      QTAFGGTS--FTDKGGLAQINKAFQGGMVLVMSLWDDYAVNMLWLDSTYPTNATA-STPG
A_bisporus_3913      KEAFGDERS-FQDRGGLSGMGSALDRGMVLVLSIWDDHAVNMLWLDSDYPLDASP-SQPG
V_volvacea_5231      KTAFNDTFS-FQQKGGMANMSEALRRGMVLVLSIWDDHAANMLWLDSITSAAACR-STPS
T_longibrachiat      SA--------Y---GGLATMGKALSSGMVLVFSIWNDNSQYMNWLDS----------G
H_jecorina_1217      SA--------Y---GGLATMGKALSSGMVLVFSIWNDNSQYMNWLDS----------G
A_oryzae_246737      ----GSA---FNRLGGLEEMGHALGRGMVLALSIWNDAGSFMQWLD----------G
consensus            k--fgds---f---ggl-qmg-al--gmVLvmSiwddhaanmlWLDs-ypt-----s-pg
```

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FIG._8H-2

```
2OVW.A              -VAGPCDATEGDPKNIVKVQPNPEVTFSNIRIGEIG-STS
1A39                -EAGPCAKGEGAPSNIVQVEPFPEVTYTNLRWGEIG-STY-------QELQ
6CEL                AVRGSCSTSSGVPAQVESQSPNAKVTFSNIKFGPIG-STG-------NPSG
1EG1.A              -NAGPCSSTEGNPSNILANNPNTHVVFSNIRWGDIG-STT
P_chrysosporium     VARGTCPQDS---ASIP-EAPTPSVVFSNIKLGDIG-TTF--G----AGSALFS
P_chrysospori_5     VARGTCPQDS---ASIP-EAPTPSVVFSNIKLGDIG-TTF--G----AGSALF-
P_chrysospori_6     VARGMCSITSGNPADVGILNPSPYVSFLNIKFGSIG-TTF-------RPA
P_chrysospori_7     VARGTCATTSGVPAQIEAQSPNAYVVFSNIKFGDLN-TTYT-GT--VSSSSV-----SS
P_chrysospori_8     VARGTCATTSGVPAQIEAQSPNAYVVFSNIKFGDLN-TTYT-GT--VSSSSV-----SS
P_chrysospori_9     VARGTCATTSGVPAQIEAQSPNAYVVFSNIKFGDLN-TTYT-GT--VSSSSV-----SS
P_chrysospor_10     VARGTCATTSGVPSDVESQVPNSQVVFSNIKFGDIG-STFS-G----TSSP-------NP
P_chrysospor_11     VARGTCATTSGVPSDVESQVPNSQVVFSNIKFGDIG-STFS-G----TSSP-------NP
P_chrysospor_12     VARGTCATTSGVPSDVESQVPNSQVVFSNIKFGDIG-STFS-G----TSSP-------NP
T_lacteus_45863     VARGTCPTTSGFPRDVESQSGSATVTYSNIKWGDLN-STFT-GTL-TTPSGS------SS
T_lacteus_45_14     VARGTCPTTSGVPSQVEGQEGSSSVIYSNIKFGDLN-STFT-GTLTNPSSPA------GP
T_lacteus_45_15     VARGTCSADSGLPTNVESQSASASVTFSNIKWGDIN-TTFT-G----TGST-------SP
A_alternata_617     TGRGECETSSGVPADVESQHADATVVYSNIKFGPLN-STF--G
L_maculans_7804     SGRGECAITSGVPADVESQHPDASVIYSNIKFGPIN-TTF--G
C_parasitica_39     KQRGTCATTSGVPADVESSDASATVIYSNIKFGPIG-ATY
C_carbonum_3913     AARGTCPTTSGKPEDVEKNSPDATVVFSNIKFGPIG-STF--A----QPA
H_grisea_134622     AERGACPTTSGVPAEVEAEAPNSNVVFSNIRFGPIG-STVA-GLPGAGNGGN------NG
H_grisea_950686     AERGACPTTSGVPAEVEAEAPNSNVVFSNIRFGPIG-STVA-GLPGAGNGGN------NG
F_oxysporum_117     SQRGSCATTSGKPSDLERDVPNSKVSFSNIKFGPIG-STY-------KSDG-------TT
C_purpurea_1906     SKRGSCPASSGRPTDVESSAPDSTVVFSNIKFGPIG-STF-------SRGK
H_thermoidea_40     GDRGPCPTTSGVPAEVEAQYPNAQVVWSNIRFGPIG-STV-------NV
```

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FIG._8I-1

```
H_thermoidea_74     GDRGPCPTTSGVPAEVEAQYPDAQVVWSNIRFGPIG-STV-------NV
L_maculans_7_26     VARRDCPTSGGKPSEVEAANPNAQVMFSNIKFGPIG-STF-------AHAA
N_crassa_729649     AYRGSGPTTSGVPAEVDANAPNSKVAFSNIKFGHLGISPFS-GGS-SGTPPS------NP
A_aculeatus_391     VARGTCPTTIGNATYVEANYPNSYVTYSNIKFGTLN-STYS-GTSSGGSSSSSTTLTTKA
A_niger_6164684     VARGTCSTDSGVPATVEAESPNAYVTYSNIKFGPIG-STYSSGS--SSGSGS------SS
P_janthinellum_     AKRGTCDISRR-PNTVESTYPNAYVIYSNIKTGPLN-STFT-GGTTSSSSTT------TT
A_niger_6164682     VARGTCDSESGVPATVEGAHPDSSVTFSNIKFGPIN-STF-------SASA
H_ceramica_1218     AVRGSCSTSSGVPAQVESQSPNAKVTFSNIKFGPIG-ST---G----NPSGG------NP
H_jecorina_2238     AVRGSCSTSSGVPAQVESQSPNAKVTFSNIKFGPIG-ST---G----NPSGG------NP
T_viride_121854     AVRGSSSTSSGVPAQLESNSPNAKVVYSNIKFGPIG-ST---G----NPSGG------NP
T_viride_406299     AVRGSCSTSSGVPAQLESNSPNAKVVYSNIKFGPIG-ST---G----NSSGG------NP
T_harzianum_710     AKRGSCSTSSGVPAQVEAQSPNSKVIYSNIRFGPIG-ST---G----GNTGS------NP
A_bisporus_3913     ISRGTCSRDSGKPEDVEANAGGVQVVYSNIKFGDIN-STF--N----NNGG---------
V_volvacea_5231     EVHATPLRESQIRSSHSRQT--RYVTFTNIKFGPFN-ST---G----TTYT-------TG
T_longibrachiat     -RAGPCSSTEGNPSNILANNPGTHVVYSNIRWGDIG-STTN-ST---GGNPP------PP
H_jecorina_1217     -NAGPCSSTEGNPSNILANNPNTHVVFSNIRWGDIG-STT--N----STAPP------PP
A_oryzae_246737     GSAGPCNATEGNPALIEKLYPDTHVKFSKIRWGDIG-STY-------RH
consensus           --rgsc-ttsgvpa-ve-q-pn--Vvfsnikfgpig-sty--g------s----------
```

FIG._8I-2

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CA 02495664 2005-02-15

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```
2OVW.A
1A39
6CEL
1EG1.A
P_chrysosporium -GRSPPGPVPGS-APAS--S----ATA---TAPP-FGSQCGGLGYAGPTGVCPSPYTCQA
P_chrysospori_5 -SGRS
P_chrysospori_6
P_chrysospori_7 SHSSTSTSSSHS-SSST--PPTQPTGV---TVPQ-WG-QCGGIGYTGST-TCASPYTCHV
P_chrysospori_8 SHSSTSTSSSHS-SSST--PPTQPTGV---TVPQ-WG-QCGGIGYTGST-TCASPYTCHV
P_chrysospori_9 SHSSTSTSSSHS-SSST--PPTQPTGV---TVPQ-WG-QCGGIGYTGST-TCASPYTCHV
P_chrysospor_10 PGGST-TSSPVT-TSPT--PP--PTGP---TVPQ-WG-QCGGIGYSGST-TCASPYTCHV
P_chrysospor_11 PGGST-TSSPVT-TSPT--PP--PTGP---TVPQ-WG-QCGGIGYSGST-TCASPYTCHV
P_chrysospor_12 PGGST-TSSPVT-TSPT--PP--PTGP---TVPQ-WG-QCGGIGYSGST-TCASPYTCHV
I_lacteus_45863 PSSPASTSGSST-SASS--SASVPTQS--GTVAQ-WA-QCGGIGYSGAT-TCVSPYTCHV
I_lacteus_45_14 PVTSSPSEPSQS-TQPS--QPAQPTQPA-GTAAQ-WA-QCGGMGFTGPT-VCASPFTCHV
I_lacteus_45_15 SSPAGPVSSSTS-VASQ--PT-QPAQG---TVAQ-WG-QCGGTGFTGPT-VCASPFTCHV
A_alternata_617
L_maculans_7804
C_parasitica_39
C_carbonum_3913
H_grisea_134622 GNPPPPTTTTSS-APAT--TTTASAGP---KAGR-WQ-QCGGIGFTGPT-QCEEPYICTK
H_grisea_950686 GNPPPPTTTTSS-APAT--TTTASAGP---KAGR-WQ-QCGGIGFTGPT-QCEEPYTCTK
F_oxysporum_117 PNPPASSSTTGS-STPT--NP--PAG----SVDQ-WG-QCGGQNYSGPT-TCKSPFTCKK
C_purpurea_1906
H_thermoidea_40
```

FIG._8J-1

```
H_thermoidea_74
L_maculans_7_26
N_crassa_729649     SSSASPTSSTAKPSSTS--TASNPSGT---GAAH-WA-QCGGIGFSGPT-TCPEPYTCAK
A_aculeatus_391     STSTTSSKTTTT-TSKT--STTSSSST---NVAQLYG-QCGGQGWTGPT-TCASG-TCTK
A_niger_6164684     SSSSTTTKATST-TLKT--TSTTSSGSSSTSAAQAYG-QCGGQGWTGPT-TCVSGYTCTY
P_janthinellum_     TSKSTSTSSSSK-TTTTVTTTTTSSGSS-GTGARDWA-QCGGNGWTGPT-TCVSPYTCTK
A_niger_6164682
H_ceramica_1218     PGGNR-GTTTTR-RPATT--TTGSSPGP---TQSH-YG-QCGGIGYSGPT-VCASGTTCQV
H_jecorina_2238     PGGNPPGTTTTT-TTSS--SZ-PPPG----AHRR-YG-QCGGIGYSGPT-VCASGTTCQV
T_viride_121854     PGGNPPGTTT-P-RPAT--STGSSPGP---TQTH-YG-QCGGIGYIGPT-VCASGSTCQV
T_viride_406299     PGGNPPGTTTTR-RPAT--STGSSPGP---TQTH-YG-QCGGIGYSGPT-VCASGSTCQV
T_harzianum_710     PGTSTTRAPPSS-TGSS------PTA----TQTH-YG-QCGGTGWTGPT-RCASGYTCQV
A_bisporus_3913     -GGGNPSPTTTR----------PNSP---AQTM-WG-QCGGQGWTGPT-ACQSPSTCHV
V_volvacea_5231     SVPTTSTSTGTT-GSST--PP-QPTGV---TVPQ--G-QCGGIGYTGPT-TCASPTTCHV
T_longibrachiat     PPPASSTTFSTT-RRSS--TTSSSPSC---TQTH-WG-QCGGIGYTGCK-TCTSGTTCQY
H_jecorina_1217     PASSTTFSTTRR-SSTT--SS--SPSC---TQTH-WG-QCGGIGYSGCK-TCTSGTTCQY
A_oryzae_246737
consensus           ----------------s-------------------fg-qcgg-gytg-t--c-s--tc--
```

FIG._8J-2

```
2OVW.A
1A39
6CEL
1EG1.A
P_chrysosporium LNIYYSQ-CI
P_chrysospori_5
P_chrysospori_6
P_chrysospori_7 LNPYYSQ-CY
P_chrysospori_8 LNPYYSQ-CY
P_chrysospori_9 LNPYYSQ-CY
P_chrysospor_10 LNPYYSQ-CY
P_chrysospor_11 LNPYYSQ-CY
P_chrysospor_12 LNPCESILSLQRSSNADQYLQTTRSATKRRLDTALQPRK
T_lacteus_45863 VNAYYSQ-CY
T_lacteus_45_14 LNPYYSQ-CY
T_lacteus_45_15 VNPYYSQ-CY
A_alternata_617
L_maculans_7804
C_parasitica_39
C_carbonum_3913
H_grisea_134622 LNDWYSQ-CL
H_grisea_950686 LNDWYSQ-CL
F_oxysporum_117 INDFYSQ-CQ
C_purpurea_1906
H_thermoidea_40
```

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FIG._8K-1

```
H_thermoidea_74
L_maculans_7_26
N_crassa_729649      DHDIYSQ-CV
A_aculeatus_391      QNDYYSQ-CL
A_niger_6164684      ENAYYSQ-CL
P_janthinellum_      QNDWYSQ-CL
A_niger_6164682
H_ceramica_1218      LNPYYSQ-CL
H_jecorina_2238      LNPYYSQ-CL
T_viride_121854      LNPYYSQ-CL
T_viride_406299      LNPYYSQ-CL
T_harzianum_710      LNPFYSQ-CL
A_bisporus_3913      INDFYSQ-CF
V_volvacea_5231      LNPYYSQ-CY
T_longibrachiat      GNDYYSQ-CL
H_jecorina_1217      SNDYYSQ-CL
A_oryzae_246737
consensus            -n-yysq-c-
```

FIG._8K-2

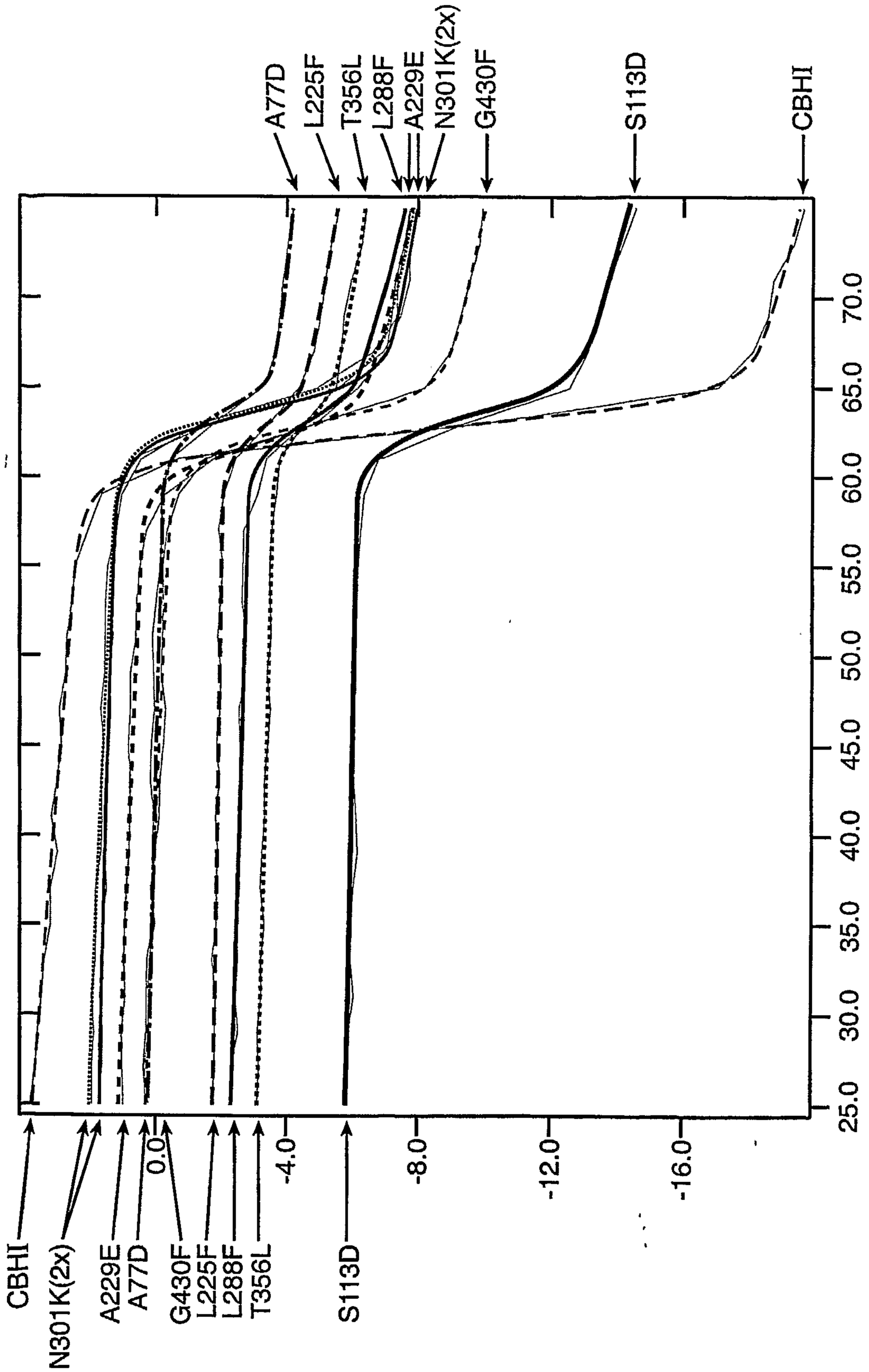
A77D
L225F
T356L
L288F
A229E
N301K(2x)
G430F
S113D
CBHI
70.0
65.0
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
0.0
-4.0
-8.0
-12.0
-16.0
CBHI
N301K(2x)
A229E
A77D
G430F
L225F
L288F
T356L
S113D

FIG._9A

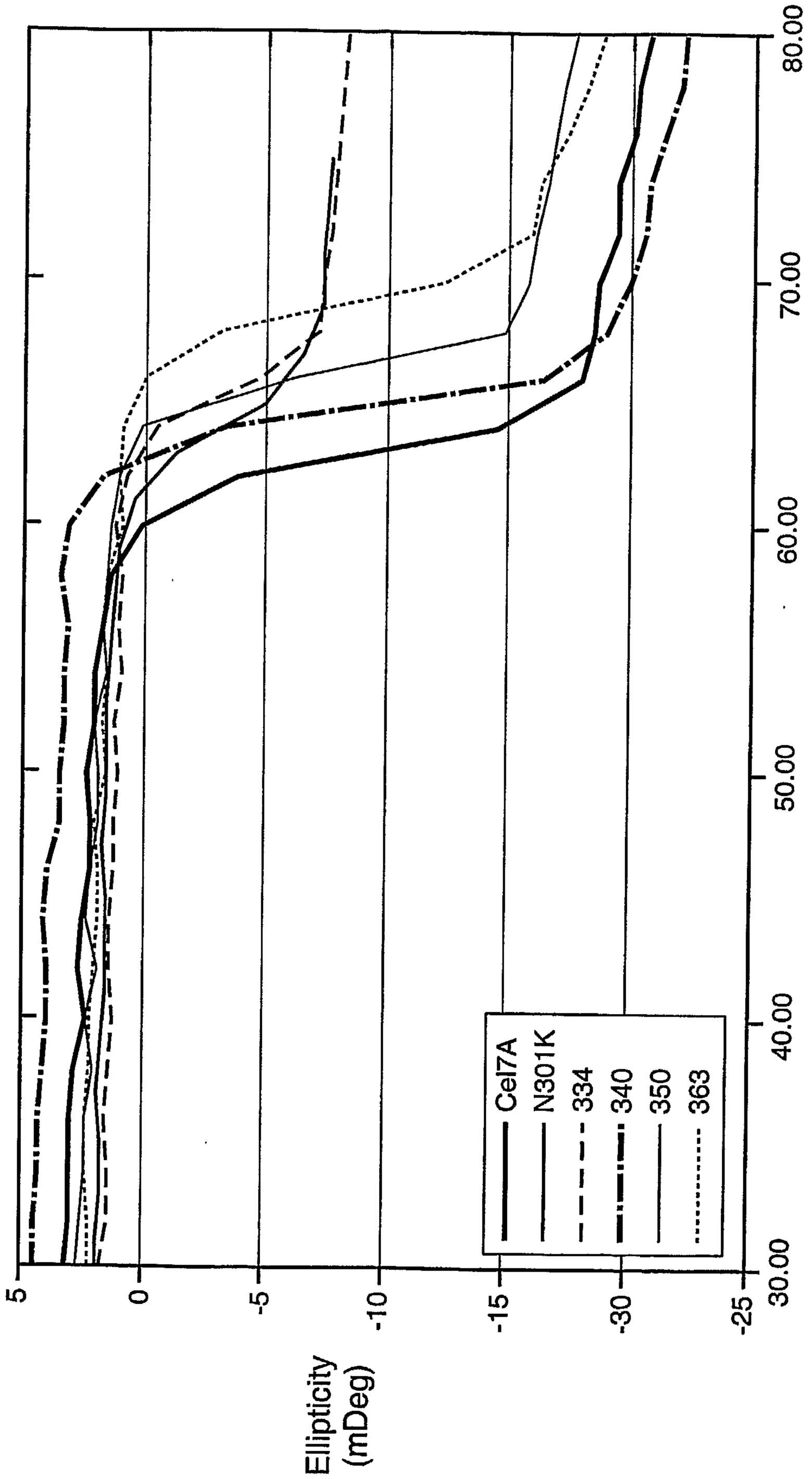
Thermal Melts of H. jecorina Cel7A and Variants
Ellipticity (mDeg)
5
0
-5
-10
-15
-30
-25
Temperature (°C)
80.00
70.00
60.00
50.00
40.00
30.00
Cel7A
N301K
334
340
350
363

FIG._9B

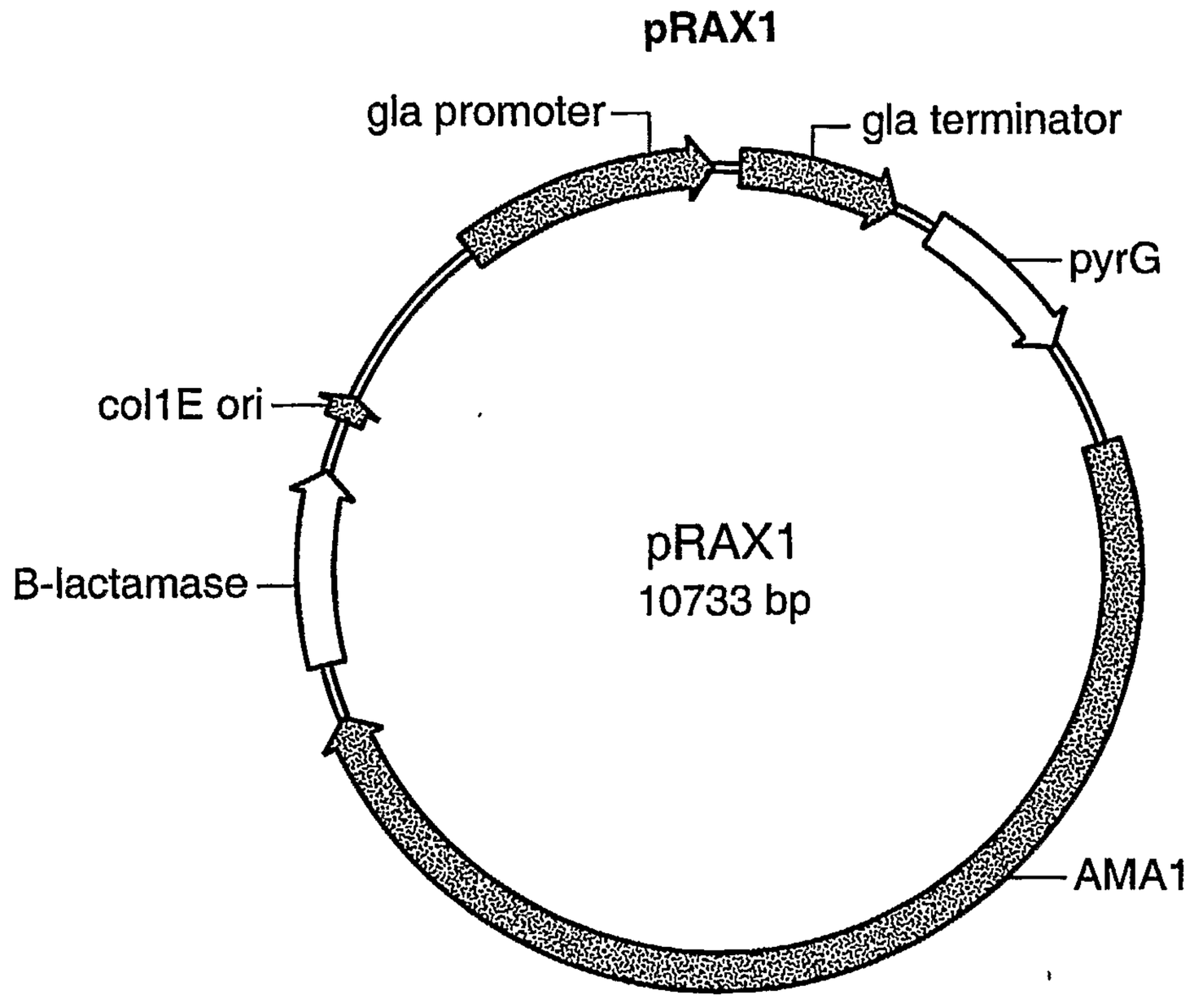
pRAX1
gla promoter
gla terminator
pyrG
col1E ori
pRAX1
10733 bp
B-lactamase
AMA1

FIG._10

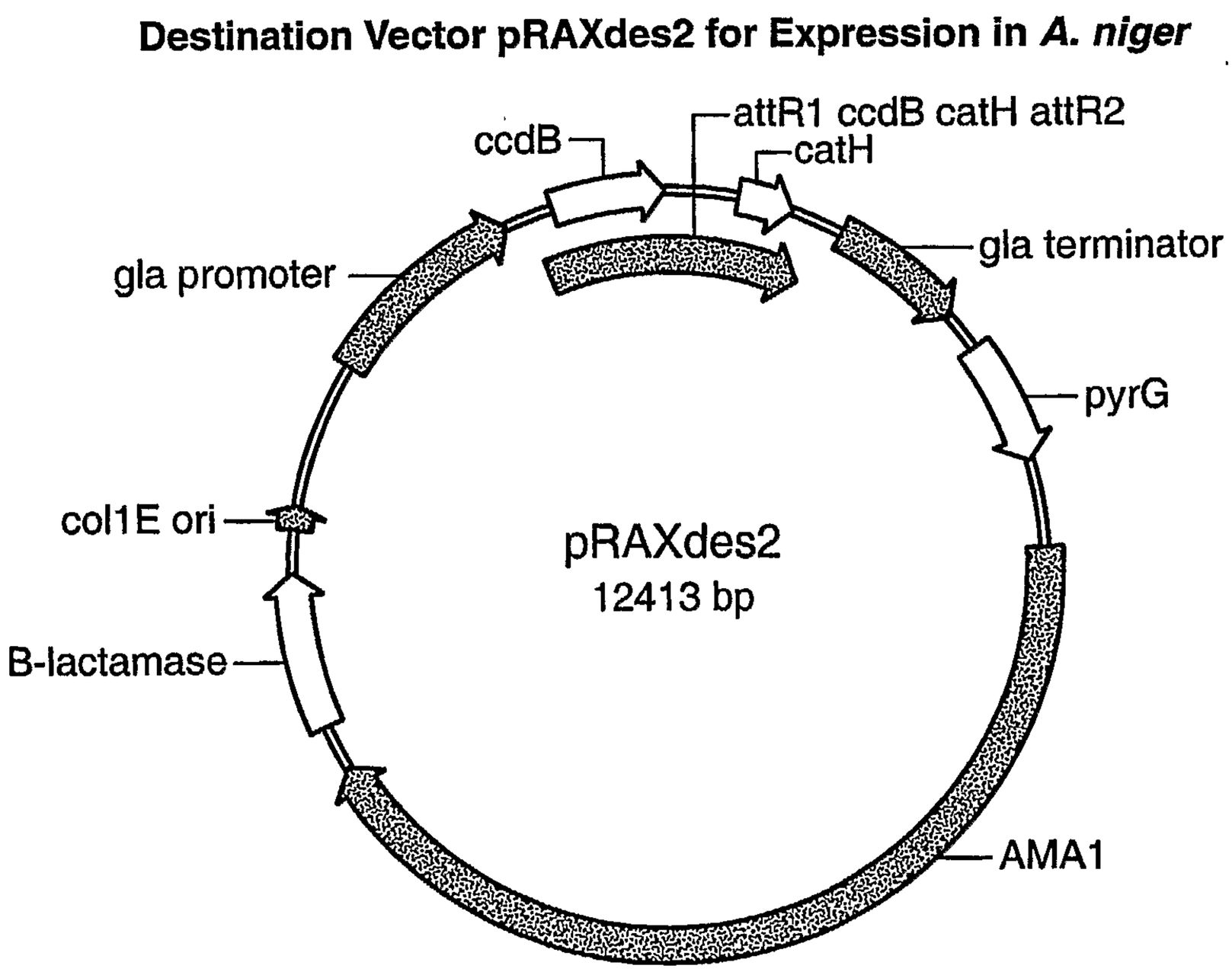
Destination Vector pRAXdes2 for Expression in A. niger
attR1 ccdB catH attR2
ccdB
catH
gla promoter
gla terminator
pyrG
col1E ori
pRAXdes2
12413 bp
B-lactamase
AMA1

FIG._11

Replicative Expression pRAXdesCBH1 Vector of CBH1 Genes Under the Control of the Glucoamylase Promoter

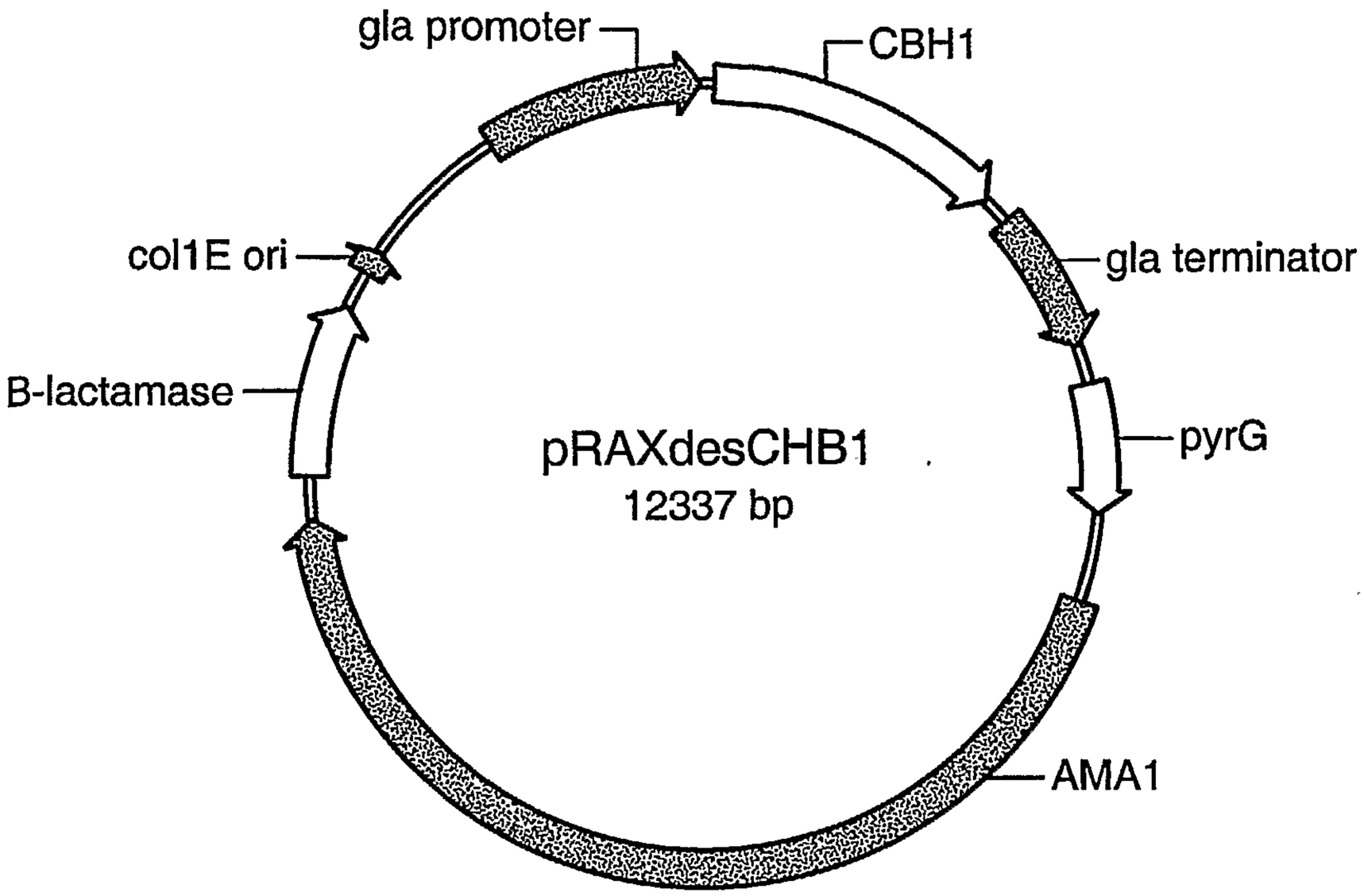

FIG._12

Replicative Expression pRAXdesCBH1 Vector of CBH1 Genes Under the Control of the Glucoamylase Promoter