(12) STANDARD PATENT (11) Application No. **AU 2019331247 B9**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Trichoderma reesei mutant strain, and method for producing protein

(51) International Patent Classification(s)
C12N 1/15 (2006.01) ***C12P 21/00*** (2006.01)
C12N 15/31 (2006.01)

(21) Application No: **2019331247** (22) Date of Filing: **2019.08.28**

(87) WIPO No: **WO20/045473**

(30) Priority Data

(31) Number	(32) Date	(33) Country
2018-160157	**2018.08.29**	**JP**

(43) Publication Date: **2020.03.05**
(44) Accepted Journal Date: **2025.01.30**
(48) Corrigenda Journal Date: **2025.02.20**

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(56) Related Art
WO 2017/170918 A1

(12) 特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局

(43) 国際公開日
2020年3月5日(05.03.2020)

WIPO | PCT

(10) 国際公開番号
WO 2020/045473 A1

(51) 国際特許分類:
C12N 1/15 (2006.01) *C12P 21/00* (2006.01)
C12N 15/31 (2006.01)

(21) 国際出願番号： PCT/JP2019/033643

(22) 国際出願日： 2019年8月28日(28.08.2019)

(25) 国際出願の言語： 日本語

(26) 国際公開の言語： 日本語

(30) 優先権データ：
特願 2018-160157 2018年8月29日(29.08.2018) JP

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(81) 指定国(表示のない限り、全ての種類の国内保護が可能): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) 指定国(表示のない限り、全ての種類の広域保護が可能): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), ユーラシア (AM, AZ, BY, KG, KZ, RU, TJ, TM), ヨーロッパ (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

添付公開書類：
— 国際調査報告（条約第21条(3)）
— 明細書の別個の部分として表した配列リスト（規則5.2(a)）

WO 2020/045473 A1

(54) **Title:** TRICHODERMA REESEI MUTANT STRAIN, AND METHOD FOR PRODUCING PROTEIN

(54) 発明の名称： トリコデルマ・リーセイ変異株およびタンパク質の製造方法

(57) **Abstract:** The present invention provides: a *Trichoderma reesei* mutant strain having such a mutation that the function of a polypeptide comprising the amino acid sequence represented by SEQ ID NO: 2 is lost or decreased; and a method for the high-throughput production of a protein, comprising culturing the mutant strain.

(57) 要約：本発明は、配列番号２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た変異を有するＴｒｉｃｈｏｄｅｒｍａ ｒｅｅｓｅｉの変異株および当該変異株を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。

DESCRIPTION

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

TECHNICAL FIELD

[0001]

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

BACKGROUND ART

[0002]

Trichoderma reesei is known to have a high protein-producing ability, and studies have heretofore been made on protein production using filamentous fungi of *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, overexpression or deletion of a factor which controls cellulase production is conducted.

[0003]

Non-Patent Literature 1 describes that a mutant strain of *Trichoderma reesei* having 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*.

CITATION LIST

NON-PATENT LITERATURE

[0004]

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

SUMMARY OF THE INVENTION

TECHNICAL PROBLEM

[0005]

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 obtain a mutant strain of *Trichoderma reesei* having a further enhanced protein-producing ability by making a search for a novel factor which controls protein production of *Trichoderma reesei*, and to provide a method for protein production using the mutant strain of *Trichoderma reesei*.

SOLUTION TO THE PROBLEM

[0006]

The present inventors thought that if a novel control factor 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 a function of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 has been eliminated or reduced. The present invention has been accomplished based on this finding.

[0007]

Specifically, the present invention includes the following (1) to (6).

(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 SEQ ID NO: 2.

(2) The mutant strain according to (1), in which the mutation is a mutation that deletes a Glycosyltransferase_GTP_type domain of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2.

(3) The mutant strain according to (2), in which the mutation is a stop codon mutation for a glutamic acid residue at the 1,523rd residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 2.

(4) A method of producing a protein, the method including a step of cultivating the mutant strain according to any one of (1) to (3).

(5) A method of producing a cellulase, the method including a step of cultivating the

mutant strain according to any one of (1) to (3).

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

a step of producing a cellulase by the method of producing a cellulase according to (5); and

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

ADVANTAGEOUS EFFECTS OF THE INVENTION

[0008]

The mutant strain of *Trichoderma reesei* in which the function of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 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

[0009]

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 SEQ ID NO: 2.

[0010]

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).

[0011]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 is a polypeptide possessed by *Trichoderma reesei* and having an overall length of 1,738 amino acid residues, and in National Center for Biotechnology Information, this polypeptide has been registered as a glycosyltransferase family 41, partial (EGR46476) that *Trichoderma reesei* QM6a strain has. The glycosyltransferase family 41, partial dimerizes to become glycosyltransferase family 41 (The EMBO Journal, 27, 2080-2788 (2008)), and has a function that a newly generated protein just after translation, during passing through the Golgi complex, undergoes a change of N-acetylgalactosamine (GalNAc) into a serine or threonine residue which is an amino acid residue (Biochemistry, Fourth edition, 11, 280-281 (1995)). Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 include the base sequence represented by SEQ ID NO: 1.

[0012]

Examples of methods for eliminating or reducing the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 include a method for introducing a mutation that causes a total deletion of a glycosyltransferase family 41, partial or a partial deletion of a glycosyltransferase family 41, partial. Specific examples thereof include a method in which a frame shift 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: 2, by a deletion, insertion, substitution, etc. of a base.

[0013]

The phrase "deletion of a glycosyltransferase family 41, partial" means a total or partial loss of the polypeptide, a change of the whole or some of the polypeptide 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: 2 comes to have a sequence identity of 80% or less with respect to the amino acid sequence of the glycosyltransferase family 41, partial. 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%.

[0014]

CDD Search Results of National Center for Biotechnology Information disclose that the 1,338th to 1,725th amino acid residues from the N-terminal side are a Glycosyltransferase_GTP_type domain. In the present invention, specific examples of the case where the function of a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 is eliminated or reduced by a mutation such as deletion, substitution, or addition, that has occurred in an amino acid sequence located in the glycosyltransferase family 41, partial include a mutation in the base sequence represented by SEQ ID NO: 1 which changes the cytosine at the 6,261st residue into adenine to thereby insert a stop codon. This mutation changes the glutamic acid residue at the 1,523rd residue in the amino acid sequence represented by SEQ ID NO: 2 into a stop codon to cause the translation to end. Since the translation thus ends in the middle of the Glycosyltransferase_GTP_type domain, which mainly performs the function of glycosyltransferase family 41, the original function of the polypeptide as a protein is eliminated.

[0015]

Furthermore, the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 may be reduced by introducing a mutation which diminishes or inhibits the expression of the polypeptide. Specifically, a mutation introduced into the promoter or terminator region of a gene encoding the amino acid sequence represented by SEQ ID NO: 2 to diminish or inhibit the expression of the polypeptide may be employed. 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. Specific examples of base sequences including a promoter and a terminator which participate in transcription of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 include the base sequence represented by SEQ ID NO: 1.

[0016]

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.

[0017]

A mutant strain of *Trichoderma reesei* in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 has been eliminated can be acquired by the following method.

[0018]

The mutant strain in which all the functions of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 has 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.

[0019]

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.

[0020]

Besides a mutation which eliminates or reduces the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2, the mutant strain of the present invention may have a mutation which improves protein production amount and/or lowers the viscosity of culture solution to inhibit the degree of saturation of oxygen dissolved in the culture solution from decreasing. Specific examples thereof include a genetic mutation which reduces the function of the polypeptide represented by any of SEQ ID NOs: 3, 5, and 7.

[0021]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 3 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

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SEQ ID NO: 3 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: 3 at least has the function of binding to a DNA and/or an RNA.

[0022]

Specific examples of genes encoding the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 3 include the base sequence represented by SEQ ID NO: 4. 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: 3 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: 3 include a mutation in the base sequence represented by SEQ ID NO: 4 which deletes any of the 1,039th to 1,044th bases.

[0023]

The polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 5 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: 5 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: 5 at least participates in transport of sugar between the inside and the outside of the fungus bodies.

[0024]

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: 6. 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: 5 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: 5 include a mutation in the base sequence represented by SEQ ID NO: 6 which inserts 11 bases at the 1,415th position.

[0025]

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 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: 7 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)).

[0026]

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 for EGR48910 include a mutation in the base sequence represented by SEQ ID NO: 8 which changes the cytosine at the 1,080th base into adenine.

[0027]

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

[0028]

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

mutant strain 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 microbes of the genus *Trichoderma* can be employed. As a nitrogen source, use can be made, for example, of polypeptone, bouillon, CSL, or soybean cake. An inducer for protein production may be added to the culture medium.

[0029]

In the case of producing a cellulase by the present invention, the mutant strain can be cultivated in a culture medium containing one or more inducers selected from the group consisting of lactose, cellulose, and xylan. Cellulose or xylan may be added by adding a biomass containing cellulose or xylan as an inducer. Specific examples of the biomass containing cellulose or xylan 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.

[0030]

The biomass containing cellulose or xylan may be a pretreated one. 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.

[0031]

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 of performing cultivation 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 condition

during the cultivation is 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.

[0032]

Although the protein to be produced in the present invention is not particularly limited, proteins 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.

[0033]

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.

[0034]

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.

[0035]

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. The obtained supernatant is diluted, and 5 μL of the diluted supernatant is added to 250 μL of Quick Start Bradford protein assay (manufactured by Bio-Rad Laboratories, Inc.). 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.

[0036]

The β-glucosidase specific activity is determined by the following method. First, for the supernatant of the culture solution, 10 μL of the enzyme dilution is added to 90 μL 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 μL 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 μmol of p-nitrophenol per minute is defined as 1 U of activity to calculate the specific activity.

[0037]

The β-xylosidase specific activity is determined by the following method. First, for the supernatant of the culture solution, 10 μL of the enzyme dilution is added to 90 μL 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 μL 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 μmol of p-nitrophenol per minute is defined as 1 U of activity to calculate the specific activity.

[0038]

The cellobiohydrolase specific activity is determined by the following method. First, for the supernatant of the culture solution, 10 μL of the enzyme dilution is added to 90 μL 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 μL 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 μmol of p-nitrophenol per minute is defined as 1 U of activity to calculate the specific activity.

[0039]

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.

[0040]

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 of *Trichoderma reesei* cannot grow therein. Examples of treatment methods for preventing the fungus bodies from growing includes heat treatment, chemical treatment, acid/alkali treatment, and UV treatment.

[0041]

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, as stated above, can be used directly as an enzyme solution.

[0042]

In the case where the protein as a target to be produced 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.

[0043]

As for the cellulose-containing biomass to be used in the present invention, the same biomass as the cellulose-containing biomass mentioned above as an inducer or the pretreated biomass can be used.

[0044]

Conditions for the saccharification reaction are not particularly limited. The saccharification reaction temperature is preferably in the range of 25-60°C, especially more preferably in the range of 30°C to 55°C. The saccharification reaction time is preferably in the range of 2 hours to 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.

[0045]

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 for removing solid matter from the saccharified solution may be conducted before the filtration. The recovered enzyme can again be used for a saccharification reaction.

EXAMPLES

[0046]

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

[0047]

<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

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

[0048]

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

(Conditions for Determination of β-Glucosidase Specific Activity)

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

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

Enzyme dilution: 10 μL

Reaction temperature: 30°C

Reaction time: 10 min

Reaction terminator: 10 μL of 2 M sodium carbonate

Absorbance: 405 nm

[0049]

(Conditions for Determination of β-Xylosidase Specific Activity)

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

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

Enzyme dilution: 10 μL

Reaction temperature: 30°C

Reaction time: 10 min

Reaction terminator: 10 μL of 2 M sodium carbonate

Absorbance: 405 nm

[0050]

(Conditions for Determination of Cellobiohydrolase Specific Activity)

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

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

Enzyme dilution: 10 μL

Reaction temperature: 30°C

Reaction time: 10 min

Reaction terminator: 10 μL of 2 M sodium carbonate

Absorbance: 405 nm

[0051]

<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 1 mL portion of a culture solution of either *Trichoderma reesei* or a *Trichoderma reesei* mutant strain, centrifuging the collected culture solution, recovering a supernatant from which the fungus bodies had been removed, and filtering the supernatant with 0.22 μm filter.

[0052]

(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

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& Sohne) or bagasse powdered to an average particle diameter of 100 μm was used as a material to be saccharified. As for enzyme solutions, an enzyme solution obtained by flask cultivation using Arbocel (registered trademark) B800 was used in an amount of 450 μL in the case of saccharifying Arbocel (registered trademark) B800 or in an amount of 400 μL in the case of saccharifying powdered bagasse. An enzyme solution obtained by flask cultivation using lactose was introduced into a measuring cylinder in an amount of 350 μL in the case of saccharifying Arbocel (registered trademark) B800 or in an amount of 400 μL in the case of saccharifying powdered bagasse, and the enzyme solution the measuring cylinder was diluted with sterilized water to 1 mL in total. The dilution was then introduced into a 2 mL tube. A saccharification reaction was conducted under temperature conditions of 50°C for 24 hours, and then 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.

[0053]

(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 μm 2.1 × 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

Preparation of *Trichoderma reesei* mutant strain in which the function of polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 has been eliminated (Method for 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: 2 has been eliminated was 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: 2 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: 9 is prepared in order to eliminate the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2, and *Trichoderma reesei* QM9414 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: 2 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 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.

[0055]

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: 10 and 11, 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: 12 and 13, and the resulting amplified fragment is treated with restriction enzymes MluI and SphI 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 SphI, respectively, to construct a plasmid for mutation introduction. The plasmid for mutation introduction is treated with restriction enzymes AflII and SphI, and the *Trichoderma reesei* QM9414 strain is transformed with the obtained DNA fragment which is shown by SEQ ID NO: 9. 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).

[0056]

(Preparation and Evaluation of the Mutant Strain)

The *Trichoderma reesei* mutant strain acquired 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.

[0057]

<Example 2>

Protein Production Test Using *Trichoderma reesei* Mutant Strain

(Preculture)

After spores of the *Trichoderma reesei* mutant strain prepared in Example 1 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 then is incubated on a shaker under the conditions of 28°C and 120 rpm for 72 hours. *Trichoderma reesei* QM9414 strain is used as a control to conduct the same experiments shown below.

[0058]

[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 $(NH_4)_2SO_4$

10 g/L KH_2PO_4

2 g/L $CaCl_2 \cdot 2H_2O$

1.5 g/L $MgSO_4 \cdot 7H_2O$

** 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 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$

5 g/L $FeCl_3 \cdot 6H_2O$

2 g/L $CuSO_4 \cdot 5H_2O$

0.4 g/L $MnCl_2 \cdot 4H_2O$

10 g/L $ZnCl_2$

[0059]

(Main Culture)

Arbocel B800 (produced by J. Rettenmaier & Sohne) 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 (produced by ABLE & Biott Co., Ltd.).

[0060]

The preculture solutions of the *Trichoderma reesei* QM9414 strain and the *Trichoderma reesei* mutant strain prepared in Example 1 are each inoculated in an amount of 200 mL into 2.5 L of the main-culture medium to which Arbocel B800 has been added.

[0061]

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.

[0062]

[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.

[0063]

(Collection of Culture Solutions)

At 120 hours after initiation of the cultivation, a 20 mL portion of each of the culture solutions is collected. A part of the collected culture solution is centrifuged under the 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.

[0064]

(Determination of Protein Concentration)

The protein concentration of each of the culture solutions which have been collected at 120 hours after initiation of the cultivation is determined under the conditions shown in Reference Example 1. As a result, the culture solution obtained by cultivating the *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 has been eliminated has a higher protein concentration than the culture solution obtained by cultivating the *Trichoderma reesei* QM9414 strain.

[0065]

(Determination of Enzyme Activities)

The culture solutions collected at 120 hours after initiation of the cultivation are examined for cellulase specific activities, i.e., 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 μmol of the substrate per minute is defined as 1 U of activity to calculate the specific activity. As a result, the culture solution obtained by cultivating the *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 has been eliminated is higher in the three specific activities than the culture solution obtained by cultivating the *Trichoderma reesei* QM9414 strain.

[0066]

(Flask Cultivation)

Spores of the *Trichoderma reesei* mutant strain I prepared in Example 1 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 (produced by J. Rettenmaier & Sohne) or lactose shown in Table 3, 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.

[0067]

Furthermore, the *Trichoderma reesei* QM9414 strain, which was the parent strain into which the mutation of mutant strain I had not been introduced, was also subjected to 120 hours incubation by the method shown above, as a control for the mutant strain.

[0068]

[Table 3]

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.

[0069]

(Collection of Culture Solutions)

At 120 hours after initiation of the 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.

[0070]

(Determination of Protein Concentration)

In the cultivation using Arbocel (registered trademark) B800, in cases when the protein concentration in the culture solution obtained by cultivating the *Trichoderma reesei* QM9414 strain was taken as 1, then the relative value of the protein concentration in the culture solution of *Trichoderma reesei* mutant strain I was 1.2. It was thus ascertained that the mutant strain had a higher protein-producing ability than the parent strain.

[0071]

Also in the cultivation using lactose, in cases when the protein concentration in the culture solution obtained by cultivating the *Trichoderma reesei* QM9414 strain was taken as 1, then the relative value of the protein concentration in the culture solution of *Trichoderma reesei* mutant strain I was 1.3. It was thus ascertained that the mutant strain had a higher

protein-producing ability than the parent strain.

[0072]

(Determination of Various Cellulase Specific Activities)

In the cultivation using Arbocel (registered trademark) B800, in cases when various cellulase specific activities of the culture solution obtained by cultivating the *Trichoderma reesei* QM9414 strain were taken as 1, then the relative values of the various cellulase specific activities of the culture solution obtained by cultivating the *Trichoderma reesei* mutant strain I were: a β-glucosidase specific activity of 1.1, a β-xylosidase specific activity of 1.5, and a cellobiohydrolase specific activity of 1.8. It was thus ascertained that the mutant strain had the unexpected effect of bringing about improvements also in various cellulase specific activities.

[0073]

Also in the cultivation using lactose, in cases when various cellulase specific activities of the culture solution obtained by cultivating the *Trichoderma reesei* QM9414 strain were taken as 1, then the relative values of the various cellulase specific activities of the culture solution obtained by cultivating the *Trichoderma reesei* mutant strain I were: a β-glucosidase specific activity of 1.8, a β-xylosidase specific activity of 1.4, and a cellobiohydrolase specific activity of 1.6. It was thus ascertained that the mutant strain had the unexpected effect of bringing about improvements also in various cellulase specific activities.

[0074]

(Saccharification Reaction Test)

In accordance with the technique described in Reference Example 3, a culture solution collected at 120 hours after initiation of the flask cultivation of *Trichoderma reesei* mutant strain I was 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.

[0075]

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 with the *Trichoderma reesei* QM9414 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

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obtained with *Trichoderma reesei* mutant strain I was 1.7. The relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained by the flask cultivation of the mutant strain using lactose was also 1.7.

[0076]

In the saccharification reaction for saccharifying powdered bagasse, in cases when the glucose concentration in the saccharified solution obtained using the cellulases obtained with the *Trichoderma reesei* QM9414 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 with *Trichoderma reesei* mutant strain I was 1.5. The relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained by the flask cultivation of the mutant strain using lactose was also 1.5.

[0077]

It was ascertained from those results that the cellulases produced by the *Trichoderma reesei* mutant strain I 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.

[0078]

<Example 3>

Preparation of *Trichoderma reesei* mutant strain in which the function of polypeptide consisting of amino acid sequence represented by SEQ ID NO: 2 has been eliminated (2)

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-C strain as a mutant strain. The genetic mutation treatment was conducted in the following 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

suspended as genetic-mutation-treated spores in 10 mL of a Tris-maleate buffer (pH 6.0). 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-C strain which had formed a large halo.

[0079]

The QM9414-C strain was genetically analyzed and, as a result, it was found that the cytosine at the 6,261st residue in the base sequence represented by SEQ ID NO: 1 had been changed to adenine. This mutation changes the glutamic acid residue at the 1,523rd residue in the amino acid sequence represented by SEQ ID NO: 2 into a stop codon and causes a deletion of a Glycosyltransferase_GTP_type domain of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2.

<Example 4>

Protein Production Test Using *Trichoderma reesei* Mutant Strain in which the function of polypeptide consisting of amino acid sequence represented by SEQ ID NO: 2 has been eliminated

(Determination of Protein Concentration and Various Cellulase Specific Activities)

The QM9414-C strain acquired in Example 3, which was a *Trichoderma reesei* mutant strain in which the function of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2 had been eliminated, was cultivated in the same manner as in Example 2, and the culture solution collected at 120 hours after initiation of the cultivation was examined for protein concentration and cellulase specific activities under the conditions shown in Reference Examples 1 and 2. As for a control, the QM9414-A strain which was the parent strain of the QM9414-C strain was used.

[0080]

The results thereof are as shown in Table 4. The culture solution of the QM9414-C strain had a relative value of protein concentration being 2.6 times that of the culture solution of QM9414-A strain. Furthermore, the various specific activities of the culture solution of the QM9414-C strain, in terms of relative value with respect to those for the QM9414-A strain, were: 3.1 times for β-glucosidase, 1.5 times for β-xylosidase, and 2.0 times for cellobiohydrolase.

[0081]

[Table 4]

	QM9414-A Strain	QM9414-C Strain
Relative value of protein concentration	1.0	2.6
Relative value of β-glucosidase specific activity	1.0	3.1
Relative value of β-xylosidase specific activity	1.0	1.5
Relative value of cellobiohydrolase specific activity	1.0	2.0

[0082]

(Saccharification Reaction Test)

In accordance with the technique described in Reference Example 3, a culture solution collected at 120 hours after initiation of the cultivation of the *Trichoderma reesei* QM9414-C strain was 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.

[0083]

As a result, in cases when the glucose concentration in the saccharified solution obtained using the cellulases obtained with the *Trichoderma reesei* QM9414-A strain was taken as 1, then the relative value of the glucose concentration in the saccharified solution obtained using the cellulases obtained with *Trichoderma reesei* QM9414-C strain was 1.1 in the saccharification of Arbocel (registered trademark) B800 and was 1.2 in the saccharification of powdered bagasse.

[0084]

It was ascertained from those results that the cellulases produced by *Trichoderma reesei* QM9414-C strain 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.

CLAIMS

[Claim 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 SEQ ID NO: 2.

[Claim 2]

The mutant strain according to claim 1, wherein the mutation is a mutation that deletes a Glycosyltransferase_GTP_type domain of the polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 2.

[Claim 3]

The mutant strain according to claim 2, wherein the mutation is a stop codon mutation for a glutamic acid residue at the 1,523rd residue from the N-terminal side in the amino acid sequence represented by SEQ ID NO: 2.

[Claim 4]

A method of producing a protein, the method comprising a step of cultivating the mutant strain according to any one of claims 1 to 3.

[Claim 5]

A method of producing a cellulase, the method comprising a step of cultivating the mutant strain according to any one of claims 1 to 3.

[Claim 6]

A method of producing a sugar, the method comprising:

a step of producing a cellulase by the method of producing a cellulase according to claim 5; and

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

SEQUENCE LISTING

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<120> Trichoderma reesei mutant strain and methods for producing proteins thereof.

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<210> 2
<211> 1738
<212> PRT

<213> Trichoderma reesei

<400> 2

```
Met Leu Ser Pro Leu Arg Ser Arg Leu Val Lys Ala Thr Phe Leu Gly
1               5                   10                  15


Thr Cys Arg Gln Val Arg Leu Ala Ser Ser Ala Arg Tyr Pro Gln Ala
            20                  25                  30


Val Ser Pro Leu Ala Phe Asp Leu His Ser Pro Ser His Pro Ser Lys
        35                  40                  45


Asp Glu Lys Thr Ala Pro Ile Ile Phe Leu His Gly Leu Phe Gly Ser
    50                  55                  60


Lys Lys Asn Asn Arg Ala Ile Ser Lys Ala Leu Ala Arg Asp Leu Lys
65                  70                  75                  80


Thr His Val Tyr Thr Val Asp Leu Arg Asn His Gly Glu Ser Pro His
                85                  90                  95


Asp Pro Arg His Asp Tyr Val Ala Met Thr Glu Asp Leu Leu Ala Phe
            100                 105                 110


Ile Asp Gln His Gly Leu Lys Glu Pro Thr Leu Ile Gly His Ser Met
        115                 120                 125


Gly Ala Lys Thr Ala Met Ser Ala Ala Leu Arg Ser Pro Glu Thr Val
    130                 135                 140


Ala Lys Val Val Ala Val Asp Asn Ala Pro Val Asp Val Thr Leu Ser
145                 150                 155                 160


Arg Thr Phe Ala Ser Tyr Val Arg Gly Met Lys Lys Ile Glu Glu Ala
                165                 170                 175


Lys Val Thr Arg Gln Ser Glu Ala Asp Ala Ile Leu Arg Asp Tyr Glu
            180                 185                 190


Glu Ser Leu Pro Ile Arg Gln Phe Leu Leu Gly Asn Leu Tyr Arg Ser
        195                 200                 205


Pro Glu Asp Gly Ile Gln Arg Phe Arg Val Pro Leu Asp Ile Leu Gly
    210                 215                 220


Arg Ser Leu Asp His Leu Gly Asp Phe Pro Tyr Lys Asn Pro Gly Glu
225                 230                 235                 240


Ala Arg Tyr Thr Lys Pro Ala Leu Phe Val Arg Gly Thr Gln Ser Lys
```

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                245                 250                 255

Tyr Val Pro Asp Asp Val Leu Pro Ile Ile Gly Gln Phe Phe Pro Arg
            260                 265                 270

Phe Gln Leu Val Asp Ile Asp Ala Gly His Trp Leu Ile Ser Glu Gln
        275                 280                 285

Pro Glu Ala Phe Arg Gln Glu Phe Tyr Tyr Leu Leu His Tyr Thr Lys
    290                 295                 300

Leu Tyr Pro Gly Arg Asp Thr Val Arg Arg Thr Asn Pro Asp Pro Val
305                 310                 315                 320

Pro Arg Glu Ala Ala Leu Asp Trp Pro Thr Gly Gly Ala Arg Ala Arg
                325                 330                 335

Leu Cys Pro Arg His Arg His Arg Pro Arg Pro Arg Pro Pro Asn Pro
            340                 345                 350

Arg Pro Pro Ser Asn Pro Pro Leu Pro Ser Val Val Gly Met Leu Pro
        355                 360                 365

Leu Val Gln Thr His Pro Arg Val Val Pro Val Pro Leu Asp Phe Glu
    370                 375                 380

Ser Phe Gly Ala Pro Ala Ala Arg Pro Glu Ala Asp Arg Arg Ala Val
385                 390                 395                 400

Ala Ser Pro Tyr His Arg Gly Val Ser His Ser Tyr Gly Gly Pro Val
                405                 410                 415

Ser Ala Ala Pro Ala Gly Gln Gly Ser Arg Asp His Asp Tyr His Leu
            420                 425                 430

Arg Arg Lys Thr Pro Arg Gly Thr Ile Asp Ala Gly Tyr Asp Gly Ser
        435                 440                 445

Pro Thr Gln Leu Ser Pro Gly Pro Pro Pro Leu Lys Gln Leu Ile Leu
    450                 455                 460

Pro Thr Pro Ser Gly Ile Tyr Pro Tyr Val Pro Pro Asn Asn Leu Pro
465                 470                 475                 480

Phe His Ala Lys Pro Arg Leu Ser Asn Thr Asp Pro Ile Ser Leu Gly
                485                 490                 495

Gln Ala Pro Ala Val Ser Pro Trp Ser Leu Ser Tyr Gly Gly Gln Asn
            500                 505                 510
```

```
Phe Val Leu Ser Ser Gly Ser Pro Met Val Pro Gln Pro Gly Pro Ser
        515                 520                 525

Trp Gln Pro Tyr Gly Tyr Pro Met Phe Ser Gln Val Pro Gly Met His
    530                 535                 540

Gln Pro Leu Met Arg Ala Ala Glu Tyr Asn Val Arg Ala Phe Cys Pro
545                 550                 555                 560

Pro Pro Ala Ser Ala Pro Asp Ala Ala Thr Phe Asn Gln Phe Gly Trp
                565                 570                 575

Gln Leu Gly Ala Ser Gln Gln Asn Leu Gly Tyr Phe Asp Gln Ser Gln
            580                 585                 590

Tyr Pro Glu Pro Arg Ser Met Leu Leu Pro Ala Ser Phe Ser His His
        595                 600                 605

Thr Ser Asp Val Gln Leu Pro Tyr Arg Pro Val Pro Asp Phe Lys Ala
    610                 615                 620

Gly Leu Glu Val Thr Ser Phe Pro Ser Gln Pro Leu Val Gln Gly Ser
625                 630                 635                 640

Tyr Leu Gly Gln Ser Ser Leu Val Glu Asp Val Pro Ala Gly Glu Gln
                645                 650                 655

Tyr Ala Val Gln Ala Ser Phe Thr Glu Lys Val Tyr Ser Lys Ala Gln
            660                 665                 670

Asp His Tyr Val Glu Leu Leu Ala Tyr Leu Gln Thr Ala Arg Arg Leu
        675                 680                 685

Asp Gln Met Ser Thr Gly Gly Asn Ser Ser Ser Ser His Val Glu Ala
    690                 695                 700

Ile Thr Asn Leu Ala Ala Thr Leu Tyr Cys Leu Asn Arg Gln Glu Glu
705                 710                 715                 720

Ala Glu Gln His Trp Leu Arg Ala Ile Lys Leu Arg Pro Asp His Leu
                725                 730                 735

Glu Ala Thr Glu Gln Leu Val Gly Leu Leu Tyr Lys Lys Arg Ser Arg
            740                 745                 750

Glu Ala Ile Asp Ile Ile Cys Phe Val Gln Gln Ala Leu Ser Leu Lys
        755                 760                 765
```

```
Arg Gly Ser Pro Asn Arg Thr Ser Tyr Pro Ala Ser Ser Asp Asp Cys
    770                 775                 780

Leu Pro Ser Gln Gln Pro Ser Lys Phe Ala Ala Pro Phe Ser Tyr His
785                 790                 795                 800

Tyr Glu Thr Ala Ser Thr Thr Ser Gly His Ser Ser Arg Arg Ser Asp
                805                 810                 815

Phe Gly Ser Ser Gly Tyr Ala Leu Pro Asn Ser Glu Asn Gly Arg Ile
            820                 825                 830

Leu Ala Leu Val His Ala Lys Gly Thr Ile Leu Tyr Gly Leu Lys Asp
        835                 840                 845

Ile Glu Arg Ala Ser Glu Ala Phe Glu Glu Ala Val Leu Ile Ser Val
    850                 855                 860

Gly Glu Arg Ile Arg Ser Val Gln Asp Leu Val Asn Arg Ile His Ala
865                 870                 875                 880

Val Leu Ala Pro Ala Gly Ser His Ser Thr Gly Ser Asp Arg Arg Pro
                885                 890                 895

Val Ser Arg Arg Pro Leu Leu Leu Pro Pro Glu Lys Ala Arg Gln Thr
            900                 905                 910

Ala His Leu Val Phe Ala Ala Asp Gly Gly Gly Ser Glu Leu Pro Gly
        915                 920                 925

Leu Ala Phe Val Pro Glu Gly Ala Ala Arg Arg Val Ala Val Gln Thr
    930                 935                 940

Thr Ser Asn Ala Leu Leu Ser Leu Ala Lys Ile Phe Gln Asp Ala Met
945                 950                 955                 960

Ser Gly Gly Ser Thr Val Pro Ser Leu Leu Arg Gln Pro Thr Gly Ile
                965                 970                 975

Gly Asp Ile Leu Ala Leu Tyr Tyr Leu Ser Leu Ser Leu Gln Glu Ser
            980                 985                 990

Pro Ser Thr Ala Asn Asn Ile Gly  Ile Leu Leu Ala Gly  Val Gln Gln
        995                 1000                1005

Thr Ala  Pro Ser Asn Val Thr  Thr Pro Gly Thr Val  Pro Pro Arg
    1010                1015                1020
```

```
Pro Ser  Leu Pro Gly Val Val  Pro Gly Ser Gly Leu  Ala Leu Ala
    1025                1030                1035

Leu Ala  Tyr Tyr Asn Tyr Gly  Leu Arg Leu Asp Pro  Arg His Val
    1040                1045                1050

His Leu  His Thr Asn Leu Gly  Ser Leu Leu Lys Asp  Val Gly Gln
    1055                1060                1065

Leu Asp  Leu Ala Ile Gln Met  Tyr Glu Arg Ala Val  Ser Cys Asp
    1070                1075                1080

Arg Thr  Phe Asp Ile Ala Leu  Thr Asn Leu Ala Asn  Ala Val Lys
    1085                1090                1095

Asp Lys  Gly Arg Ile Lys Asp  Ala Ile Ala Tyr Tyr  Arg Arg Ala
    1100                1105                1110

Val Asp  Ser Asn Pro Asp Phe  Ala Glu Ala Val Cys  Gly Leu Leu
    1115                1120                1125

Thr Ala  Leu Asn Ser Val Cys  Asp Trp Arg Gly Arg  Gly Gly Ala
    1130                1135                1140

Leu Leu  Glu Ser Gly Lys Tyr  Asp Arg Trp His Val  Asp Asp Glu
    1145                1150                1155

Gly Thr  Leu Val Asp Val Arg  Thr Ala Met His Gly  Ser Gly Leu
    1160                1165                1170

Thr Gln  Arg Val Ile Gly Ile  Ile Ser Gln Gln Leu  Asp Asp Ala
    1175                1180                1185

Ser Gln  Trp Gly Arg Gly Ile  Leu Gln Gln Pro Thr  Ile His Gly
    1190                1195                1200

Leu Val  Glu Gln Leu Gln Glu  Phe Cys His Asn Pro  Lys Phe Asp
    1205                1210                1215

Leu Glu  Asn Ala Ile Arg Ala  Trp Ala His Lys Ser  Trp Glu Gly
    1220                1225                1230

Ser Arg  Leu Val Arg Leu Val  Glu Arg Ala Thr Arg  Val Ile Leu
    1235                1240                1245

Trp Lys  Trp Tyr His Asp Arg  His Val Ala Gln Arg  Lys Ala Thr
    1250                1255                1260

Pro Ser  Gln Tyr Tyr Arg Pro  Gln Leu Pro Ser Ser  Leu Thr Ile
```

```
    1265                1270                1275

Pro Ser  Ala Pro Thr Val Leu  Pro Phe His Thr Phe  Thr Tyr Pro
    1280                1285                1290

Leu Ser  Ala Lys Asp Ile Arg  Ser Ile Ser Gln Arg  Asn Ala Met
    1295                1300                1305

Arg Ile  Ser Cys Ser Thr Leu  Arg Ala Pro Trp Leu  Pro Pro Thr
    1310                1315                1320

Val Tyr  Pro Pro Pro Pro Pro  Pro Asn Pro Tyr Leu  Asn Val Gly
    1325                1330                1335

Tyr Val  Ser Ser Asp Phe Asn  Asn His Pro Leu Ala  His Leu Met
    1340                1345                1350

Gln Ser  Val Phe Gly Phe His  Asn Pro Gln Arg Ala  Arg Ala Phe
    1355                1360                1365

Cys Tyr  Ala Thr Thr Pro Ser  Asp Lys Ser Ile His  Arg Gln Gln
    1370                1375                1380

Ile Glu  Arg Glu Ala Pro Val  Phe Arg Asp Val Ser  Ser Trp Pro
    1385                1390                1395

Ala Glu  Lys Leu Ile Glu Gln  Ile Val Arg Asp Glu  Ile His Ile
    1400                1405                1410

Leu Val  Asn Leu Asn Gly Tyr  Thr Arg Gly Ala Arg  Asn Glu Ile
    1415                1420                1425

Phe Ala  Ala Arg Pro Ala Pro  Ile Gln Met Ser Phe  Met Gly Phe
    1430                1435                1440

Ala Gly  Thr Leu Gly Ala Glu  Trp Cys Asp Tyr Leu  Leu Ala Asp
    1445                1450                1455

Thr Thr  Ala Val Pro Pro Ser  Thr Leu Arg Pro Trp  Arg Asn Asn
    1460                1465                1470

Thr Thr  Ile Glu Asp Val Phe  Gln Asp Met Thr Glu  Gly Asp Glu
    1475                1480                1485

Arg Gln  Trp Met Tyr Ser Glu  Asn Ile Ile Phe Cys  Arg Asp Thr
    1490                1495                1500

Phe Phe  Cys Cys Asp His Ala  Gln Ser Cys Asp Asp  Asn Glu Arg
    1505                1510                1515
```

```
Asp Met  Thr Trp Glu Asp Glu  Glu Arg Arg Arg Trp  Lys Met Arg
    1520                1525                1530

Lys Glu  Leu Phe Pro Thr Ile  Ala Asp Asp Ala Ile  Ile Leu Ala
    1535                1540                1545

Asn Phe  Asn Gln Leu Tyr Lys  Ala Arg Glu Thr Asn  Leu Arg Gln
    1550                1555                1560

Thr Ala  Glu Ala Trp Ala Gly  Ala Glu Val Ala Ser  Arg Leu Val
    1565                1570                1575

Phe Thr  Asp Val Ala Pro Lys  Asn Gln His Ile Asn  Arg Ala Arg
    1580                1585                1590

Val Cys  Asp Leu Phe Leu Asp  Thr Ala Glu Cys Asn  Ala His Thr
    1595                1600                1605

Thr Ala  Ala Asp Val Leu Trp  Ser Ser Thr Pro Leu  Leu Thr Leu
    1610                1615                1620

Pro Arg  Tyr Ser Tyr Lys Met  Cys Ser Arg Met Ala  Ala Ser Ile
    1625                1630                1635

Leu Arg  Gly Ala Leu Pro Lys  Ser Ala Glu Gly Gln  Gln Ala Ala
    1640                1645                1650

Leu Glu  Leu Ile Ala Asp Gly  Glu Thr Glu Tyr Glu  Asp Gln Ala
    1655                1660                1665

Ala Glu  Leu Ala Gly Gly Leu  Thr Tyr Val Met Thr  Asp Glu Gly
    1670                1675                1680

Tyr Gly  Arg Gly Lys Gly Arg  Leu Ala Glu Leu Arg  Lys Leu Leu
    1685                1690                1695

Trp Asp  Ser Arg Trp Ser Cys  Gly Leu Phe Asn Thr  Arg Arg Trp
    1700                1705                1710

Val Asn  Asp Leu Glu Arg Ala  Tyr Glu Glu Ala Trp  Arg Arg Trp
    1715                1720                1725

Val Ala  Gly Lys Gly Gly Asp  Ile Tyr Leu
    1730                1735
```

<210> 3
<211> 859
<212> PRT

<213> Trichoderma reesei

<400> 3

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Met Pro His Arg Glu Arg Gly Lys Gln Arg Glu Gly Gly Asp Ser Tyr
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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
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Leu Pro Val Lys Arg Arg Arg Tyr Asp Ser Glu Ser Val Ser Arg Ser
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Pro Pro Pro Leu Lys Arg Gly Arg Arg Asp Asn
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<210> 4
<211> 2580
<212> DNA
<213> Trichoderma reesei

<400> 4

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ggcggcacgt acatcccgcc ggcgaggctg agggcgctgc aggcgcagat cacggacaag      240

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cacgagatgc tggccggcca gatcctgctg ctgctgctgc acaagccgac cgacgacagc      660

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gagtgggagg agcatgagga ggcatacaag aggctcaagg cggagattct gggcgaagcc     1020
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<213> Trichoderma reesei

<400> 5

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Leu Tyr Lys Thr Thr Leu Leu Asn Thr Val Cys Leu Val Ala Gly Leu

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Ser Ile Phe Phe Phe Gly Tyr Asp Gln Gly Leu Met Gly Gly Val Asn
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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

Asp Asn Thr Ser Ser Phe Ile Trp Arg Phe Pro Val Ala Phe Gln Ile
        195                 200                 205

Leu Pro Leu Ile Leu Leu Phe Leu Ile Ile Trp Ile Met Pro Glu Ser
    210                 215                 220

Pro Arg Trp Leu Val Lys Val Gly Arg Glu Glu Glu Ala Arg Phe Ile
225                 230                 235                 240

Leu Gly Arg Leu Arg Gly Asn Glu Gly Glu Asp Gly Leu Lys Ala Glu
                245                 250                 255

Ala Glu Tyr Asn Asp Ile Val Asn Ile His Lys Leu Glu Val Asp Thr
            260                 265                 270

Ala Lys Gln Gln Ser Tyr Phe Ser Met Phe Phe Gly Ile Gly Ser Gly
        275                 280                 285
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Lys Leu His Thr Gly Arg Arg Val Gln Leu Val Ile Trp Leu Gln Ile
    290                 295                 300

Leu Gln Glu Trp Ile Gly Ile Ala Gly Ile Thr Ile Tyr Gly Pro Glu
305                 310                 315                 320

Ile Phe Thr Ile Ala Gly Ile Ser Ala Lys Asp Arg Leu Trp Val Ser
                325                 330                 335

Gly Ile Asn Asn Ile Thr Tyr Met Phe Ala Thr Leu Ile Cys Val Phe
            340                 345                 350

Thr Ile Asp Arg Ile Gly Arg Arg Trp Thr Leu Tyr Trp Gly Ala Val
        355                 360                 365

Gly Gln Gly Ile Cys Met Phe Val Ala Gly Gly Leu Ala Arg Ala Thr
    370                 375                 380

Ile Asn Ala Ser Gly Lys Ala Ser Gln Ser His Ile Gly Gly Ala Ala
385                 390                 395                 400

Thr Phe Phe Val Phe Leu Tyr Thr Ala Ile Phe Gly Ala Thr Trp Leu
                405                 410                 415

Thr Val Pro Trp Leu Tyr Pro Ala Glu Ile Phe Pro Leu Gln Val Arg
            420                 425                 430

Ala Lys Gly Asn Ala Trp Gly Val Val Gly Trp Ser Ile Gly Asn Gly
        435                 440                 445

Trp Cys Val Leu Leu Leu Pro Thr Ile Phe Lys Ala Leu Asn Glu Lys
    450                 455                 460

Thr Leu Tyr Ile Phe Gly Ala Val Asn Ala Leu Ser Ile Leu Val Val
465                 470                 475                 480

Trp Ala Leu Tyr Pro Glu Ser Asn Gln Arg Thr Leu Glu Glu Met Asp
                485                 490                 495

Leu Val Phe Ala Ser Asp Ser Ile Trp Ala Trp Glu Ala Glu Arg Asn
            500                 505                 510

Phe Ala Lys Leu Lys Ala Glu Asn Pro Asp Leu Val Gln Gly Ser Thr
        515                 520                 525

Asn His Gly Val Val Asp Ile Glu Gln Val Ala Glu Pro Lys Glu
    530                 535                 540
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<212> DNA
<213> Trichoderma reesei

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gccctgcagg cctcagctat gaatgcgaac tggatgtttt gcggtatgtc gatgattctt 420

ggacaatcac aaccgaacta ttactgatga tgagatgaaa cagcccgcgt tctgaacggc 480

gtcggcactg gaatcttgaa cgcaatcacg cctgtgtggg caaccgagac tgctgctcac 540

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gcctactggc tggaattgta cgtgcctcct cactcaggat ccccagtctt gtggaaagtc 660

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aaaggagtag                                                            1870
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<210> 7
<211> 735
<212> PRT
<213> Trichoderma reesei

<400> 7

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            20                  25                  30

Arg Lys Glu Ser Ile Gln Lys Thr Ile Met Ala Met Thr Leu Gly Lys
        35                  40                  45

Asp Val Ser Ala Leu Phe Pro Asp Val Leu Lys Asn Ile Ala Thr Ser
    50                  55                  60

Asp Leu Asp Gln Lys Lys Leu Val Tyr Leu Tyr Leu Met Asn Tyr Ala
65                  70                  75                  80

Lys Thr His Pro Asp Leu Cys Ile Leu Ala Val Asn Thr Phe Val Gln
                85                  90                  95

Asp Ser Glu Asp Pro Asn Pro Leu Val Arg Ala Leu Ala Ile Arg Thr
            100                 105                 110

Met Gly Cys Ile Arg Val Asp Lys Met Val Asp Tyr Met Glu Glu Pro
        115                 120                 125

Leu Arg Lys Thr Leu Arg Asp Glu Ser Pro Tyr Val Arg Lys Thr Ala
    130                 135                 140

Ala Ile Cys Val Ala Lys Leu Phe Asp Leu Asn Pro Ala Met Cys Ile
145                 150                 155                 160

Glu Asn Gly Phe Ile Glu Thr Leu Gln Glu Met Ile Gly Asp Pro Asn
                165                 170                 175

Pro Met Val Val Ala Asn Ser Val Gln Ala Leu Ala Glu Ile Ser Glu
            180                 185                 190

Thr Ala Pro Glu Thr Arg Ala Leu Leu Val Thr Pro Pro Val Leu Lys
        195                 200                 205
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Lys Leu Leu Met Ala Met Asn Glu Cys Thr Glu Trp Gly Arg Ile Thr
    210                 215                 220

Ile Leu Thr Val Leu Ala Asp Tyr Ala Ala Thr Asp Val Lys Glu Ser
225                 230                 235                 240

Glu His Ile Cys Glu Arg Val Ile Pro Gln Phe Gln His Val Asn Pro
                245                 250                 255

Ser Val Val Leu Ala Ala Val Lys Val Val Phe Ile His Met Lys Ser
            260                 265                 270

Ile Asn Pro Glu Leu Val Arg Ser Tyr Leu Lys Lys Met Ala Pro Pro
        275                 280                 285

Leu Val Thr Leu Val Ala Ser Ala Pro Glu Val Gln Tyr Val Ala Leu
    290                 295                 300

Arg Asn Ile Asp Leu Leu Leu Gln Ala Lys Pro Asp Ile Leu Ser Lys
305                 310                 315                 320

Glu Leu Arg Val Phe Phe Cys Lys Tyr Asn Asp Pro Pro Tyr Val Lys
                325                 330                 335

Met Gln Lys Leu Glu Ile Met Val Arg Ile Ala Asn Glu Lys Asn Tyr
            340                 345                 350

Glu Gln Leu Leu Ser Glu Leu Lys Glu Tyr Ala Leu Glu Val Asp Met
        355                 360                 365

Asp Phe Val Arg Arg Ala Ile Lys Ala Ile Gly Gln Val Ala Ile Lys
    370                 375                 380

Ile Glu Glu Ala Ser Gly Lys Cys Val Gln Ala Leu Glu Asp Leu Leu
385                 390                 395                 400

Ala Thr Lys Val Asn Tyr Val Val Gln Glu Val Val Val Val Ile Lys
                405                 410                 415

Asp Ile Leu Arg Lys Tyr Pro Gly Tyr Glu Gly Val Ile Pro Ser Leu
            420                 425                 430

Cys Asn Tyr Ile Asp Glu Leu Asp Glu Ala Asn Ala Arg Gly Ser Leu
        435                 440                 445

Ile Trp Ile Val Gly Glu Tyr Ala Glu Lys Ile Ser Asn Ala Glu Glu
    450                 455                 460
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Ile Leu Glu Gly Phe Val Asp Thr Phe Leu Glu Glu Phe Thr Gln Thr
465                 470                 475                 480


Gln Leu Gln Ile Leu Thr Ala Val Val Lys Leu Phe Leu Lys Lys Pro
                485                 490                 495


Ser Gly Ala Gln Gly Leu Val Gln Lys Val Leu Gln Glu Ala Thr Thr
            500                 505                 510


Asn Asn Asp Asn Pro Asp Ile Arg Asp Arg Ala Tyr Val Tyr Trp Arg
        515                 520                 525


Leu Leu Ser Gly Asp Leu Glu Val Ala Lys Asn Ile Val Leu Ser Gln
    530                 535                 540


Lys Pro Thr Ile Ser Thr Thr Met Thr Ser Leu Pro Thr Ala Leu Leu
545                 550                 555                 560


Glu Gln Leu Leu Ser Glu Leu Ser Thr Leu Ala Ser Val Tyr His Lys
                565                 570                 575


Pro Pro Glu Ala Phe Val Gly Lys Gly Arg Phe Gly Ala Asp Glu Ile
            580                 585                 590


Gln Arg Ala Ala Ile Gln Glu Gln Arg Gln Asn Ala Ala Glu Asn Pro
        595                 600                 605


Ile Ala Ala Ser Val Ala Ala Ala Ala Ala Asn Gly Ser Ser Ser Val
    610                 615                 620


Ser Gln Asn Asn Ile Glu Asn Leu Leu Asp Ile Asp Phe Asp Gly Ala
625                 630                 635                 640


Ala Pro Ala Ser Gln Glu Gln Asn Ser Ala Ala Gly Thr Pro Asp Arg
                645                 650                 655


Val Ser Ser Pro Ala Thr Gly Gly Met Ala Asp Met Met Ser Met Phe
            660                 665                 670


Asp Ala Pro Pro Ala Gly Ser Ser Gly Gly Ala Pro Ser Gly Gly Met
        675                 680                 685


Asn Asp Leu Met Asn Gly Phe Glu Gly Leu Asn Phe Gly Ala Thr Ser
    690                 695                 700


Thr Asn Gln Pro Leu Pro Ala Ala Met Gln Leu His Asn Ala Gln Gly
705                 710                 715                 720


Gly Ser Gln Pro Lys Lys Asp Ser Asp Asp Leu Leu Gly Leu Leu
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725 730 735

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<213> Trichoderma reesei

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<211> 5723
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA fragment for transformation

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