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(54) Title: MUTANT STRAIN OF TRICHODERMA REESEI AND METHOD FOR PRODUCING PROTEIN USING SAME

(54) 発明の名称: トリコデルマ・リーセイの変異株およびそれを用いたタンパク質の製造方法

(57) Abstract: The present invention is a mutant strain of Trichoderma reesei in which the function of a polypeptide comprising an amino acid sequence represented by any of SEQ ID NOS: 4-6 is deleted or decreased, and a method for producing a high level of protein by culturing the mutant strain in medium containing lactose.

(57) 要約: 本発明は、配列番号4~6のいずれかで表されるアミノ酸配列からなるポリペプチドの機能が欠損または低下している Trichoderma reesei の変異株および当該変異株をラクトースを含む培地で培養することによりタンパク質を高生産する方法である。



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DESCRIPTION

TITLE OF INVENTION: MUTANT STRAIN OF TRICHODERMA REESEI AND
METHOD FOR PRODUCING PROTEIN USING SAME

5

TECHNICAL FIELD

[0001]

The present invention relates to a *Trichoderma reesei* mutant strain having an enhanced protein-producing ability and to a method of protein production using the mutant strain.

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BACKGROUND ART

[0002]

Trichoderma reesei is known to have a high protein-producing ability, and studies have heretofore been made on protein production using *Trichoderma reesei*. *Trichoderma reesei* is especially excellent in terms of the ability to produce a cellulase, which is classified as a saccharifying enzyme, among proteins. For example, in order to further enhance cellulase production amount, investigations such as overexpression or deletion of a factor which controls cellulase production and investigations on cultivation conditions for cellulase production are being conducted.

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[0003]

Non-Patent Literature 1 describes that a mutant strain of *Trichoderma reesei* which has a high cellulase-producing ability was acquired by reducing the function of Cre1, which is a transcription factor repressing cellulase production, among the cellulase-production-controlling factors of *Trichoderma reesei*.

25

[0004]

Meanwhile, Non-Patent Literature 2 describes a method of cultivating *Trichoderma reesei* in a culture medium to which glucose or lactose has been added, as a method of improving the production amount of a cellulase of *Trichoderma reesei*.

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CITATION LIST

NON-PATENT LITERATURE

[0005]

Non-Patent Literature 1: Juliano P, Single nucleotide polymorphism analysis of a *Trichoderma reesei* hyper-cellulolytic mutant developed in Japan, Bioscience, Biotechnology, and Biochemistry, Volume 77, 2013, Issue 3, P534-543

Non-Patent Literature 2: Antonella A, Regulation of cellulase and hemicellulase gene expression in fungi, Current genomics, Volume 14, 2013, P230-249

SUMMARY OF THE INVENTION

TECHNICAL PROBLEM

[0006]

As described above, a transcription factor which is one of protein-production-controlling factors in *Trichoderma reesei* has been identified, but this is considered to be merely a part of the control mechanism. Then, an object of the present invention is to acquire a mutant strain of *Trichoderma reesei* which has a further enhanced protein-producing ability by making a search for a novel mechanism controlling protein production by *Trichoderma reesei*, and to provide a method of protein production using the mutant strain of *Trichoderma reesei*.

SOLUTION TO THE PROBLEM

[0007]

The present inventors thought that if a gene which had been unknown and was capable of bringing about an increase in protein production could be specified, then the amount of proteins to be produced by *Trichoderma reesei* could be further increased. The inventors diligently made investigations and, as a result, have discovered that an improvement in protein-producing ability can be attained by cultivating a mutant strain of *Trichoderma reesei* in which the function of one or more polypeptides selected from polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 4, 5, and 6 has been eliminated or reduced. The present invention has been accomplished based on this finding.

[0008]

Specifically, the present invention includes the following (1) to (15).

(1) A mutant strain of *Trichoderma reesei*, the mutant strain having a mutation that eliminates or reduces a function of a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 4 to 6.

(2) The mutant strain according to (1), in which the mutation is a mutation that

deletes an HSF-type DNA-binding domain of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4.

(3) The mutant strain according to (2), in which the mutation is a frameshift mutation accompanying a mutation in a region nearer to the N-terminal side than the HSF-type DNA-binding domain.

(4) The mutant strain according to (3), in which the mutation is a frameshift mutation due to a mutation in which a histidine residue at the 30th residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 4 is changed to a residue of an amino acid other than histidine.

(5) The mutant strain according to (1), in which the mutation is a mutation that deletes a TLD domain of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5.

(6) The mutant strain according to (5), in which the mutation is a frameshift mutation accompanying a mutation in a region nearer to the N-terminal side than the TLD domain.

(7) The mutant strain according to (6), in which the mutation is a frameshift mutation due to a mutation in which a glutamine residue at the 3rd residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 5 is changed to a residue of an amino acid other than glutamine.

(8) The mutant strain according to (1), in which the mutation is a mutation of an amino acid sequence of an F-box domain region of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6.

(9) The mutant strain according to (8), in which the mutation is a deletion of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6, the deletion being caused by a frameshift mutation accompanying a mutation of the amino acid sequence of the F-box domain region.

(10) The mutant strain according to (9), in which the mutation is a frameshift mutation due to a mutation in which an alanine residue at the 167th residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 6 is changed to a residue of an amino acid other than alanine.

(11) A method of producing a protein, the method including a step of cultivating the mutant strain according to any one of (1) to (10).

(12) A method of producing a protein, the method including a step of cultivating the

mutant strain according to any one of (1) to (10), in a culture medium at least including lactose.

(13) A method of producing a cellulase, the method including a step of cultivating the mutant strain according to any one of (1) to (10).

5 (14) A method of producing a cellulase, the method including a step of cultivating the mutant strain according to any one of (1) to (10), in a culture medium at least including lactose.

(15) A method of producing a sugar, the method including:

a step of producing a cellulase by the method of producing a cellulase according to

10 (13) or (14); and

a step of saccharifying a cellulose-containing biomass using the cellulase obtained in the step.

ADVANTAGEOUS EFFECTS OF THE INVENTION

15 [0009]

The mutant strain of *Trichoderma reesei* in which the function of a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 4 to 6 has been eliminated or reduced has an improved protein-producing ability and is capable of highly efficiently producing a protein, as compared with the parent strain into which the mutation has not been introduced. Furthermore, in the case where the produced proteins are cellulases, an unexpected effect that the cellulases have improved various specific activities is also obtained.

DESCRIPTION OF EMBODIMENTS

[0010]

25 The present invention is characterized in that a mutation is introduced into a parent strain of *Trichoderma reesei*, which is a microorganism originally having an excellent protein-producing ability, to thereby further enhance the protein-producing ability. Specifically, the present invention relates to a mutant strain of *Trichoderma reesei*, the mutant strain being characterized by having a mutation which eliminates or reduces the function of a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 4 to 6.

30 [0011]

The parent strain of *Trichoderma reesei* to be used in the present invention is not limited to wild strains, and mutant strains which have been improved so as to have an

increased protein-producing ability can also be favorably used as the parent strain. For example, a mutant strain having an improved protein production property obtained by performing a mutation treatment with a mutagen, UV irradiation, etc. can be utilized as the parent strain. Specific examples of mutant strains usable as the parent strain include the following known mutant strains belonging to *Trichoderma reesei*: QM6a strain (NBRC31326), QM9123 strain (ATCC24449), QM9414 strain (NBRC31329), PC-3-7 strain (ATCC66589), QM9123 strain (NBRC31327), RutC-30 strain (ATCC56765), CL-847 strain (Enzyme. Microbiol. Technol., 10, 341-346 (1988)), MCG77 strain (Biotechnol. Bioeng. Symp., 8, 89 (1978)), and MCG80 strain (Biotechnol. Bioeng., 12, 451-459 (1982)). QM6a strain, QM9414 strain, and QM9123 strain are available from NBRC (NITE Biological Resource Center), and PC-3-7 strain and RutC-30 strain are available from ATCC (American Type Culture Collection).

[0012]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 is a polypeptide possessed by *Trichoderma reesei*, and in National Center for Biotechnology Information, this polypeptide has been registered as a predicted protein, partial (EGR45828) that *Trichoderma reesei* QM6a strain has. The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 is a polypeptide whose function is unknown, but Conserved Domain Architecture Retrieval Tool of National Center for Biotechnology Information discloses that the 86th to 186th amino acid residues from the N-terminal side are a heat shock factor (HSF)-type DNA-binding domain. The HSF-type DNA-binding domain is known to have the function of binding to an upstream region of a gene encoding an HSF, which is a transcription factor controlling the expression of heat shock proteins (Cell, 65(3), 363-366 (1991)). Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 include the base sequence represented by SEQ ID NO: 1.

[0013]

Examples of methods for eliminating or reducing the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 include a method for introducing a mutation that causes a total deletion of an HSF-type DNA-binding domain, a partial deletion of an HSF-type DNA-binding domain, or a total deletion of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4. Specific examples thereof include a method in which a frameshift mutation or a stop codon mutation is

introduced into a gene sequence encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4, by a deletion, insertion, substitution, etc. of a base. [0014]

The phrase “deletion of an HSF-type DNA-binding domain” means a total or partial loss of the domain, a change of the whole or some of the domain into different amino acid(s), or a combination of these. More specifically, that phrase means that the amino acid sequence represented by SEQ ID NO: 4 comes to have a sequence identity of 80% or less with respect to the amino acid sequence of the HSF-type DNA-binding domain. The sequence identity thereto is preferably 50% or less, more preferably 20% or less, more preferably 10% or less, more preferably 5% or less, more preferably 3% or less, more preferably 1% or less, and most preferably 0%. [0015]

Specific examples of the case where the function of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 is eliminated or reduced by a mutation such as deletion, substitution, or addition, that has occurred in an amino acid sequence located in the HSF-type DNA-binding domain include a mutation in the amino acid sequence represented by SEQ ID NO: 4 which results in a partial or total loss of the region ranging from the 86th to 186th residues from the N-terminal side, which corresponds to the HSF-type DNA-binding domain. Specific examples of such mutations include a mutation in the base sequence represented by SEQ ID NO: 1 which causes a frameshift including insertion of one base residue of guanine into the 85th position. This mutation changes the 30th amino acid residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 4 from histidine to threonine. The succeeding frameshift causes the translation to end at the 90th amino acid residue from the N-terminal side and results in loss of the amino acid sequence constituting the HSF-type DNA-binding domain. [0016]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 is a polypeptide possessed by *Trichoderma reesei*, and in National Center for Biotechnology Information, this polypeptide has been registered as a predicted protein (EGR47155) that *Trichoderma reesei* QM6a strain has. The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 is a polypeptide whose function is unknown, but Conserved Domain Architecture Retrieval Tool of National Center for Biotechnology Information discloses that the 362nd to 553rd amino acid residues from the N-

are a TLD domain. The function of the TLD domain is unknown. Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 include the base sequence represented by SEQ ID NO: 2.

[0017]

5 Examples of methods for reducing the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 include a method for introducing a mutation that causes a total deletion of a TLD domain, a partial deletion of a TLD domain, or a total deletion of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5. Specific examples thereof include a method in which a frameshift mutation or a
10 stop codon mutation is introduced into a gene sequence encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5, by a deletion, insertion, substitution, etc. of a base.

[0018]

The phrase “deletion of a TLD domain” means a total or partial loss of the domain,
15 a change of the whole or some of the domain into different amino acid(s), or a combination of these. More specifically, that phrase means that the amino acid sequence represented by SEQ ID NO: 5 comes to have a sequence identity of 80% or less with respect to the amino acid sequence of the TLD domain. The sequence identity thereto is preferably 50% or less, more preferably 20% or less, more preferably 10% or less, more preferably 5% or less, more
20 preferably 3% or less, more preferably 1% or less, and most preferably 0%.

[0019]

Specific examples of the case where the function of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 is eliminated by a mutation such as deletion, substitution, or addition, that has occurred in an amino acid sequence located in the
25 TLD domain include a mutation in the amino acid sequence represented by SEQ ID NO: 5 which results in a partial or total loss of the region ranging from the 362nd to 553rd residues from the N-terminal side, which corresponds to the TLD domain. Specific examples of such mutations include a frameshift mutation in the base sequence represented by SEQ ID NO: 2 which causes insertion of the 46 base residues represented by SEQ ID NO: 27 into the 6th
30 position. This mutation changes the glutamine residue at the 3rd residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 5 into arginine. The translation ends at this position, and the amino acid sequence constituting the TLD domain is lost.

[0020]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 is a polypeptide possessed by *Trichoderma reesei*, and in National Center for Biotechnology Information, this polypeptide has been registered as a predicted protein (EGR48056) that *Trichoderma reesei* QM6a strain has. The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 is a polypeptide whose function is unknown, but Conserved Domain Architecture Retrieval Tool of National Center for Biotechnology Information discloses that the 130th to 172nd amino acid residues from the N-terminal side are an F-box domain. The F-box domain is known to be a domain present in proteins which control the cell cycle (Proc. Natl. Acad. Sci., 95, 2417-2422 (1998)). Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 include the base sequence represented by SEQ ID NO: 3.

[0021]

Examples of methods for reducing the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 include a method for introducing a mutation that causes a total deletion of an F-box domain, a partial deletion of an F-box domain, or a total deletion of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6. Specific examples thereof include a method in which a frameshift mutation or a stop codon mutation is introduced into a gene sequence encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6, by a deletion, insertion, substitution, etc. of a base.

[0022]

The phrase "deletion of an F-box domain" means a total or partial loss of the domain, a change of the whole or some of the domain into different amino acid(s), or a combination of these. More specifically, that phrase means that the amino acid sequence represented by SEQ ID NO: 6 comes to have a sequence identity of 80% or less with respect to the amino acid sequence of the F-box domain. The sequence identity thereto is preferably 50% or less, more preferably 20% or less, more preferably 10% or less, more preferably 5% or less, more preferably 3% or less, more preferably 1% or less, and most preferably 0%.

[0023]

Specific examples of the case where the function of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 is eliminated by a mutation such as

F-box domain include a frameshift mutation in the base sequence represented by SEQ ID NO: 3 which results in a loss of one cytosine base residue which is the 499th residue. This mutation changes the 167th amino acid residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 6 from arginine to alanine. The succeeding frameshift causes the translation to end at the 193rd residue from the N-terminal side, resulting in loss of the amino acid sequence constituting the F-box domain.

[0024]

Another method which may be used for reducing the function of the polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 4 to 6 is to introduce a mutation which diminishes or inhibits the expression of the polypeptide. Specifically, the mutation which diminishes or inhibits the expression of the polypeptide may be one introduced into the promoter or terminator region of a gene encoding the amino acid sequence represented by any of SEQ ID NOs: 4 to 6. In general, the promoter and terminator regions correspond to a region of hundreds of bases in length before and after the gene participating in transcription.

[0025]

For introducing such mutations into the gene, use can be made of existing genetic mutation methods such as a mutation treatment with a mutagen known to a person skilled in the art or with UV irradiation, etc., gene recombination such as homologous recombination using a selection marker, and a mutation by a transposon.

[0026]

The mutant strain of the present invention is only required to satisfy that the function of at least one or more polypeptides among polypeptides consisting of the amino acid sequences of SEQ ID NOs: 4 to 6 has been eliminated or reduced. The function of two or all of these polypeptides may have been eliminated or reduced. There are no particular limitations on combinations of polypeptides whose functions have been eliminated or reduced, and any of the following mutant strains are included in the mutant strain of the present invention: a mutant strain of *Trichoderma reesei* in which the functions of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 4 and 5 have been eliminated or reduced; a mutant strain of *Trichoderma reesei* in which the functions of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 4 and 6 have been eliminated or reduced; and a mutant strain of *Trichoderma reesei* in which the functions of the polypeptides consisting of the amino acid sequences represented by SEQ

ID NOs: 5 and 6 have been eliminated or reduced.

[0027]

The mutant strain of the present invention may be a mutant strain in which the functions of all the three polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 4 to 6 have been eliminated or reduced. The mutant strain in which the functions of all the three polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 4 to 6 have been eliminated or reduced can be acquired by subjecting spores of *Trichoderma reesei* as a parent strain to a genetic mutation treatment with nitrosoguanidine (NTG), ethylmethanesulfonic acid (EMS), UV, etc., and analyzing the genes of the resultant mutant strains to collect a mutant strain having the mutation by screening.

[0028]

Since the mutant strain of the present invention has an enhanced protein-producing ability as compared with the parent strain into which the mutation has not been introduced, a culture solution of the mutant strain of the present invention has a higher protein concentration than a culture solution obtained by cultivating the parent strain not having the mutation under the same cultivation conditions. In the case where the protein is an enzyme, the enzyme has enhanced specific activity. The increasing rate in protein concentration and the increasing rate in enzyme specific activity are not particularly limited so long as the concentration and the specific activity have increased. It is, however, preferable that the increasing rates are 20% or larger.

[0029]

Besides having a mutation which eliminates or reduces the function of the polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 4 to 6, the mutant strain of the present invention may have a mutation which improves protein production amount and/or lowers the viscosity of culture solutions to inhibit the degree of saturation of oxygen dissolved in the culture solutions from decreasing. Specific examples thereof include a genetic mutation which reduces the function of the polypeptide represented by any of SEQ ID NOs: 7, 9, and 11.

[0030]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 7 is a polypeptide possessed by *Trichoderma reesei* and has been registered at National Center for Biotechnology Information as predicted protein EGR50654 possessed by *Trichoderma reesei* QM6a strain. The polypeptide consisting of the amino acid sequence represented by

deletion, substitution, or addition, that has occurred in an amino acid sequence located in the SEQ ID NO: 7 is a polypeptide whose function is unknown, but Conserved Domain Architecture Retrieval Tool of National Center for Biotechnology Information discloses that the 95th to 277th amino acid residues from the N-terminal side have Middle domain of eukaryotic initiation factor 4G domain (hereinafter referred to as MIF4G domain) and the 380th to 485th amino acid residues from the N-terminal side have MA-3 domain. The two domains, MIF4G and MA-3, are known to have the function of binding to DNAs or RNAs (Biochem., 44, 12265-12272 (2005); Mol. Cell. Biol., 1, 147-156 (2007)). It is presumed from those disclosures that the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 7 at least has the function of binding to a DNA and/or an RNA.

[0031]

Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 7 include the base sequence represented by SEQ ID NO: 8. Examples of genetic mutations which reduce the function of EGR50654 include a total deletion of the MIF4G domain and/or MA-3 domain possessed by EGR50654, a partial deletion of the MIF4G domain and/or MA-3 domain, and a genetic mutation which changes the configuration relationship between the MIF4G domain and the MA-3 domain. Furthermore, the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 7 can be reduced also by introducing a mutation which diminishes or inhibits the expression of the polypeptide. Specific examples of the deletion of the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 7 include a mutation in the base sequence represented by SEQ ID NO: 8 which deletes any of the 1,039th to 1,044th bases.

[0032]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 9 is a polypeptide possessed by *Trichoderma reesei* and has been registered at National Center for Biotechnology Information as predicted protein EGR44419 possessed by *Trichoderma reesei* QM6a strain. The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 9 is a polypeptide whose function is unknown, but Conserved Domain Architecture Retrieval Tool of National Center for Biotechnology Information discloses that the 26th to 499th amino acid residues from the N-terminal side have a Sugar (and other) Transporter domain. It is presumed from this disclosure that the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 9 at least participates in transport of sugar between the inside and the outside of the fungus bodies.

[0033]

Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 9 include the base sequence represented by SEQ ID NO: 10. Examples of genetic mutations which reduce the function of EGR44419 include a total deletion of the Sugar (and other) Transporter domain possessed by EGR44419, a partial deletion of the Sugar (and other) Transporter domain, and a genetic mutation which changes the configuration relationship of the Sugar (and other) Transporter domain. Furthermore, the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 9 can be reduced also by introducing a mutation which diminishes or inhibits the expression of the polypeptide. Specific examples of the deletion of the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 9 include a mutation in the base sequence represented by SEQ ID NO: 10 which inserts 11 bases at the 1,415th position.

[0034]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 11 is a polypeptide possessed by *Trichoderma reesei* and has been registered at National Center for Biotechnology Information as EGR48910 of a beta-adaptin large subunit possessed by *Trichoderma reesei* QM6a strain. The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 11 is one of the proteins that constitute adaptor proteins, which bind to clathrin which is widely conserved in eucaryotes, and which constitute vesicles that take part in transport inside and outside the cells and inside and outside the fungus bodies (Proc. Nati. Acad. Sci. USA., 101, 14108-14113 (2004)).

[0035]

Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 11 include the base sequence represented by SEQ ID NO: 12. Examples of genetic mutations for EGR48910 include a mutation in the base sequence represented by SEQ ID NO: 12 which changes the cytosine at the 1,080th base into adenine.

[0036]

The present invention further relates to a method of protein production including a step of cultivating the mutant strain.

[0037]

In the method of protein production of the present invention, the protein-producing

ability is further enhanced by cultivating the mutant strain in a culture medium containing an inducer. Examples of inducers improving the producibility of, in particular, cellulases include lactose, glucose, cellulose, cellobiose, and xylan. Preferred of these are lactose and/or glucose. Especially preferred is lactose.

5 [0038]

In the case of adding an inducer to the culture medium, the inducer may be added at any timing, for example, at initiation of the cultivation or in the middle of the cultivation. However, especially in cases when lactose or glucose is added as an inducer, it is preferred to add the inducer in the middle of the cultivation because this addition enables the effect of improving the protein-producing ability to continue during the cultivation. The amount of lactose to be added per day per L of the culture solution is preferably about 1 g to 50 g, more preferably about 3-25 g, especially preferably about 6-20 g. The amount of glucose to be added per day per L of the culture solution is preferably about 1-200 g, more preferably about 5-100 g, especially preferably about 20-80 g.

15 [0039]

In the case of adding cellulose, cellobiose, or xylan as an inducer, biomass containing any of these may be added as an inducer. Specific examples of such biomass include not only plants such as seed plant, pteridophyte, bryophyte, algae, and water plant, but also waste building materials. The seed plants are classified into gymnosperms and angiosperms, and both can be used favorably. The angiosperms are further classified into monocotyledons and dicotyledons. Specific examples of the monocotyledons include bagasse, switchgrass, napier grass, erianthus, corn stover, corncob, rice straw, and wheat straw, and preferred specific examples of the dicotyledons include beet pulp, eucalyptus, oak, and white birch.

25 [0040]

In the case of using such biomass as an inducer, the biomass may be a pretreated to be used. Methods for the pretreatment are not particularly limited, but, for example, known methods such as acid treatment, sulfuric acid treatment, dilute sulfuric acid treatment, alkali treatment, hydrothermal treatment, subcritical treatment, fine grinding treatment, and steaming treatment can be used. Pulp may be used as the biomass containing cellulose or xylan, which has been subjected to such a pretreatment.

[0041]

The composition of the culture medium to be used in the step of cultivating the

mutant strain of *Trichoderma reesei* of the present invention is not particularly limited as long as it is a culture medium composition where the *Trichoderma reesei* can produce a protein, and a known culture medium composition for filamentous fungi of the genus *Trichoderma* can be employed. As a nitrogen source, use can be made, for example, of polypeptone, bouillon, CSL, or soybean cake. The inducer for protein production may be added to the culture medium.

[0042]

Methods for the cultivation are not particularly limited. For example, the mutant strain can be cultivated by liquid culture in which a centrifuge tube, flask, jar fermenter, tank, or the like is used or solid culture in which a plate or the like is used. It is preferred to cultivate *Trichoderma reesei* under aerobic conditions, and especially preferred among those cultivation methods is submerged culture performed in a jar fermenter or a tank while conducting aeration or stirring. The air flow rate is preferably about 0.1-2.0 vvm, more preferably 0.3-1.5 vvm, especially preferably 0.5-1.0 vvm. The cultivation temperature is preferably about 25-35°C, more preferably 25-31°C. The pH conditions during the cultivation are preferably pH 3.0-7.0, more preferably pH 4.0-6.0. As for cultivation time, the cultivation is conducted under conditions capable of protein production, until the protein is accumulated in a recoverable amount. The cultivation period is usually 24-288 hours, preferably 24-240 hours, more preferably 36-240 hours, still more preferably 36-192 hours.

[0043]

Although the protein to be produced in the present invention is not particularly limited, proteins to be excreted from the fungus bodies can be efficiently produced. Preferred of these are enzymes. More preferred are saccharifying enzymes such as cellulases, amylases, invertases, chitinases, and pectinases. Still more preferred are cellulases.

[0044]

Cellulases that can be produced in the present invention include various hydrolases, which include enzymes having a decomposition activity against xylan, cellulose, and hemicellulose. Specific examples thereof include cellobiohydrolase (EC 3.2.1.91) which produces cellobiose by hydrolyzing cellulose chains, endoglucanase (EC 3.2.1.4) which hydrolyzes cellulose chains from central portions thereof, β -glucosidase (EC 3.2.1.21) which hydrolyzes cellooligosaccharide and cellobiose, xylanase (EC 3.2.1.8) which is characterized by acting on hemicellulose and, in particular, on xylan, and β -xylosidase (EC 3.2.1.37) which

hydrolyzes xylooligosaccharide.

[0045]

Improvement in protein-producing ability or improvement in cellulase specific activity of the *Trichoderma reesei* mutant strain of the present invention as compared with the parent strain is ascertained by comparing culture solutions obtained by cultivating the mutant strain and the parent strain under the same conditions in protein concentration or in one or more specific activities selected from the group consisting of β -glucosidase specific activity, β -xylosidase specific activity, and cellobiohydrolase specific activity, the protein concentration and the specific activities being determined by the following methods.

[0046]

The protein concentration is determined in the following manner. Culture solutions of the mutant strain and parent strain are each centrifuged at 15,000×g for 10 minutes to obtain a supernatant. To 250 μ L of Quick Start Bradford protein assay (manufactured by Bio-Rad Laboratories, Inc.) is added 5 μ L of a diluted cellulase solution. The mixture is allowed to stand still at room temperature for 15 minutes and then examined for absorbance at 595 nm. The concentration of the protein contained in the saccharifying-enzyme solution is calculated on the basis of a calibration curve obtained using bovine serum albumin solutions as reference solutions.

[0047]

The β -glucosidase specific activity is determined by the following method. First, for the supernatant of the culture solution, 10 μ L of an enzyme dilution is added to 90 μ L of 50 mM acetate buffer containing 1 mM p-nitrophenyl- β -glucopyranoside (produced by Sigma-Aldrich Japan), and the mixture is allowed to react at 30°C for 10 minutes. Then, 10 μ L of 2 M sodium carbonate is added and mixed well to stop the reaction, and the increase in absorbance at 405 nm is measured. Finally, release of 1 μ mol of p-nitrophenol per minute is defined as 1 U of activity to calculate the specific activity.

[0048]

The β -xylosidase specific activity is determined by the following method. First, for the supernatant of the culture solution, 10 μ L of an enzyme dilution is added to 90 μ L of 50 mM acetate buffer containing 1 mM p-nitrophenyl- β -xylopyranoside (produced by Sigma-Aldrich Japan), and the mixture is allowed to react at 30°C for 30 minutes. Then, 10 μ L of 2 M sodium carbonate is added and mixed well to stop the reaction, and the increase in absorbance at 405 nm is measured. Finally, release of 1 μ mol of p-nitrophenol per minute is

defined as 1 U of activity to calculate the specific activity.

[0049]

The cellobiohydrolase specific activity is determined by the following method.

First, for the supernatant of the culture solution, 10 μ L of an enzyme dilution is added to 90 μ L of 50 mM acetate buffer containing 1 mM p-nitrophenyl- β -lactopyranoside (produced by Sigma-Aldrich Japan), and the mixture is allowed to react at 30°C for 60 minutes. Then, 10 μ L of 2 M sodium carbonate is added and mixed well to stop the reaction, and the increase in absorbance at 405 nm is measured. Finally, release of 1 μ mol of p-nitrophenol per minute is defined as 1 U of activity to calculate the specific activity.

[0050]

Methods for recovering a protein contained in the culture solution where the mutant strain has been cultivated are not particularly limited, but the protein can be recovered by removing the fungus bodies of the mutant strain from the culture solution. Examples of methods for removing the fungus bodies include centrifugation, membrane separation, and filter press.

[0051]

Furthermore, in the case where the culture solution in which the mutant strain has been cultivated is used as a protein solution without removing the fungus bodies therefrom, the culture solution is preferably treated so that the mutant strain cannot grow therein.

Examples of treatment methods for preventing the fungus bodies from growing include heat treatment, chemical treatment, acid/alkali treatment, and UV treatment.

[0052]

In the case where the protein is an enzyme, the culture solution from which the fungus bodies have been removed or which has been treated so that the fungus bodies cannot grow therein, as stated above, can be used directly as an enzyme solution.

[0053]

In the case where the protein produced by the method of the present invention is a cellulase, this cellulase can be used to saccharify cellulose-containing biomass to produce a sugar. The cellulase obtained by cultivating the mutant strain is high especially in β -glucosidase specific activity as compared with the cellulase obtained by cultivating the parent strain into which the mutation has not been introduced, and can hence efficiently decompose the cellulose-containing biomass to obtain a sugar solution having a high glucose concentration. Thus, a larger quantity of sugar can be obtained.

[0054]

The cellulose-containing biomass to be used in the present invention can be either the same biomass as the cellulose-containing biomass mentioned above as an inducer or a pretreated biomass.

5 [0055]

Conditions for the saccharification reaction are not particularly limited. The saccharification reaction temperature is preferably in the range of 25-60°C, more preferably in the range of 30-55°C. The saccharification reaction time is preferably in the range of 2-200 hours. The pH in the saccharification reaction is preferably in the range of 3.0-7.0, more preferably in the range of 4.0-6.0. In the case of cellulases derived from the genus *Trichoderma*, the optimal pH for the reaction is 5.0. Furthermore, since the pH changes during the hydrolysis, it is preferred to add a buffer to the reaction solution or to conduct the reaction while keeping the pH constant by using an acid or an alkali.

[0056]

15 In the case where the enzyme is separated and recovered from the saccharified solution, use can be made of a method in which the saccharified solution is filtered with an ultrafiltration membrane or the like to recover the enzyme on the non-permeation side. According to need, a step of removing solid matter from the saccharified solution may be conducted before the filtration. The recovered enzyme can again be used for a
20 saccharification reaction.

EXAMPLES

[0057]

The present invention is described specifically below by referring to Examples.

25 [0058]

<Reference Example 1> Conditions for Protein Concentration Measurement

Protein concentration measuring reagent used: Quick Start Bradford protein assay (produced by Bio-Rad Laboratories, Inc.)

Measuring conditions

30 Measuring temperature: room temperature

Protein concentration measuring reagent: 250 µL

Culture solution of filamentous fungus: 5 µL

Reaction time: 5 min

Absorbance: 595 nm

Standard: BSA

[0059]

<Reference Example 2> Conditions for Determination of Specific Activity of Cellulases

5 (Conditions for Determination of β -Glucosidase Specific Activity)

Substrate: p-nitrophenyl- β -glucopyranoside (produced by Sigma-Aldrich Japan)

Reaction solution: 90 μ L of 50 mM acetate buffer containing 1 mM p-nitrophenyl- β -glucopyranoside

Enzyme dilution: 10 μ L

10 Reaction temperature: 30°C

Reaction time: 10 min

Reaction terminator: 10 μ L of 2 M sodium carbonate

Absorbance: 405 nm

[0060]

15 (Conditions for Determination of β -Xylosidase Specific Activity)

Substrate: p-nitrophenyl- β -xylopyranoside (produced by Sigma-Aldrich Japan)

Reaction solution: 90 μ L of 50 mM acetate buffer containing 1 mM p-nitrophenyl- β -xylopyranoside

Enzyme dilution: 10 μ L

20 Reaction temperature: 30°C

Reaction time: 10 min

Reaction terminator: 10 μ L of 2 M sodium carbonate

Absorbance: 405 nm

[0061]

25 (Conditions for Determination of Cellobiohydrolase Specific Activity)

Substrate: p-nitrophenyl- β -lactopyranoside (produced by Sigma-Aldrich Japan)

Reaction solution: 90 μ L of 50 mM acetate buffer containing 1 mM p-nitrophenyl- β -lactopyranoside

Enzyme dilution: 10 μ L

30 Reaction temperature: 30°C

Reaction time: 10 min

Reaction terminator: 10 μ L of 2 M sodium carbonate

Absorbance: 405 nm

[0062]

<Reference Example 3> Saccharification Test of Cellulose-Containing Biomass

As cellulose-containing biomass, use was made of Arbocel (registered trademark) B800 (produced by J. Rettenmaier & Sohne) or bagasse powdered to an average particle diameter of 100 μm . As an enzyme solution, use was made of a filtrate obtained by collecting a 1 mL portion of a culture solution of either a *Trichoderma reesei* mutant strain or the *Trichoderma reesei* parent strain into which the mutation had not been introduced, centrifuging the collected culture solution, recovering a supernatant from which the fungus bodies had been removed, and filtering the supernatant with a 0.22- μm filter.

[0063]

(Saccharification Reaction)

100 μL of a 1 M sodium acetate buffer was used as a buffer for saccharification reaction; 2 μL of 50 g/L erythromycin solution was used for preventing the propagation of various germs; and 0.1 g of Arbocel (registered trademark) B800 (produced by J. Rettenmaier & Sohne) or bagasse powdered to an average particle diameter of 100 μm was used as a material to be saccharified. Enzyme solutions were prepared in the following manner. An enzyme solution obtained by flask cultivation using Arbocel (registered trademark) B800 was introduced in an amount of 150 μL into a measuring cylinder. An enzyme solution obtained by flask cultivation using lactose was introduced in an amount of 300 μL into a measuring cylinder. An enzyme solution obtained by 5 L jar fermenter cultivation was introduced into a measuring cylinder in such an amount as to result in a protein concentration of 0.8 mg. The enzyme solution in each measuring cylinder was diluted with sterilized water to 1 mL in total, and the dilution was introduced into a 2 mL tube. A saccharification reaction was conducted under temperature conditions of 50°C for 24 hours, and the saccharification mixture was centrifuged. The resultant supernatant was recovered as a saccharified solution, and the enzymatic reaction was terminated by adding 1 N NaOH solution in an amount of one-tenth the amount of the recovered saccharified solution. The glucose concentration in the saccharified solution after the termination of the reaction was determined by the UPLC shown below.

[0064]

(Determination of Glucose Concentration)

Glucose was quantitatively analyzed under the following conditions using ACQUITY UPLC System (Waters). The quantitative analysis was performed on the basis of

a calibration curve drawn with standard solutions of glucose.

Column: ACQUITY UPLC BEH Amide 1.7 μ m 2.1 $\times$ 100 mm Column

Separation method: HILIC

Mobile phase: mobile phase A: 80% acetonitrile, 0.2% aqueous TEA solution, and mobile phase B: 30% acetonitrile, 0.2% aqueous TEA solution, in accordance with the following gradient. The gradient was a linear gradient reaching the mixing ratio corresponding to the time below.

Initiation condition: (A 99.90%, B 0.10%), 2 minutes after initiation: (A 96.70%, B 3.30%), 3.5 minutes after initiation: (A 95.00%, B 5.00%), 3.55 minutes after initiation: (A 99.90%, B 0.10%), 6 minutes after initiation: (A 99.90%, B 0.10%)

Detection method: ELSD (evaporative light scattering detector)

Flow rate: 0.3 mL/min

Temperature: 55°C

[0065]

<Example 1>

Preparation of *Trichoderma reesei* mutant strain in which the function of polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 has been eliminated (Method of preparing Mutant Strain)

A *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 has been eliminated is prepared in the following manner. A gene represented by SEQ ID NO: 1 which encodes the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 is destroyed by replacing the gene with acetamide as a selection marker and with acetamidase (AmdS) gene (amdS) capable of decomposing acetamide as a selection marker gene. A DNA fragment consisting of the gene sequence represented by SEQ ID NO: 13 is prepared in order to eliminate the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4, and *Trichoderma reesei* ATCC66589 strain is transformed with the DNA fragment, thereby preparing the *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4 has been eliminated. By this method, a *Trichoderma reesei* mutant strain is obtained in which the base sequence represented by SEQ ID NO: 1 has been deleted. For allowing a DNA fragment consisting of the base sequence represented by SEQ ID NO: 1 to be introduced

introduction is prepared so as to add a portion homologous to the gene sequence of the *Trichoderma reesei* QM9414 strain.

[0066]

Specifically, PCR is conducted using genomic DNA extracted in a usual manner from the *Trichoderma reesei* QM9414 strain and oligo DNAs represented by SEQ ID NOs: 14 and 15, and the resulting amplified fragment is treated with restriction enzymes AflII and NotI to obtain a DNA fragment for use as the upstream DNA fragment. In addition, PCR is conducted using oligo DNAs represented by SEQ ID NOs: 16 and 17, and the resulting amplified fragment is treated with restriction enzymes MluI and SpeI to obtain a DNA fragment for use as the downstream DNA fragment. The upstream and downstream DNA fragments are introduced into a plasmid to which amdS has been inserted by using restriction enzymes AflII and NotI and restriction enzymes MluI and SpeI, respectively, to construct a plasmid for mutation introduction. The plasmid for mutation introduction is treated with restriction enzymes AflII and SpeI, and the *Trichoderma reesei* ATCC66589 strain is transformed with the obtained DNA fragment which is shown by SEQ ID NO: 13. The manipulations involving the molecular biological technique are performed as described in Molecular cloning, laboratory manual, 1st, 2nd, 3rd (1989). In addition, the transformation is carried out using a standard technique, i.e., a protoplast PEG method, and specifically, is performed as described in Gene, 61, 165-176 (1987).

[0067]

(Preparation and Evaluation of the Mutant Strain)

The *Trichoderma reesei* mutant strain obtained by the method described above was used as *Trichoderma reesei* mutant strain I in the following protein production test and experiments for determining protein concentration and cellulase specific activity.

[0068]

<Example 2>

Preparation of *Trichoderma reesei* mutant strain in which the function of polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 has been eliminated (Method of preparing Mutant Strain)

A *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 has been eliminated is prepared in the following manner. A gene represented by SEQ ID NO: 2 which encodes the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 is destroyed

by replacing the gene with acetamide as a selection marker and with acetamidase (AmdS) gene (amdS) capable of decomposing acetamide as a selection marker gene. A DNA fragment consisting of the gene sequence represented by SEQ ID NO: 18 is prepared in order to eliminate the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5, and *Trichoderma reesei* ATCC66589 strain is transformed with the DNA fragment, thereby preparing the *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 has been eliminated. By this method, a *Trichoderma reesei* mutant strain is obtained in which the base sequence represented by SEQ ID NO: 2 has been deleted. For allowing a DNA fragment consisting of the base sequence represented by SEQ ID NO: 2 to be introduced upstream and downstream an amdS-containing DNA sequence, a plasmid for mutation introduction is prepared so as to add a portion homologous to the gene sequence of the *Trichoderma reesei* QM9414 strain.

[0069]

Specifically, a synthesized DNA fragment shown by SEQ ID NO: 19 is treated with restriction enzymes AflII and NotI to obtain a DNA fragment for use as the upstream DNA fragment. In addition, PCR is conducted using genomic DNA extracted in a usual manner from the *Trichoderma reesei* QM9414 strain and oligo DNAs represented by SEQ ID NOs: 20 and 21, and the resulting amplified fragment is treated with restriction enzymes MluI and SpeI to obtain a DNA fragment for use as the downstream DNA fragment. The upstream and downstream DNA fragments are introduced into a plasmid to which amdS has been inserted by using restriction enzymes AflII and NotI and restriction enzymes MluI and SpeI, respectively, to construct a plasmid for mutation introduction. The plasmid for mutation introduction is treated with restriction enzymes AflII and SpeI, and the *Trichoderma reesei* ATCC66589 strain is transformed with the obtained DNA fragment which is shown by SEQ ID NO: 18. The manipulations involving the molecular biological technique are performed as described in Molecular cloning, laboratory manual, 1st, 2nd, 3rd (1989). In addition, the transformation is carried out using a standard technique, i.e., a protoplast PEG method, and specifically, is performed as described in Gene, 61, 165-176 (1987).

[0070]

(Preparation and Evaluation of the Mutant Strain)

The *Trichoderma reesei* mutant strain obtained by the method described above was used as *Trichoderma reesei* mutant strain II in the following protein production test and

experiments for determining protein concentration and cellulase specific activity.

[0071]

<Example 3>

Preparation of *Trichoderma reesei* mutant strain in which the function of polypeptide

- 5 consisting of the amino acid sequence represented by SEQ ID NO: 6 has been eliminated
(Method of preparing Mutant Strain)

A *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 has been eliminated is prepared in the following manner. A gene represented by SEQ ID NO: 3 which encodes the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 is destroyed
10 by replacing the gene with acetamide as a selection marker and with acetamidase (AmdS) gene (amdS) capable of decomposing acetamide as a selection marker gene. A DNA fragment consisting of the gene sequence represented by SEQ ID NO: 22 is prepared in order to eliminate the function of the polypeptide consisting of the amino acid sequence represented
15 by SEQ ID NO: 6, and *Trichoderma reesei* ATCC66589 strain is transformed with the DNA fragment, thereby preparing the *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6 has been eliminated. By this method, a *Trichoderma reesei* mutant strain is obtained in which the base sequence represented by SEQ ID NO: 3 has been deleted. For allowing a DNA
20 fragment consisting of the base sequence represented by SEQ ID NO: 3 to be introduced upstream and downstream an amdS-containing DNA sequence, a plasmid for mutation introduction is prepared so as to add a portion homologous to the gene sequence of the *Trichoderma reesei* QM9414 strain.

[0072]

- 25 Specifically, PCR is conducted using genomic DNA extracted in a usual manner from the *Trichoderma reesei* QM9414 strain and oligo DNAs represented by SEQ ID NOs: 23 and 24, and the resulting amplified fragment is treated with restriction enzymes AflII and NotI to obtain a DNA fragment for use as the upstream DNA fragment. In addition, PCR is conducted using oligo DNAs represented by SEQ ID NOs: 25 and 26, and the resulting
30 amplified fragment is treated with restriction enzymes MluI and XhoI to obtain a DNA fragment for use as the downstream DNA fragment. The upstream and downstream DNA fragments are introduced into a plasmid to which amdS has been inserted by using restriction enzymes AflII and NotI and restriction enzymes MluI and XhoI, respectively, to construct a

plasmid for mutation introduction. The plasmid for mutation introduction is treated with restriction enzymes AflII and SpeI, and the *Trichoderma reesei* ATCC66589 strain is transformed with the obtained DNA fragment which is shown by SEQ ID NO: 22. The manipulations involving the molecular biological technique are performed as described in Molecular cloning, laboratory manual, 1st, 2nd, 3rd (1989). In addition, the transformation is carried out using a standard technique, i.e., a protoplast PEG method, and specifically, is performed as described in Gene, 61, 165-176 (1987).

[0073]

(Preparation and Evaluation of the Mutant Strain)

The *Trichoderma reesei* mutant strain obtained by the method described above was used as *Trichoderma reesei* mutant strain III in the following protein production test and experiments for determining protein concentration and cellulase specific activity.

[0074]

<Example 4>

Protein Production Test Using *Trichoderma reesei* Mutant Strains

(Preculture)

After spores of each of the *Trichoderma reesei* mutant strains prepared in Examples 1 to 3 are diluted with physiological saline to be 1.0×10^7 /mL, 2.5 mL of the diluted spore solution is inoculated into 250 mL of the preculture medium shown in Table 1 which has been placed in a 1 L baffled flask, and is incubated on a shaker under the conditions of 28°C and 120 rpm for 72 hours. *Trichoderma reesei* ATCC66589 strain is used as a control to conduct the same experiments shown below.

[0075]

[Table 1]

Glucose	20 g
5× Mandel's solution*	200 mL
10× Ammonium tartrate solution**	100 mL
Corn steep liquor	50 g
Trace element solution***	1 mL
Tween 80	0.5 mL
PE-M	1 mL
	(per 1 L)

* The 5×Mandel's solution has the following composition.

7 g/L $(\text{NH}_4)_2\text{SO}_4$

10 g/L KH_2PO_4

2 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

1.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

5 ** The 10× Ammonium tartrate solution contains 92 g/L ammonium tartrate.

*** The trace element solution has the following composition.

0.3 g/L H_3BO_3

1.3 g/L $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$

5 g/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$

10 2 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

0.4 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$

10 g/L ZnCl_2

[0076]

(Main Culture)

15 Arbocel (registered trademark) B800 is added to the main-culture medium shown in Table 2, and an investigation of submerged culture is conducted using a 5 L jar fermenter (manufactured by ABLE & Biott Co., Ltd.).

[0077]

20 The preculture solutions of the *Trichoderma reesei* ATCC66589 strain and the *Trichoderma reesei* mutant strains prepared in Examples 1, 2, and 3 are each inoculated in an amount of 200 mL into 2 L of the main-culture medium to which Arbocel (registered tradename) B800 has been added.

[0078]

25 After the inoculation of each preculture medium into the main-culture medium, submerged culture is performed under the cultivation conditions of 28°C, 700 rpm, and an air flow rate of 100 mL/min while regulating the pH to 5.0.

[0079]

[Table 2]

Arbocel B800 (produced by J. Rettenmaier & Sohne)	100 g
5× Mandel's solution*	200 mL
Corn steep liquor	25 g
Trace element solution***	1 mL

Tween 80	0.5 mL
PE-M	1 mL
	(per 1 L)

* Same as in Table 1.

*** Same as in Table 1.

[0080]

(Addition of Liquid Sugar during Main Culture)

5 At 40 hours after initiation of the main culture, the liquid sugar culture medium shown in Table 3 is continuously added to the main-culture solution in an amount of 250 mL per day.

[0081]

[Table 3]

Glucose	500 g
Lactose	125 g
(NH ₄) ₂ SO ₄	8.75 g
	(per 1 L)

10 [0082]

(Collection of Culture Solutions)

 At each of given time periods after initiation of the cultivation, 20 mL portion of each of the main-culture solutions of the *Trichoderma reesei* mutant strains prepared in Examples 1 to 3 is collected. A part of the collected culture solution is centrifuged under the
15 conditions of 15,000×g and 4°C for 10 minutes to obtain a supernatant. The supernatant is filtered with a 0.22 μm filter, and the filtrate is used as a cellulase solution in the following experiments.

[0083]

(Determination of Protein Concentration)

20 The protein concentration of each of the culture solutions of Examples 1 to 3 which have been collected at intervals after initiation of the cultivation is determined under the conditions shown in Reference Example 1. As a result, the *Trichoderma reesei* mutant strains prepared in Examples 1 to 3 give culture solutions having higher protein concentrations, in terms of relative value, than that of the *Trichoderma reesei* ATCC66589
25 strain.

[0084]

(Determination of Enzyme Activities)

The culture solutions of Examples 1 to 3 which have been collected at intervals after initiation of the cultivation are used as enzyme solutions to determine the specific activities of β -glycosidase, β -xylosidase, and cellobiohydrolase under the conditions shown in Reference Example 2. In determining the specific activity, an increase in absorbance at 405 nm is measured, and release of 1 μ mol of the substrate per minute is defined as 1 U of activity to calculate the specific activity. As a result, the culture solutions of the *Trichoderma reesei* mutant strains prepared in Examples 1 to 3 are higher in the three specific activities than the culture solution of the *Trichoderma reesei* ATCC66589 strain.

[0085]

(Flask Cultivation)

Spores of each of the *Trichoderma reesei* mutant strains I to III prepared in Examples 1 to 3 were diluted with physiological saline to be 1.0×10^7 /mL, and 0.1 mL of the resultant spore dilution was inoculated into 10 mL of the flask culture medium containing Arbocel (registered trademark) B800 or lactose shown in Table 4, that had been placed in a 50 mL baffled flask. This spore dilution was incubated on a shaker under the conditions of 28°C and 120 rpm for 120 hours.

[0086]

Furthermore, the *Trichoderma reesei* ATCC66589 strain, which was the parent strain into which any of the mutations of mutant strains I to III had not been introduced, was subjected to 120 hours incubation by the method shown above, as a control for the mutant strains.

[0087]

[Table 4]

Arbocel B800 (produced by J. Rettenmaier & Sohne)	20 g
or	
Lactose (produced by Kanto Chemical Co., Inc.)	20 g
5× Mandel's solution*	200 mL
10× Ammonium tartrate solution**	100 mL
Corn steep liquor	50 g
Trace element solution***	1 mL
Tween 80	0.5 mL
PE-M	1 mL

(per 1 L)

* Same as in Table 1.

** Same as in Table 1.

*** Same as in Table 1.

[0088]

5 (Collection of Culture Solutions)

At 120 hours after initiation of the flask cultivation, 1 mL portion of each culture solution was collected. The culture solution was centrifuged under the conditions of 15,000×g and 4°C for 10 minutes to obtain a supernatant. The supernatant was filtered with a 0.22 µm filter, and the filtrate was used in the following experiments.

10 [0089]

(Determination of Protein Concentration)

In the flask cultivation using Arbocel (registered trademark) B800, in cases when the protein concentration in the culture solution obtained by cultivating the *Trichoderma reesei* ATCC66589 strain was taken as 1, then the relative values of the protein concentrations in the culture solutions of *Trichoderma reesei* mutant strains I, II, and III were 1.1 each. It was thus ascertained that the mutant strains had a higher protein-producing ability than the parent strain.

[0090]

Also in the flask cultivation using lactose, in cases when the protein concentration in the culture solution obtained by cultivating the *Trichoderma reesei* ATCC66589 strain was taken as 1, then the protein concentration for *Trichoderma reesei* mutant strain I was 1.2, for mutant strain II was 1.3, and for mutant strain III was 1.2. It was thus ascertained that the mutant strains had a higher protein-producing ability than the parent strain.

[0091]

25 (Determination of Various Cellulase Specific Activities)

In the flask cultivation using Arbocel (registered trademark) B800, in cases when various cellulase specific activities of the culture solution obtained by cultivating the *Trichoderma reesei* ATCC66589 strain were taken as 1, then the β-glucosidase specific activities were 1.1 for *Trichoderma reesei* mutant strain I, 1.2 for mutant strain II, and 1.1 for mutant strain III, the β-xylosidase specific activity was 1.1 for all the *Trichoderma reesei* mutant strains I, II, and III, and the cellobiohydrolase specific activity was also 1.1 for all the mutant strains I, II, and III. It was thus ascertained that the mutant strains had the

unexpected effect of bringing about improvements in various cellulase specific activities.

[0092]

Also in the flask cultivation using lactose, in cases when various cellulase specific activities of the culture solution obtained by cultivating the *Trichoderma reesei* ATCC66589 strain were taken as 1, then the β -glucosidase specific activities were 1.1 for *Trichoderma reesei* mutant strain I, 1.2 for mutant strain II, and 1.4 for mutant strain III, the β -xylosidase specific activities were 1.1 for *Trichoderma reesei* mutant strain I, 1.4 for mutant strain II, and 1.4 for mutant strain III, and the cellobiohydrolase specific activities were 1.2 for mutant strain I, 1.1 for mutant strain II, and 1.1 for mutant strain III. It was thus ascertained that the mutant strains had the unexpected effect of bringing about improvements in various cellulase specific activities.

[0093]

(Saccharification Reaction Test)

In accordance with the technique described in Reference Example 3, culture solutions collected at 120 hours after initiation of the flask cultivation of *Trichoderma reesei* mutant strains I, II, and III were used as cellulases to conduct a saccharification reaction test of cellulose-containing biomass. As the cellulose-containing biomass, Arbocel (registered trademark) B800 or powdered bagasse was used.

[0094]

As a result, in the saccharification reaction for saccharifying Arbocel (registered trademark) B800, in cases when the glucose concentration in the saccharified solution obtained using the cellulases obtained from the *Trichoderma reesei* ATCC66589 strain by the flask cultivation using lactose was taken as 1, then the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from *Trichoderma reesei* mutant strain I was 1.2, the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from mutant strain II was 1.3, and the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from mutant strain III was 1.1. Meanwhile, in cases when the glucose concentration in the saccharified solution obtained using the cellulases obtained from *Trichoderma reesei* ATCC66589 strain by the flask cultivation using Arbocel (registered trademark) B800 was taken as 1, then the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from *Trichoderma reesei* mutant strain I was 1.1, the relative value of the glucose concentration in the saccharified solution

upstream and downstream an amdS-containing DNA sequence, a plasmid for mutation obtained using the cellulases obtained from mutant strain II was 1.2, and the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from mutant strain III was 1.1.

[0095]

In the saccharification reaction for saccharifying powdered bagasse, in cases when the glucose concentration in the saccharified solution obtained using the cellulases obtained from the *Trichoderma reesei* ATCC66589 strain by the flask cultivation using lactose was taken as 1, then the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from *Trichoderma reesei* mutant strain I was 1.3, the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from mutant strain II was 1.3, and the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from mutant strain III was 1.1. Meanwhile, in cases when the glucose concentration in the saccharified solution obtained using the cellulases obtained from *Trichoderma reesei* ATCC66589 strain by the flask cultivation using Arbocel (registered trademark) B800 was taken as 1, then the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from *Trichoderma reesei* mutant strain I was 1.2, the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from mutant strain II was 1.3, and the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained from mutant strain III was 1.1.

[0096]

It was ascertained from those results that the cellulases produced by each of the *Trichoderma reesei* mutant strains I to III were superior in enzymatic activity to the cellulases produced by the parent strain and hence had an excellent ability to produce glucose from cellulose-containing biomass.

[0097]

<Example 5>

Preparation of *Trichoderma reesei* mutant strain in which the functions of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 4, 5, and 6 have been eliminated

A QM9414-A strain, which was a strain obtained by passage culture of *Trichoderma reesei* QM9414 strain, was subjected to a genetic mutation treatment to acquire a QM9414-B strain as a mutant strain. The genetic mutation treatment was

manner. Spores of the QM9414-A strain were inoculated into the preculture medium shown in Table 1 so that 1.0×10^5 spores were inoculated per mL of the preculture medium. 15 mL of the preculture medium was incubated for a half day and then centrifuged to recover the spores. The recovered spores were suspended in a Tris-maleate buffer (pH 6.0) so as to give a 10 mL spore solution, and 0.5 mL of an NTG solution obtained by dissolution with a Tris-maleate buffer (pH 6.0) so as to result in a concentration of 1.0 g/L was added thereto. The resultant mixture was held at 28°C for 100 minutes to perform the genetic mutation treatment. The spores which had undergone the genetic mutation treatment were recovered by centrifuging, subsequently rinsed with a Tris-maleate buffer (pH 6.0) three times, and finally suspended as genetic-mutation-treated spores in 10 mL of a Tris-maleate buffer (pH 6.0). Subsequently, the genetic-mutation-treated spores were added to an agar medium prepared by adding crystalline cellulose. The size of halos, which surrounded colonies and indicated regions where the crystalline cellulose had been decomposed by cellulases, was used as an index to select a QM9414-B strain which had formed a large halo.

[0098]

The QM9414-B strain was genetically analyzed and, as a result, was ascertained to have undergone the following mutations in the base sequences represented by SEQ ID NOs: 1, 2, and 3.

[0099]

In the base sequence represented by SEQ ID NO: 1, one guanine base residue had been inserted into the 85th position. This mutation changed the 30th amino acid residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 4 from histidine to threonine, and the succeeding frameshifts caused the translation to end at the 90th amino acid residue from the N-terminal side.

[0100]

In the base sequence represented by SEQ ID NO: 2, 46 base residues represented by SEQ ID NO: 27 had been inserted into the 6th position. This mutation changed the glutamine residue at the 3rd residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 5 into arginine and caused the translation to end at that position.

[0101]

In the base sequence represented by SEQ ID NO: 3, the one cytosine base residue at the 499th residue had been deleted. This mutation changed the 167th amino acid residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 6 from

alanine to arginine, and the succeeding frameshifts caused the translation to end at the 193rd position from the N-terminal side.

[0102]

<Example 6>

Protein Production Test Using *Trichoderma reesei* QM9414-B Strain

The mutant strain QM9414-B strain obtained in Example 5 was cultivated in accordance with the (Preculture), (Main Culture), (Addition of Liquid Sugar during Main Culture), and (Collection of Culture Solutions) described in Example 4, and the protein concentration was determined under the conditions shown in Reference Example 1. The parent strain QM9414-A strain was used as a control and cultivated in the same manner as for the QM9414-B strain, and the protein concentration was determined under the conditions shown in Reference Example 1.

[0103]

The produced-protein concentrations at 120 hours after initiation of the cultivation were determined and, as a result, the QM9414-B strain gave an increased relative value which was 1.2 times higher than that of the QM9414-A strain. Likewise, the protein concentrations at 200 hours after initiation of the cultivation were determined. As a result, the QM9414-A strain gave a protein concentration which was unchanged from that obtained at 120 hours after initiation of the cultivation, whereas the QM9414-B strain gave an increased protein concentration which was 1.3 times higher than the protein concentration obtained at 120 hours after initiation of the cultivation.

[0104]

In cases when the protein concentration given by the QM9414-A strain at 120 hours after initiation of the cultivation was taken as 1, the relative value of the protein concentration given by the QM9414-B strain was 1.8. In cases when the protein concentration given by the QM9414-A strain at 185 hours after initiation of the cultivation was taken as 1, the relative value of the protein concentration given by the QM9414-B strain was 2.7.

[0105]

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0106]

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

CLAIMS

[Claim 1]

A mutant strain of *Trichoderma reesei*, the mutant strain having a mutation in a gene encoding a polypeptide consisting of an amino acid sequence represented by SEQ ID NOs:4 to 6 that eliminates expression of a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 4 to 6.

[Claim 2]

The mutant strain according to claim 1, wherein the mutation is a mutation that deletes an HSF-type DNA-binding domain of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 4.

[Claim 3]

The mutant strain according to claim 2, wherein the mutation is a frameshift mutation accompanying a mutation in a region nearer to the N-terminal side than the HSF-type DNA-binding domain.

[Claim 4]

The mutant strain according to claim 3, wherein the mutation is a frameshift mutation due to a mutation in which a histidine residue at the 30th residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 4 is changed to a residue of an amino acid other than histidine.

[Claim 5]

The mutant strain according to claim 1, wherein the mutation is a mutation that deletes a TLD domain of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5.

[Claim 6]

The mutant strain according to claim 5, wherein the mutation is a frameshift mutation accompanying a mutation in a region nearer to the N-terminal side than the TLD domain.

[Claim 7]

The mutant strain according to claim 6, wherein the mutation is a frameshift mutation due to a mutation in which a glutamine residue at the 3rd residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 5 is changed to a residue of an amino acid other than glutamine.

[Claim 8]

The mutant strain according to claim 1, wherein the mutation is a mutation of an amino acid sequence of an F-box domain region of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6.

[Claim 9]

The mutant strain according to claim 8, wherein the mutation is a deletion of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 6, the deletion being caused by a frameshift mutation accompanying a mutation of the amino acid sequence of the F-box domain region.

[Claim 10]

The mutant strain according to claim 9, wherein the mutation is a frameshift mutation due to a mutation in which an arginine residue at the 167th residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 6 is changed to a residue of an amino acid other than arginine.

[Claim 11]

A method of producing a protein, the method comprising a step of cultivating the mutant strain according to any one of claims 1 to 10.

[Claim 12]

A method of producing a protein, the method comprising a step of cultivating the mutant strain according to any one of claims 1 to 10, in a culture medium at least comprising lactose.

[Claim 13]

The method of producing a protein according to claim 11 or 12, wherein, in the cultivation step, at least one selected from the group consisting of lactose and glucose is added to a culture medium in the middle of a cultivation.

[Claim 14]

A method of producing a cellulase, the method comprising a step of cultivating the mutant strain according to any one of claims 1 to 10.

[Claim 15]

A method of producing a cellulase, the method comprising a step of cultivating the mutant strain according to any one of claims 1 to 10, in a culture medium at least comprising lactose.

[Claim 16]

The method of producing a cellulase according to claim 14 or 15, wherein, in the cultivation step, at least one selected from the group consisting of lactose and glucose is added to a culture medium in the middle of a cultivation.

[Claim 17]

A method of producing a sugar, the method comprising:

a step of producing a cellulase by the method of producing a cellulase according to any one of claims 14 to 16; and

a step of saccharifying a cellulose-containing biomass using the cellulase obtained in the step.

SEQUENCE LISTING

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accacgtaga ggagcagcga cgggccagcg attcggcagc cgacgccagc ctggacaccg	3000
cctcttcctt tagcgagaat accgtcgaga ctctgacacc tgatccgagc gtcgccgatc	3060
cggtcgcttc ggaaacgagc ggcatggccg aggatgacga gtacgagcag cagattcagc	3120
aggctatacg tctgtctctg ctagaaggcg tcaacaacgg cgtggaacag tcacctgtgg	3180
attcctcacg gggcaacagc tctgttgatt tcgaccaacc ggtcaatgtc aagtacaagc	3240
ccaagggcgg gaagaagggg aagcaatcag gggcttcttc tgggtggctca ccgtctgcga	3300
gccacacgcc cgttggtggt ggtgcttctt cttcgcggct gagcacgact gaagatgagg	3360
atntagcgat tgctctgagt ctgagcatgc aggaccaggg agggggatac tcgccgccgg	3420
gaatgtcgtc gtcgacgatg atggggagga gtgcttactt ggaggctgct gctggtgtcg	3480
atgaggagga tgagtttccg tctttgcctg gtgaaggga ggggaagggg gtgcagagat	3540
ggtga	3545

<210> 4
 <211> 553
 <212> PRT
 <213> Trichoderma reesei

<400> 4

Pro	Thr	Ile	Ser	Met	Ser	Pro	Pro	Ser	Pro	Val	Asp	His	Pro	Met	Glu
1				5					10					15	

Gly	Thr	Ser	Pro	Glu	Asp	Asn	Ser	Pro	Thr	Gly	His	Gly	His	Gly	Ser
			20					25					30		

Gly	Ser	Asp	Pro	Glu	Gly	Ser	Ser	Asn	Gly	Lys	Pro	Pro	Val	Ser	Arg
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

35

40

45

Ala Gly Asn Ala Ser Pro Gln Glu His Gly Ala Leu Thr Thr Ser Ser
 50 55 60

Asn Met Pro Ala Pro Pro Pro Ala Ala Ala Ala Val His Gln Pro
 65 70 75 80

Lys Ile Val Gln Thr Ala Phe Ile His Lys Leu Tyr Asn Met Leu Glu
 85 90 95

Asp Thr Ser Ile Gln His Leu Ile Ser Trp Ser Ser Ser Ala Glu Ser
 100 105 110

Phe Val Met Ser Pro Ser Ala Asp Phe Ser Lys Val Leu Ser Gln Tyr
 115 120 125

Phe Lys His Thr Asn Ile Ser Ser Phe Val Arg Gln Leu Asn Met Tyr
 130 135 140

Gly Phe His Lys Glu Arg Asp Val Phe His Thr Gly Asn Pro Glu Thr
 145 150 155 160

Thr Leu Trp Glu Phe Lys His Gly Asn Gly Asn Phe Lys Arg Gly Asp
 165 170 175

Leu Val Gly Leu Arg Glu Ile Lys Arg Arg Ala Ser Arg His Ala Leu
 180 185 190

Val Asn Arg Glu Asn Thr Phe Pro Lys Thr Ser Thr Ser Gln Pro Gly
 195 200 205

Thr Pro Ile Glu Pro Val Gln Val Pro Pro Asp Ser Ile Glu Ala Arg
 210 215 220

Ile Ala Asn Leu Glu His Ser Leu Tyr Asp Thr Ala Ala Arg Leu Gln
 225 230 235 240

Arg Ser Glu Glu Ser Ala His Tyr Met His Val Lys Asn Gln Ala Ile
245 250 255

Met Glu Thr Leu Asn Arg Leu Leu Phe Phe Asn Gln Glu Leu Ser Lys
260 265 270

Gly Ile Leu Ser Leu Val Pro Pro Asp Asn Pro Val His Arg Asp Val
275 280 285

Met Thr Leu Gln Gly Glu Met Val Arg Gln Ala Glu Met Leu Arg Ser
290 295 300

Leu Asp Glu Pro His Glu Pro Val Tyr Ser Ala Arg Gln Gln Phe Phe
305 310 315 320

Gly Thr Val Asp Asn Ala Pro Val Ser Pro Arg Gln Leu Pro Gln Asp
325 330 335

Asp Asn Arg Arg Val Thr Leu Asn Val Pro Gln Gly Arg Ser Gln Pro
340 345 350

Ser Tyr Arg Pro Ala Val Pro Ser Asn Leu Ser Ala Gly Thr Arg Arg
355 360 365

Pro Tyr Gly Ser Ile Ser Gly Gly Ala Gly Ser Ser Pro Leu Arg Asn
370 375 380

Ala Ala Pro Ala Pro Pro Ala Gly Pro His Pro Leu Ser Asn Val Glu
385 390 395 400

Thr Val Pro Ser Asn Leu Ala Arg Arg His Thr Ala Ala Asp Ile Arg
405 410 415

Ala His Gly Trp Gln Pro Asn Ala Pro Pro Tyr Pro Pro Gly Ala Val
420 425 430

Pro Pro Pro Val Trp Pro Gln Ser Pro Asn Arg Pro Glu Asp Gln Arg

435

440

445

Ile Arg Asp Ser Leu Ser Ser Phe Ser Leu Gln Ala Pro Ser Ala His
 450 455 460

Gly His Pro His Ser Arg Pro Ala Thr Pro Pro His Ala Pro Phe Ser
 465 470 475 480

Asn Gly Ala Ser Gly Gly Ser Asp Thr Phe Gly Ser Trp Ser Trp Gly
 485 490 495

Ala Ala Ser Asn Arg Glu Ser Lys Thr Ile Gly Ala Leu Lys Glu Ser
 500 505 510

Ser Ala Pro Pro Thr Arg Arg Gly Ser Met Ala His Ile Leu Asn Pro
 515 520 525

Ser Asp Thr Ala Glu Arg Ser Asp Glu Asp Glu Asp Pro Arg Gly Asp
 530 535 540

Asp Asp Arg Lys Arg Lys Arg Arg Gln
 545 550

<210> 5

<211> 609

<212> PRT

<213> Trichoderma reesei

<400> 5

Met Gly Gln Ala Pro Ser Gln Thr Arg Arg Thr Arg Arg Thr His Glu
 1 5 10 15

Glu Leu Thr Gln Glu Leu Ala Tyr Arg Phe Lys Glu Lys Cys Phe Thr
 20 25 30

Ser Leu Glu Tyr Tyr Ser Leu Lys Asp Val Phe Lys Lys Leu Ala Asp
 35 40 45

Gln Gln Gly Asp Ile Arg Tyr Leu Lys Glu Asp Thr Ile Ala Arg Phe
50 55 60

Leu Glu Ile Pro Asp Ile Leu Gly Ala Ser Pro Val Ile Phe His Met
65 70 75 80

Ile Ser Tyr Leu Gly Ala Phe Pro Phe Leu Gln Glu Ala Pro Val Val
85 90 95

Leu Glu Leu Ala Gln Leu Ile Met Val Val Val Ile Met Thr Glu Arg
100 105 110

Tyr Lys Arg Val Leu Ala Lys Gly Ser Thr Asp Arg Thr Lys Leu Phe
115 120 125

Phe Lys Ser Leu Ala Val Tyr Asp Arg Lys Val Met Glu Glu Thr Gly
130 135 140

Ser Ser Pro Arg Asn Ser Thr Ser Lys Asp Ala Thr Ala Arg Pro Ser
145 150 155 160

Ala Asn Thr Arg Gly Phe Ala Ile Asp Glu Pro Met Ala Glu Asp Glu
165 170 175

Asp Asp Gly Asp Asp Asp Asp Asp Asp Leu Val Ile Thr Ala Phe Glu
180 185 190

Leu Leu Asp Ile Asp Glu Ala Thr Lys His Gly Glu Ala Ala Ala Ile
195 200 205

Lys Glu Ala Ile Ile Pro Thr Asp Asn Phe Arg Lys Leu Ile Met Leu
210 215 220

Leu Leu Leu Ile Ala Pro Leu Asp Ala Gln Glu Ser Leu Ser Gln Tyr
225 230 235 240

Ser Ser Arg Val Ala Gly Pro Glu Leu Glu Ser Leu Arg Ala Thr Ala
245 250 255

Glu Cys Val Leu Ala Ser Phe Val Asp Val Glu Thr Ser Pro Gly Ile
260 265 270

Gly Tyr Thr Arg Phe Lys Thr Val Ile Pro Val Leu Phe Pro Asn Leu
275 280 285

Phe Ala Gly Phe Asn Gly Leu Phe Glu His Phe Leu Phe Ser Lys Asp
290 295 300

Leu Asp Phe Ser Lys His Lys Val Glu Lys Pro Gly Asp Glu Gln Leu
305 310 315 320

Ile Ile Gly Lys Ile Ala Gln Pro Leu Leu Pro Thr Pro Gly Asp Ile
325 330 335

Met Thr Glu His Thr Leu Ser Gln Leu Ser Leu Phe Leu Pro Gly Ser
340 345 350

Ser Leu Phe Arg Arg Val Arg Leu Leu Tyr Ser Gly Asn Asp Ala Gly
355 360 365

Phe Ser Met Gly Ser Leu Gln Thr Lys Val Phe Asn Trp Arg Ala Pro
370 375 380

Thr Ile Leu Leu Val Ser Gly Ser Arg Leu Ala Asp Val Pro Glu Gly
385 390 395 400

Gly Gln Glu Ala Ser Phe Ala Ser Ser Leu Pro Thr Lys Arg Phe Pro
405 410 415

His Gly Ser Lys Ser Glu Arg Val Thr Phe Gly Val Tyr Val Arg Glu
420 425 430

Pro Trp Lys His Thr His Lys Glu Cys Phe Gly Asn Ser Glu Thr Ile
435 440 445

Leu Phe Gln Leu Glu Pro Ile His Asp Val Phe Pro Ala Ser Thr Ile
 450 455 460

Asn Thr Asp Tyr Val Thr Phe Thr Lys Pro Pro Gly Asn Arg Pro Cys
 465 470 475 480

Leu Ala Phe Gly Cys Pro His Pro Lys Pro Thr Gln Ser His Arg Lys
 485 490 495

Glu Gly Ile His Ala Leu Gly Ala Val Ser Leu Leu Phe Asp Glu Ser
 500 505 510

Phe Glu Phe Gly Val Phe Asn His Asp Tyr Lys Ser Arg Gly Gly Ala
 515 520 525

Phe His Thr Ser Ile Val Arg Lys Tyr Asp Phe Gln Asp Arg Phe Arg
 530 535 540

Ile Glu Asn Met Glu Val Trp Gly Cys Gly Gly Asp Glu Glu Ala Lys
 545 550 555 560

Ala Gln Ala Glu Arg Trp Ala Trp Glu Glu Arg Glu Ala Glu Ala Arg
 565 570 575

Arg Arg Ile Asn Leu Gly Thr Gly Asp Ile Glu Ala Asp Arg Ala Leu
 580 585 590

Leu Glu Met Ala Gly Leu Val Gly Gly Asn Arg Ser Gly Gly Ser Met
 595 600 605

Gly

<210> 6
 <211> 1162
 <212> PRT
 <213> Trichoderma reesei

<400> 6

Met Thr Gln Glu Pro Leu Lys Thr Pro Ala Gln Leu Pro Ala Asn Asn
 1 5 10 15

His Leu Asp Ser Leu Gln Leu Pro Gln His Gly Ser Leu His Arg Asp
 20 25 30

Ala Ser Gly Pro Gly Arg Gly Leu Pro Gly Phe Ser Leu Gly Ser Gln
 35 40 45

Arg Leu Arg Leu Asp Pro Ala Glu His Gln Gly Arg Glu Ala Gly Val
 50 55 60

Asn Ala Glu Arg Leu Asp Glu Lys Leu Gln Gln Leu Ser Leu Asp Gly
 65 70 75 80

Ala His Asp Gly Arg Pro Ser Val Pro Gly Gln Arg Val Tyr Glu Tyr
 85 90 95

Glu Lys Ala Ser Thr Pro Gln Ala Ser Lys Gln Gly Leu Gly Phe Gln
 100 105 110

Val Ile Lys Arg Ser Glu Pro His Ala Asp Gly Leu Ser Leu Glu Asp
 115 120 125

Phe Pro Asn Glu Ile Leu Thr His Ile Phe Ser His Leu Pro Pro Asp
 130 135 140

Cys His Ser Ser Val Ala Leu Val Ser Lys Arg Phe Trp Ala Leu Val
 145 150 155 160

Thr Thr His His Ala Trp Arg Met Ala Phe Met Arg Phe Phe Pro Gly
 165 170 175

His Thr Ile Leu Glu Asn Asn Gly Lys Ala Ala Ala Ala Thr His Ser
 180 185 190

Met Thr Gly Pro Ser Ser Asp Val Val Arg Phe Glu Thr Arg Tyr Phe

205

Pro	Asp	Ile	Pro	Lys	Ile	Pro	Glu	Met	Ser	Asp	Ala	Ile	Cys	Ser	Val
385					390					395					400

Trp Ile Ala Lys Ser Ser Thr Val Thr Ala Thr Thr Gln Ser Met Cys
405 410 415

Gly Met Leu Thr Gly Ser Ala Leu Gly Ile Ile Thr Ala Tyr Ser Leu
420 425 430

Gly Trp Asp Thr Thr Gly Pro Arg Tyr Ala Asn Gly Asp Val Thr Ala
435 440 445

Arg Trp Val Val Ser Pro Gly Val Pro Ile Ile Ser Leu Lys Val Asp
450 455 460

Asp Gly Phe Asn Gln Lys Arg Lys Ser Ser Ser Arg Val Trp Ala Val
465 470 475 480

Ala Leu Asn Ala Leu Gly Glu Val Tyr Tyr Leu Cys Asp Val Pro Val
485 490 495

Gly Lys Pro Gly Arg Thr Thr Gly Glu Asp Met Thr Arg Asn Ala Trp
500 505 510

Tyr Ala Gly Arg Thr Ala Tyr Trp His Leu Leu Glu Ala Thr Arg Arg
515 520 525

Val Ala Arg Glu Asp Glu Ala Asp Lys Asn Ala Thr Arg Gly Gly Tyr
530 535 540

Ser Pro Arg Ser Pro Ser Leu Asp Met His Leu Ser Lys Glu Gln Ile
545 550 555 560

Val Ala Glu Ala Arg Glu Ile Glu Lys Phe Met Arg Tyr Arg Pro Ser
565 570 575

His Phe Arg Lys Val Cys Glu Gly Trp Asp Met Gln Arg Lys Leu Glu
580 585 590

Val Asp Phe Ala Ser Asp Asp Gly Lys Gly Ala Gly Glu Ser Ile Phe

595		600		605											
Val	Ile	Asp	Cys	Gly	Leu	Ala	Glu	Asn	Arg	Pro	Val	Ser	Ile	Gln	Arg
610						615					620				
Tyr	Ser	Arg	Ser	Leu	Ile	Pro	Val	Gln	Gly	Thr	Pro	Ser	Glu	Ser	Ser
625					630					635					640
Thr	Pro	Leu	Ala	Pro	Val	Pro	Thr	Ser	Leu	Phe	Gly	Ser	Ile	Gly	Gly
				645					650					655	
Phe	Ala	Asn	Arg	Ile	Ser	Pro	Val	Ser	Glu	Ser	Gln	Ala	Pro	Leu	Ser
			660						665				670		
Pro	Pro	Pro	Thr	Pro	Lys	Ser	Pro	Pro	Val	Pro	Ser	Thr	Val	Leu	His
			675				680					685			
Asp	Trp	Phe	Lys	Gln	Gly	Phe	Glu	Phe	Lys	Gly	His	Gly	His	Asp	Thr
690						695					700				
Ile	Leu	Ser	Val	Ala	Leu	Asp	Asn	Ser	Leu	Thr	Ser	Leu	Tyr	Thr	Leu
705					710					715					720
Gly	Glu	Asp	Pro	Leu	His	Thr	Ala	Asn	Glu	Ala	Ser	Ser	Thr	Thr	Ser
				725					730					735	
Pro	Trp	Ala	Glu	His	Gly	Ala	Arg	Glu	Ile	Pro	Gly	Arg	Arg	Thr	Arg
			740					745					750		
Phe	Ile	Ile	Ala	Gly	Thr	Asn	Ser	Gly	Ala	Val	Leu	Val	Trp	Asn	Ala
		755					760					765			
Arg	Asp	Asp	Asp	Arg	Thr	Arg	Asp	Ile	Gln	Pro	Leu	Arg	Ile	Leu	Gln
770						775					780				
Thr	Glu	Ser	Pro	Glu	Val	Ser	Ala	Val	Ala	Ala	Ser	Gly	Leu	Tyr	Leu
785					790					795					800

Val His Gly Gly Ser Asp Gly Leu Val Gln Ala Trp Asp Pro Leu Ala
805 810 815

Ser Thr Thr Asp Pro Ile Arg Thr Ile Asn Ala Arg Ser Asn Gly Arg
820 825 830

Val Pro Arg His Met Leu Val Met Asn Pro Ala Leu Gln Glu Glu Thr
835 840 845

Tyr Ser Ala Ala Lys Ala Ile Tyr Leu Asp Pro Asp Ser Thr Thr Leu
850 855 860

Gln Gly Val Val Ser Phe Gly Ala Phe Leu Arg Tyr Trp Ser Tyr Gly
865 870 875 880

Ser Asn Gly His Ala Thr Gly Arg Lys Arg Arg Val Arg His Ala Asp
885 890 895

Met Asp Ala Arg Leu Ala Ser Arg Arg Gln Gly His Ala Val Ser Gly
900 905 910

Tyr Ile Ala Ser Glu Glu Ala Glu Met Arg Arg Glu Asp Glu Gln Gln
915 920 925

Ala Arg Glu His Asn Arg Arg Leu Lys Arg Phe Gly Ala Leu Gly Asp
930 935 940

Leu Thr Glu Glu Glu Ala Leu Leu Tyr Ala Gln Met Val Ser Gln Glu
945 950 955 960

Ala Tyr His Val Glu Glu Gln Arg Arg Ala Ser Asp Ser Ala Ala Asp
965 970 975

Ala Ser Leu Asp Thr Ala Ser Ser Phe Ser Glu Asn Thr Val Glu Thr
980 985 990

Leu Thr Pro Asp Pro Ser Val Ala Asp Pro Val Ala Ser Glu Thr Ser

995	1000	1005
Gly Met Ala Glu Asp Asp 1010	Glu Tyr Glu Gln Gln 1015	Ile Gln Gln Ala 1020
Ile Arg Leu Ser Leu Leu 1025	Glu Gly Val Asn Asn 1030	Gly Val Glu Gln 1035
Ser Pro Val Asp Ser Ser 1040	Arg Gly Asn Ser Ser 1045	Val Asp Phe Asp 1050
Gln Pro Val Asn Val Lys 1055	Tyr Lys Pro Lys Gly 1060	Gly Lys Lys Gly 1065
Lys Gln Ser Gly Ala Ser 1070	Ser Gly Gly Ser Pro 1075	Ser Ala Ser His 1080
Thr Pro Val Gly Gly Gly 1085	Ala Ser Ser Ser Arg 1090	Leu Ser Thr Thr 1095
Glu Asp Glu Asp Leu Ala 1100	Ile Ala Leu Ser Leu 1105	Ser Met Gln Asp 1110
Gln Gly Gly Gly Tyr Ser 1115	Pro Pro Gly Met Ser 1120	Ser Ser Thr Met 1125
Met Gly Arg Ser Ala Tyr 1130	Leu Glu Ala Ala Ala 1135	Gly Val Asp Glu 1140
Glu Asp Glu Phe Pro Ser 1145	Leu Pro Gly Glu Gly 1150	Lys Gly Lys Gly 1155
Val Gln Arg Trp 1160		

<210> 7
 <211> 859
 <212> PRT

<213> Trichoderma reesei

<400> 7

Met Pro His Arg Glu Arg Gly Lys Gln Arg Glu Gly Gly Asp Ser Tyr
1 5 10 15

Arg Pro Ser Arg Pro Ala Arg Ser Arg Ser Arg Ser Arg Ser Pro Pro
20 25 30

Arg Ala Pro Val Pro Val Arg Thr Glu Glu Glu Lys Gln Ala Ala Ala
35 40 45

Lys Ala Glu Tyr Glu Lys Leu Leu Asn Met Arg Ser Gly Gly Thr Tyr
50 55 60

Ile Pro Pro Ala Arg Leu Arg Ala Leu Gln Ala Gln Ile Thr Asp Lys
65 70 75 80

Ser Ser Lys Glu Tyr Gln Arg Met Ala Trp Glu Ala Leu Lys Lys Ser
85 90 95

Ile Asn Gly Leu Ile Asn Lys Val Asn Thr Ala Asn Ile Lys His Ile
100 105 110

Val Pro Glu Leu Phe Gly Glu Asn Leu Val Arg Gly Arg Gly Leu Phe
115 120 125

Cys Arg Ser Ile Met Lys Ala Gln Ala Ala Ser Leu Pro Phe Thr Pro
130 135 140

Ile Tyr Ala Ala Met Ala Ala Ile Val Asn Thr Lys Leu Pro Gln Val
145 150 155 160

Gly Glu Leu Leu Val Lys Arg Leu Ile Met Gln Phe Arg Lys Gly Phe
165 170 175

Lys Arg Asn Asp Lys Ala Val Cys Leu Ser Ser Thr Thr Phe Leu Ala
180 185 190

His Leu Ile Asn Gln Gln Val Gln His Glu Met Leu Ala Gly Gln Ile
195 200 205

Leu Leu Leu Leu Leu His Lys Pro Thr Asp Asp Ser Val Glu Ile Ala
210 215 220

Val Gly Phe Cys Lys Glu Val Gly Gln Tyr Leu Glu Glu Met Gln Pro
225 230 235 240

Ala Ile Ser Met Ala Val Phe Asp Gln Phe Arg Asn Ile Leu His Glu
245 250 255

Ser Asp Ile Asp Lys Arg Thr Gln Tyr Met Ile Glu Val Leu Phe Gln
260 265 270

Ile Arg Lys Asp Lys Phe Lys Asp His Pro Ala Ile Lys Glu Glu Leu
275 280 285

Asp Leu Val Glu Glu Glu Asp Gln Ile Thr His Lys Val Glu Leu Asp
290 295 300

Gly Glu Ile Asp Val Gln Asp Gly Leu Asn Ile Phe Lys Tyr Asp Pro
305 310 315 320

Glu Trp Glu Glu His Glu Glu Ala Tyr Lys Arg Leu Lys Ala Glu Ile
325 330 335

Leu Gly Glu Ala Ser Asp Asp Glu Glu Gly Asp Glu Asp Glu Asp Glu
340 345 350

Asp Glu Ser Ser Glu Asp Glu Glu Asn Glu Glu Thr Lys Ala Met Glu
355 360 365

Ile Lys Asp Gln Ser Asn Ala Asp Leu Val Asn Leu Arg Arg Thr Ile
370 375 380

Tyr Leu Thr Ile Met Ser Ser Ala Asp Pro Glu Glu Ala Val His Lys
 385 390 395 400

Leu Met Lys Ile Asn Leu Pro Val Gly Gln Glu Pro Glu Leu Pro Ser
 405 410 415

Met Ile Val Glu Cys Cys Ser Gln Glu Lys Thr Tyr Thr Lys Phe Phe
 420 425 430

Gly Leu Ile Gly Glu Arg Phe Ala Lys Ile Asn Arg Leu Trp Cys Asp
 435 440 445

Leu Phe Glu Gln Ala Phe Val Lys Tyr Tyr Glu Thr Ile His Arg Tyr
 450 455 460

Glu Asn Asn Lys Leu Arg Asn Ile Ala Met Leu Phe Gly His Met Phe
 465 470 475 480

Ala Ser Asp Ala Leu Gly Trp His Cys Leu Ser Val Ile His Leu Asn
 485 490 495

Glu Glu Glu Thr Thr Ser Ser Ser Arg Ile Phe Ile Lys Ile Leu Phe
 500 505 510

Gln His Ile Ser Glu Glu Ile Gly Leu Ala Lys Leu Arg Ala Arg Met
 515 520 525

Thr Asp Glu Thr Leu Arg Pro Ser Leu Glu Gly Leu Phe Pro Arg Glu
 530 535 540

Asn Pro Arg Asn Ile Arg Phe Ser Ile Asn Tyr Phe Thr Ser Ile Gly
 545 550 555 560

Met Gly Val Leu Thr Glu Glu Met Arg Glu His Leu Met Asn Met Pro
 565 570 575

Lys Pro Ala Leu Pro Ala Pro Ala Ala Gln Asp Arg Ser Asp Thr Asp
 580 585 590

Ser Val Ser Ser Tyr Ser Ser Tyr Thr His Ser Ser Tyr Ser Ser Arg
595 600 605

Ser Arg Ser Arg Ser Arg Ser Val Gly Arg Arg Ser Gly Gly Arg Gly
610 615 620

Arg Ser Leu Ser Arg Thr Pro Pro Arg Arg Gly Ala Arg Ser Arg Ser
625 630 635 640

Tyr Ser Asp Asp Ser Arg Ser Pro Ser Arg Ser Arg Ser Arg Ser Arg
645 650 655

Ser Asp Ser Val Ser Thr Arg Gly Arg Arg Arg Ala Ser Tyr Ser Ala
660 665 670

Ser Pro Pro Arg Arg Gly Gly Arg Arg Val Ala Ser Arg Ser Arg Ser
675 680 685

Tyr Ser Ser Gly Ser Ser Arg Ser Pro Pro Pro Arg Asn Arg Gly Arg
690 695 700

Ala Arg Ser Asn Ser Tyr Ser Ser Tyr Ser Arg Ser Pro Ser Ser Ser
705 710 715 720

Pro Arg Arg Gly Arg Asp Ala Asp Ser Ala Ser Pro Pro Pro Arg Arg
725 730 735

Gly Arg Pro Arg Gln Ser Pro Pro Gly Gly Pro Ala Gly Arg Arg Asn
740 745 750

Ser Ser Ser Val Gly Ser Gly Gly Pro Arg Lys Lys Pro Arg Arg Asp
755 760 765

Ser Arg Ser Pro Ser Arg Asp Tyr Ser Ser Arg Ser Pro Ser Arg Ser
770 775 780

Pro Ser Arg Ser Arg Ser Pro Pro Pro Ala Ala Arg Gly Arg Arg Gly
785 790 795 800

Ser Tyr Thr Pro Ser Arg Ser Arg Ser Pro Pro Pro Arg Arg Val Arg
805 810 815

Asp Gly Ser Pro Gly Arg Leu Arg Gly Gly Arg Ser Pro Ser Pro Pro
820 825 830

Leu Pro Val Lys Arg Arg Arg Tyr Asp Ser Glu Ser Val Ser Arg Ser
835 840 845

Pro Pro Pro Leu Lys Arg Gly Arg Arg Asp Asn
850 855

<210> 8
<211> 2580
<212> DNA
<213> Trichoderma reesei

<400> 8
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gaggaggaga agcaggcggc ggcaaaggcc gactacgaga agctgctcaa catgcggctc 180
ggcggcacgt acatcccgcc ggcgaggctg agggcgctgc aggcgcagat cacggacaag 240
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cccttcacgc ccatctacgc cgccatggcc gccattgtca acaccaagct gccgcaggtc 480
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aaggccgtct gtctgtcgtc gaccaccttc ctcgcccacc tcataacca gcaggtgcag 600
cacgagatgc tggccggcca gatcctgctg ctgctgctgc acaagccgac cgacgacagc 660
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gccattttcca tggccgtctt cgaccagttc cgcaacatcc tccacgagtc cgacattgac	780
aagcgaacgc agtacatgat tgagggtgctc ttccagatca ggaaggacaa gttcaaggat	840
cacccggcca tcaaggagga gctggacttg gtggaggagg aggaccagat cacgcacaag	900
gtggagcttg atggcgagat tgatgtgcag gacggactca acatcttcaa gtacgacccg	960
gagtgggagg agcatgagga ggcatacaag aggctcaagg cggagattct gggcgaagcc	1020
agcgatgacg aggagggcga cgaggacgag gacgaggacg agagctccga agatgaagaa	1080
aacgaagaga caaaggccat ggagatcaag gaccagtcta acgccgactt ggtcaaccta	1140
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tggtgctcgc aggagaagac gtacaccaag ttctttggct tgatcggcga gcgtttcgcc	1320
aagatcaatc ggctgtggtg cgacctcttt gagcaggcct ttgtcaagta ctacgagacg	1380
atccaccgat acgaaaacaa caagctgcgg aacattgcc a tgctgtttgg ccacatgttt	1440
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acgtcgagca gccgcatctt catcaagatt ctgttccagc acatttccga ggaaatcggc	1560
ctggctaagc tccgggcacg catgactgac gagacgtgc ggcccagcct cgaaggcctc	1620
ttccccagag agaaccctcg caacatccga ttctccatca actacttcac cagcatcggc	1680
atgggtgtac tgaccgagga gatgcgagag cacctcatga acatgcccaa gcctgcgctg	1740
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actcactcat catactcttc ccgctcgcgc tcacgggtccc gatctgtggg tcgtcggagc	1860
ggcggtcgag gccgatcgct ttcccgaact ccgcctcgac gtggcgcaag gagccgatcc	1920
tactctgacg actcacggtc accgtcgcgg tcaagatcac gatcccgtc cgattccgtc	1980
tctactcgtg ggcaaggcg agcgtcgtac tcggccagtc ctccccggcg tggtagccgt	2040
cgggttgcca gcagaagccg aagctactcg tcgggctcct cacggtctcc gccaccacgg	2100
aaccgcggtc gcgcacgaag caactcgtat agttcctaca gccgctctcc atcttcttca	2160
ccacgacgcg gcagagacgc agactcggcc agcccgcctc cgcaagggg tcgaccgcgc	2220

cagagccac caggcgtcc cgcaggtcga aggaacagct cgtctgtcgg cagcggaggg 2280
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 ggctgtctga ggggtgggag gtcgcctagt cctcctttgc cgggtgaagag gaggcggtat 2520
 gatagcgaga gtgtttctcg gtcgccgcct cttttgaagc gcgggagaag ggataactaa 2580

<210> 9
 <211> 543
 <212> PRT
 <213> Trichoderma reesei

<400> 9

Met Thr Val Leu Thr Ser Pro Leu Ala Ser Tyr Asn Val Ala Asn Lys
 1 5 10 15

Leu Tyr Lys Thr Thr Leu Leu Asn Thr Val Cys Leu Val Ala Gly Leu
 20 25 30

Ser Ile Phe Phe Phe Gly Tyr Asp Gln Gly Leu Met Gly Gly Val Asn
 35 40 45

Thr Thr Arg Asp Tyr Ala Glu Arg Met Gly Phe Gly His Trp Asp Glu
 50 55 60

Asp Gln Asn Ile Val Val Val Asp Lys Pro Leu Leu Gln Gly Gly Ile
 65 70 75 80

Val Ala Val Tyr Tyr Leu Pro Gly Thr Leu Cys Gly Cys Leu Leu Gly
 85 90 95

Gly Trp Leu Gly Asp Arg Tyr Gly Arg Ile Lys Thr Ile Ala Ile Ala
 100 105 110

Cys Ala Trp Ser Val Cys Ala Ala Ala Leu Gln Ala Ser Ala Met Asn
 115 120 125

Ala Asn Trp Met Phe Cys Ala Arg Val Leu Asn Gly Val Gly Thr Gly
130 135 140

Ile Leu Asn Ala Ile Thr Pro Val Trp Ala Thr Glu Thr Ala Ala His
145 150 155 160

Thr Ser Arg Gly Gln Phe Val Ser Ile Glu Phe Thr Leu Asn Ile Leu
165 170 175

Gly Val Val Val Ala Tyr Trp Leu Glu Phe Gly Thr Ser Lys Tyr His
180 185 190

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Pro Arg Trp Leu Val Lys Val Gly Arg Glu Glu Glu Ala Arg Phe Ile
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Ala Glu Tyr Asn Asp Ile Val Asn Ile His Lys Leu Glu Val Asp Thr
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Lys Leu His Thr Gly Arg Arg Val Gln Leu Val Ile Trp Leu Gln Ile
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Glu Leu Arg Val Phe Phe Cys Lys Tyr Asn Asp Pro Pro Tyr Val Lys
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Glu Gln Leu Leu Ser Glu Leu Lys Glu Tyr Ala Leu Glu Val Asp Met
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Ala Thr Lys Val Asn Tyr Val Val Gln Glu Val Val Val Val Ile Lys
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Asp Ile Leu Arg Lys Tyr Pro Gly Tyr Glu Gly Val Ile Pro Ser Leu
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<223> DNA fragment for transformation

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<212> DNA

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<223> DNA fragment for transformation

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