The invention relates to novel variants of fungal endoglucanases, their production and means for their production. Especially the invention relates to variants of Acremonium thermophilum Cel45A. The invention further relates to enzyme preparations and detergent compositions comprising at least one novel variant endoglucanase as well as to processes for treating cellulosic material therewith. The novel variant endoglucanase polypeptides have improved performance in textile applications, especially in biofinishing and biostoning, and in detergent applications, in fabric care and color maintenance, especially in prevention and removal of fuzz and pills, in color care and revival.
FUNERAL ENDOGLUCANASE VARIANTS, THEIR PRODUCTION AND USE

FIELD OF THE INVENTION

The invention relates to novel variants of fungal endoglucanases, their production and means for their production. The invention further relates to enzyme preparations comprising at least one of the novel endoglucanase variants as well as to processes for treating cellulosic material therewith. The novel endoglucanase variants are especially useful in textile treatment and detergent applications.

BACKGROUND OF THE INVENTION

Cellulose is a linear polysaccharide of glucose residues connected by β-1,4 linkages. It is the main component of plant cell walls, and the basic building block for many textiles and for paper. It gives plant cells remarkable strength helping them to resist mechanical stress and osmotic pressure. Cotton is the purest natural form of cellulose.

Cellulases or cellulolytic enzymes are a group of glycoside hydrolase enzymes that catalyze the hydrolysis of beta-1,4 glycosidic linkages in the cellulose polymer. Cellulases are known to be produced by a large number of bacteria, yeast, and fungi. Cellulases comprise a catalytic domain/core (CD) expressing cellulase activity. In addition to the catalytic domain, the cellulase molecule may comprise one or more cellulose binding domains (CBDs), also named as carbohydrate binding domains/modules (CBD/CBM). The CBD enhances the binding of the enzyme to a cellulose-containing fiber and increases the efficacy of the catalytic domain.

Cellulases are utilized, based on their properties, in various industrial fields. In the textile industry, cellulases are used in denim finishing for creating a fashionable stone washed appearance in denim cloths in a biostoning process, and they are also used, for instance, to clean fuzz and prevent formation of pills on the surface of cotton garments. In detergent industry, cellulases are used to brighten colors, to prevent graying and pilling of garments and to improve cleaning. Cellulases are further used in food industry, including baking, and animal feed manufacturing, and they have a great potential in the pulp and paper industry, for instance, in deinking to release ink from fiber surfaces, in improving pulp drainage and fiber modification, in energy reduction, in refining and drying stages of paper, board and pulp production. Cellulases are also utilized in hydrolysis of lignocellulosic material for, e.g. bioethanol production.
The practical use of cellulases is hampered by the nature of the cellulase compositions, which often are enzyme mixtures having a variety of activities and substrate specificities. The unique properties of each cellulase make some more suitable for certain purposes than others. The wide spectrum of industrial uses for cellulases has established a need for commercial cellulase products containing different cellulase components and functioning in different pH and temperature ranges. The production costs of microbiologically produced enzymes are tightly connected with the productivity of the enzyme producing strain and the final activity yield in the fermentation broth. There exists a need in the art to identify enzyme variants and enzymatic compositions that have improved efficacy and capacity to act on a greater variety of cellulosic materials.

US201 1250674 (A1) provides a method for improving the properties of a cellulolytic enzyme i.e., an endo-1,4-glucanase, by amino acid substitution, deletion or insertion. The invention discloses Humicola insolens cellulase variants, which have been improved with respect to activity; and/or sensitivity to anionic tensides; and/or pH optimum and pH profile as well as stability. US201 30244292 relates to a family 5 glycoside hydrolase variant having endoglucanase activity and to polynucleotides encoding the polypeptides.

Although cellulolytic enzymes have been used successfully in commercial applications for many years, a need still exists for new cellulolytic enzymes with altered properties, such as improved performance, in varying industrial applications.

BRIEF DESCRIPTION OF THE INVENTION

An object of the present invention is thus to provide novel variants of cellulolytic enzymes, which show improved performance, when compared to the parental enzyme.

The objects of the invention are achieved by a variant endoglucanase polypeptide comprising an amino acid sequence having at least 95% sequence identity with amino acids 1 to 214 of SEQ ID NO: 1 and an amino acid substitution at one or more positions selected from the group consisting of 51, 75, 77, 82, 109, 116, 118, 135, 150 and 205, the positions being numbered with reference to SEQ ID NO: 1, wherein the variant polypeptide has endoglucanase activity.

The invention is further directed to an isolated polynucleotide selected from the group consisting of:
a) a polynucleotide or complementary DNA encoding an endoglucanase polypeptide variant of claim 1;
b) a polynucleotide or complementary DNA encoding an endoglucanase polypeptide variant of claim 8;
c) a polynucleotide encoding a fragment of a polypeptide encoded by a polynucleotide of a) or b) wherein said fragment is having endoglucanase activity;
d) a polynucleotide comprising a nucleotide sequence which is degenerate to the nucleotide sequence of a polynucleotide sequence of a), b) or c); or the complementary strand of such a polynucleotide.

The invention is still further directed to an expression vector comprising said polynucleotide and a host cell comprising said expression vector.

Still further the invention is directed to a method for the production of the endoglucanase polypeptide variant, comprising the steps of transforming a host cell with an expression vector encoding said polypeptide, and culturing said host cell under conditions enabling expression of said polypeptide, and optionally recovering and purifying said polypeptide.

The invention is further directed to an enzyme preparation comprising at least one novel endoglucanase polypeptide variant of the invention and the use of said enzyme preparation especially in textile and detergent industry, but also in biomass processing, preferably in biofuel, starch, pulp and paper, food, baking, feed or beverage industries.

In one aspect, the present invention relates to a detergent composition comprising an endoglucanase polypeptide variant or an enzyme preparation of the invention and optionally auxiliaries, such as surface active agents, surfactants, bleaching agents, builders, stabilizers, buffers, mediators of an oxidase, anti-corrosion agents, polymers/antiredeposition agents, optical brighteners, dyes, pigments, caustics, abrasives and preservatives, perfumes, etc.

The invention also relates a process for treating cellulosic material, wherein said process comprises contacting the cellulosic material with the endoglucanase polypeptide variant or enzyme preparation of the invention. The treating of cellulosic material involves detergent applications, biostoning or bio-finishing.

Specific embodiments of the invention are set forth in the dependent claims. Other objects, details and advantages of the present invention will become apparent from the following drawings, detailed description and examples.
It should be understood, however, that the embodiments given in the description, drawings and in the examples are for illustrative purposes only, and that various changes and modifications are possible within the scope of the claims.

The present invention describes improved, higher performance cellulases for use in various industrial processes. The invention discloses a number of amino acid residue positions important for the properties of an endoglucanase enzyme and thereby for the performance thereof. Particularly, the present invention discloses variant endoglucanase polypeptides having improved performance in textile applications, especially in biofinishing and biostoning, and in detergent applications, in fiber care and color maintenance, especially in prevention and removal of fuzz and pills, in color care and revival. The variant endoglucanases of the invention perform well at broad pH range and notably at neutral and alkaline pH. This enables biofinishing treatment simultaneously with dyeing, leading to considerable cost savings. The color fastness is often better at neutral conditions. These novel endoglucanases are also effective at smaller enzyme dosages than the reference enzymes.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following the invention will be described in greater detail by means of preferred embodiments with reference to the attached drawings, in which

Figure 1 illustrates a schematic picture of the expression cassettes used in the transformation of Trichoderma reesei A152 for production of Acremonium thermophilum cellulases of the invention. The recombinant genes were under control of T. reesei ceUA promoter and transcription termination was ensured with the addition of the T. reesei cel7A terminator. The amdS gene (amdS) was included for selection of the transformants.

Figure 2 shows the performance of A. thermophilum ACMO variants in detergent application as an increase of darkness (sum of -AL of 4 stripes) after 10 washing and tumbling cycles of test monitors. Commercial preparation Carezyme® and ACMO cellulase were used for comparison. Washing conditions in Launder-Ometer were: 40°C, 60 min, 16°dH, commercial liquid detergent 5 g/l, pH approx. 8.5, enzyme dosage 0.2 mg AEP/l;

Figure 2A shows results of prepilled test monitor E-253. Pilling removal/color revival effect;
Figure 2B shows results of unpilled test monitor (E-252). Color care/antipilling effect.

Figure 3 shows the performance of A. thermophilum ACMO variants ACM72 and ACM88 in detergent application as an increase of darkness (sum of -AL° of 4 stripes) after 10 washing and tumbling cycles of test monitors. Commercial preparation Carezyme® and the ACMO cellulase were used for comparison. Washing conditions in Launder-Ometer were: 40°C, 60 min, 16°dH, commercial liquid detergent 5 g/l, pH approx. 8.5, enzyme dosage 0.1 mg AEP/l;

Figure 3A shows results of prepilled test monitor E-253. Pilling removal/color revival effect;

Figure 3B shows results of unpilled test monitor (E-252). Color care/antipilling effect.

Figure 4 shows the performance of A. thermophilum ACMO variant ACM88 in biofinishing (defuzzing) treatment in washing machine at 50°C, pH 6 or without adjustment (pH approx. 8), 60 min, enzyme dosage 0.025 or 0.05 mg AEP per weight (g) of the fabric. The ACMO cellulase was used for comparison.

Figure 5 shows the performance of A. thermophilum Cel45A cellulase variants ACM90 and ACM91 in detergent application as an increase of darkness (sum of -AL° of 4 stripes) after 10 washing and tumbling cycles of test monitors. At Cel45A cellulase was used for comparison. Washing conditions in Launder-Ometer were: 40°C, 60 min, 16°dH, commercial liquid detergent 5 g/l, pH approx. 8.5, enzyme dosage 0 - 0.2 or 0 - 0.4 mg AEP/l;

Figure 5A shows results of prepilled test monitor E-253. Pilling removal/color revival effect;

Figure 5B shows results of unpilled test monitor (E-252). Color care/antipilling effect.

DETAILED DESCRIPTION OF THE INVENTION

Cellulolytic enzymes or cellulases are enzymes having cellulolytic activity, which means that they are capable of hydrolyzing cellulosic substrates or derivatives thereof into smaller saccharides. Cellulolytic enzymes thus include both cellulases and hemicellulases. Cellulases include (1) endoglucanases (EG, EC 3.2.1.4) which cut internal beta-1,4-glucosidic bonds; (2) exoglucanases or cellobiohydrolases (CBH, EC 3.2.1.176, EC 3.2.1.91) that cut the disaccharide
cellulbiose from the reducing or non-reducing end of the crystalline cellulose polymer chain and (3) beta-1,4-glucosidases (BG, EC 3.2.1.21) which hydrolyze the cellulbiose and other short cello-oligosaccharides to glucose.

The present invention relates in particular to endoglucanases. Specifically, the present invention relates to fungal endoglucanases belonging to glycosyl hydrolase family 45, especially to variants of these endoglucanases. More specifically the invention relates to variants of Acremonium thermophilum Cel45A endoglucanase polypeptide. "Glycosyl hydrolase family 45" refers to the glycosyl hydrolase family as defined by Henrissat 1991, and Henrissat and Bai- roch 1993, 1996.

The variants of the invention were designed by protein engineering techniques on the basis of amino acid sequence comparison between the catalytic core domains of Acremonium thermophilum Cel45A and Geomyces pannorum Cel45A (SEQ ID NO:1 and SEQ ID NO:2, respectively) and their three-dimensional structures. The amino acid and nucleotide sequences of Acremonium thermophilum Cel45A and Geomyces pannorum Cel45A and their isolation are disclosed in patent applications FI20055692 and FI20086253 (AB Enzymes Oy, FI).

The invention relates to a variant endoglucanase polypeptide or an enzymatically active fragment of it, comprising an amino acid sequence having at least 95 % sequence identity with amino acids 1 to 214 of SEQ ID NO: 1 and an amino acid substitution at one or more positions selected from the group consisting of 51, 75, 77, 82, 109, 116, 118, 135, 150 and 205, the positions being numbered with reference to SEQ ID NO: 1, wherein the variant polypeptide has endoglucanase activity. Especially, the variant endoglucanase polypeptide has at least 96, 97, 98 or 99% identity with amino acids 1 to 214 of SEQ ID NO: 1. The variant endoglucanase polypeptide may contain any combination of said substitutions.

By the term "identity" is here meant the identity between two amino acid sequences. The degree of identity is determined by using EMBOSS Water pairwise sequence alignment program at EBI (European Bioinformatics Institute) http://www.ebi.ac.uk/Tools/psa/emboss_water/ with the following parameters: BLOSUM62, Gap open 10, Gap extend 0.5. The algorithm is described in Smith and Waterman (1981).
In one embodiment of the invention the polypeptide variant comprises a substitution at a position corresponding to one or more of S51 N, A75S, A77S, S82Q, S109N, S116Q, S116E, Q118H, A135Q, S150Q and S205N.

In another embodiment the polypeptide variant has a substitution at the positions corresponding to S51 N, S82Q, S109N, S116E, Q118H, A135Q, S150Q and S205N.

Still in another embodiment the polypeptide variant has a substitution at the positions corresponding to S51 N, S82Q, S116E, Q118H, A135Q, S150Q and S205N.

According to one embodiment the variant endoglucanase polypeptide has an amino acid sequence of SEQ ID NO: 12 or 13 and an amino acid substitution at one or more positions selected from the group consisting of 51, 75, 77, 82, 109, 116, 118, 135, 150 and 205, the positions being numbered with reference to SEQ ID NO: 1.

According to one embodiment the variant endoglucanase polypeptide has an amino acid sequence of SEQ ID NO: 12 and a substitution at the positions corresponding to S51 N, S82Q, S109N, S116E, Q118H, A135Q, S150Q and S205N.

According to another embodiment the variant endoglucanase polypeptide has an amino acid sequence of SEQ ID NO: 12 and a substitution at the positions corresponding to S51 N, S82Q, S116E, Q118H, A135Q, S150Q and S205N.

According to one embodiment the variant endoglucanase polypeptide has an amino acid sequence of SEQ ID NO: 13 and a substitution at the positions corresponding to S51 N, S82Q, S109N, S116E, Q118H, A135Q, S150Q and S205N.

According to another embodiment the variant endoglucanase polypeptide has an amino acid sequence of SEQ ID NO: 13 and a substitution at the positions corresponding to S51 N, S82Q, S116E, Q118H, A135Q, S150Q and S205N.

In the present invention the polypeptide variants are derived from a parental molecule ce/45A-ACM0 (SEQ ID NO: 3), which is a polynucleotide containing a region encoding for the catalytic core domain of A. thermophilum Cel45A attached to the linker and CBM region of T. reesei Cel7A, or from a parental molecule ce/45A (SEQ ID NO: 9), which is a polynucleotide containing
a region encoding for the catalytic core domain of *A. thermophilum* Cel45A endoglucanase and having the natural linker and CBM region. However, any polynucleotide sequence encoding for the catalytic core domain of *A. thermophilum* Cel45A may be used for creating the polypeptide variants of the invention. As used herein, a "variant" is a polypeptide having an amino acid substitution, deletion or insertion at one or more positions. Preferably the variants have a substitution. The variants are generated by mutagenesis i.e. by deliberately introducing changes in DNA to produce mutant gene products i.e. proteins. The changes or modifications of the parental nucleotide sequence may be introduced by several methods including e.g. site-directed and random mutagenesis. For site-directed mutagenesis a protein structure and good understanding of the structure-function relationship is beneficial. In the absence of such deep understanding, methods based on random mutagenesis may be used.

A variant may be obtained e.g. by altering hydrogen bond contacts, altering charge distribution, introduction of a salt bridge, introduction of metal binding sites, filling an internal structural cavity with one or more amino acids with bulkier side groups (in e.g. regions which are structurally mobile), substitution of histidine residues with other amino acids, removal of a deamination site, or by helix capping. Stability of the protein may be improved by substitution of at least one amino acid with cysteine residue or insertion of one or more cysteine residues which creates at least one disulfide bridge.

The endoglucanase polypeptide variants of the invention are preferably recombinantly produced fusion proteins. They are conveniently prepared using the generally known recombinant DNA technology. Briefly, the polynucleotide encoding the endoglucanase is cloned and inserted into an expression vector, transformed into a host cell and expressed. Methods for protein production by recombinant technology in different host systems are well known in the art (Sambrook and Russel, 2001; Coen, 2001; Gellissen, 2005). Preferably, the polypeptide variants are produced as extracellular proteins that are secreted into the culture medium, from which they can easily be recovered and isolated.

The endoglucanase polypeptide variants may comprise in addition to the catalytic core domain, which forms the active or functional site of the enzyme, one or more "cellulose binding domains" ("CBDs"), also named as carbohydrate binding domains/modules (CBD/CBM) located either at the N- or C-terminus of the catalytic domain. CBMs have carbohydrate-binding activity and they mediate the binding of cellulase to crystalline cellulose but have little or no...
effect on hydrolytic activity of the enzyme on soluble substrates. The endoglucanase variants of the invention may optionally also contain a signal sequence and a linker connecting the CBM and catalytic domain via a flexible and highly glycosylated region. The modular structure of cellulases containing carbohydrate binding module and/or the linker region is well known in the art. The carbohydrate binding module and the linker region may be heterologous or homologous. "Heterologous" as used in the present context means that the CBM and/or the possible linker part of the variant endoglucanase polypeptide are obtained from a different organism than the cellulolytically active core domain. "Homologous" as used herein means that the CBM and/or the possible linker part of the variant are from the same organism as the cellulolytically active core. The invention discloses that any linker or CBM may be used in the variant endoglucanase polypeptide. The performance of the endoglucanase polypeptide variants of the invention is improved as compared to the parental enzyme regardless of the origin of the linker or CBM region (Examples 3 and 6).

"Enzymatically active fragment" refers to the part of a specific amino acid sequence that is long enough to have the desired enzymatic activity. In other words a fragment may be e.g. only the mature part of the polypeptide or even a subsequence of the mature part. It may or may not contain a linker and CBM domain. The enzymatic activity refers herein to cellulolytic activity meaning catalytic ability of the polypeptide to hydrolyse cellulose or derivatives thereof. The enzymatic activity may be determined as described in Example 1.

The present invention relates further to novel polynucleotides which comprise nucleotide sequences having SEQ ID NO: 4 to 8 (Table 1), or fragments thereof long enough to encode an enzymatically active endoglucanase variants, or a sequence encoding a novel polypeptide variant as defined above, including complementary strands thereof. The polynucleotides of the invention are recombinant molecules containing genetically engineered non-naturally occurring sequences. "Polynucleotide" as used herein refers to both RNA and DNA, and it may be single stranded or double stranded. It may also be complementary DNA (cDNA). With cDNA is meant a DNA molecule synthesized from a messenger RNA template obtained from a eukaryotic or prokaryotic organism. Further, the polynucleotide may be degenerate as a result of the genetic code to any one of the sequences as defined above. This means that different codons may code for the same amino acid.
The present invention relates to a recombinant expression "vector" comprising a polynucleotide encoding the endoglucanase polypeptide variants as characterized above, operably linked to regulatory sequences, which are capable of directing the expression of a gene encoding said endoglucanase polypeptide variants in a suitable host. Said regulatory sequences may originate from the host organism or from another organism. The expression vector may further comprise marker genes for selection of the transformant strains or the selection marker may be introduced to the host in another vector construct by co-transformation.

Still the present invention relates to a production "host", which can be any organism capable of expressing the desired polypeptide. Preferably the host is a microbial cell, more preferably a fungus. Most preferably the host is a filamentous fungus, such as Trichoderma, Aspergillus, Fusarium, Humicola, Chrysosporium, Neurospora, Rhizopus, Penicillium, Myceliophthora, Sporotrichum, and Mortierella. The endoglucanase polypeptide variants may be produced in a heterologous or homologous host. The host may or may not be genetically modified. Preferred hosts for producing the polypeptides of the invention are in particular strains from the genus Trichoderma. Preferably the recombinant host is modified to express and secrete the endoglucanase polypeptide variants of the invention as its main activity or one of its main activities. This can be done by deleting genes encoding major endogenous secreted enzymes e.g. the four major cellulases of Trichoderma and by integrating heterologous genes to a locus with high expression and production levels.

The present invention relates also to a method for producing variant endoglucanase polypeptides of the invention, said method comprising the steps of transforming a host cell with an expression vector encoding said polypeptide, and culturing said host cell under conditions enabling production of said polypeptide, and optionally recovering and purifying said polypeptide. The production medium may be a medium suitable for growing the host organism and containing inducers for efficient gene expression.

The present invention relates to an enzyme preparation comprising the variant endoglucanase polypeptides of the invention. As used in the present context the "enzyme preparation" refers to any enzyme product or composition, which comprises at least one of the novel variant endoglucanase polypeptides described herein. Such an enzyme preparation may be a spent culture medium or filtrate containing one or more variant endoglucanase polypeptides, or one or
more variant endoglucanase polypeptides and one or more other enzymes. Spent culture medium means the culture medium of the host comprising the produced enzymes. Preferably the host cells are separated from said medium after the production. The enzyme preparation or composition may be a "whole culture broth" obtained, optionally after inactivating the production host(s) or microorganism(s) without any biomass separation, down-stream processing or purification of the desired cellulosytic enzyme(s), because the variant endoglucanase polypeptides can be secreted into the culture medium, and they display activity in the ambient conditions of the spent culture medium.

The enzyme preparation may contain the enzymes in at least partially purified and isolated form. It may even essentially consist of the desired enzyme or enzymes. If desired, the enzyme preparations may be dried, spray-dried or lyophilized, granulated or the enzymatic activity may be otherwise concentrated and/or stabilized for storage. If required, a desired enzyme may be crystallized or isolated or purified in accordance with conventional methods, such as filtration, extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like.

In addition to one or more variant endoglucanase polypeptides, the enzyme preparation may comprise one or more other enzymes, which may be for example other cellulases, amylases, lipases, proteases, hemicellulases, ligninases, pectinolytic enzymes and/or oxidative enzymes. More specifically, the enzyme preparation may comprise at least one further enzyme selected from a group of cellbiohydrolase, endoglucanase, beta-glucanase, beta-glucosidase, serine protease, xylanase, beta-xylosidase, mannanase, beta-mannosidase, endopectinlyase, pectate lyase, pectinesterase, laccase, cutinase, peroxidase and copper-dependent lytic polysaccharide monooxygenase i.e. glycosyl hydrolase family 61 enzymes. The enzyme preparation may contain any combination of these enzymes and the variant endoglucanase polypeptides of the invention, but the enzymes are not limited to those described herein. They can for example also be commercially available enzyme preparations. It depends on the application what other enzymes are included in the enzyme preparation or used in the enzyme treatment.

In addition to the variant endoglucanase polypeptides, the enzyme preparation of the invention may comprise one or more suitable additives selected from the group consisting of surfactants or surface active agents, buffers,
anti-corrosion agents, stabilizers, bleaching agents, mediators, builders, caustics, abrasives and preservatives, optical brighteners, antiredeposition agents, dyes, pigments, perfumes etc.

The enzyme preparations may be provided as a liquid or as a solid, for example, as a dried powder or granular, especially non-dusting granules, a stabilized liquid, tablet, crystal or crystal slurry. It is envisioned that the enzyme preparations can be further enriched to satisfy the requirements of a specific utility in various applications e.g. in the textile industry. A mixture of enzymes secreted by a host can be advantageous in a particular industrial application, for example in biofinishing and biostoning.

The present invention relates further to a detergent composition comprising at least one of the novel variant endoglucanase polypeptides or an enzyme preparation thereof, a surfactant and optionally one or more additives selected from the group consisting of stabilizers, buffers, surface active agents, builders, cobuilders, bleaching agents, bleach activators, other detergent enzymes, mediators of enzymes (e.g. for oxidases, peroxidases, laccases), anti-corrosion agents, antiredeposition agents and soil release polymers, caustics, abrasives, optical brighteners, dyes, pigments, perfumes and preservatives. As used in the present context, the expression "detergent" means a substance or material intended to assist cleaning or having cleaning properties. Suitable listing of the contents of detergents is given in U.S. Patent No. 5,443,750 or in WO2013/131964 and a suitable list of surfactants is given in U.S. Patent No. 3,664,961.

Surfactants are useful in emulsifying grease and wetting surfaces. The surfactant may be a non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic. Buffers may be added to the enzyme preparation or composition to modify pH or affect performance or stability of other ingredients. Suitable stabilizers include polyols such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or boric acid derivatives, peptides, etc. Bleaching agent is used to oxidize and degrade organic compounds. Examples of suitable chemical bleaching systems are H2O2 sources, such as perborate or percarbonate with or without peracid-forming bleach activators such as tetraacetylethylene diamine, or alternatively peroxycacids, e.g. amide, imide or sulfone type. Chemical oxidizers may be replaced partially or completely by using oxidizing enzymes, such as laccases or peroxidases. Many laccases do not function effectively in the absence of mediators. Builders or complexing agents
include substances, such as zeolite, diphosphate, triphosphate, carbonate, citrate, etc. The detergent composition may further comprise one or more polymers, such as carboxymethylcellulose, poly(ethylene glycol), poly(vinylalcohol), polyvinyl pyrrolidone), etc. Also, softeners, caustics, preservatives for preventing spoilage of other ingredients, abrasives and substances modifying the foaming and viscosity properties can be added.

The detergent composition of the invention may comprise one or more components selected from the group consisting of anionic surfactants (0 - 40% by weight), nonionic surfactants (0 - 40% by weight), and phosphonates (0 - 15% by weight) in addition to the effective amount of the variant endoglucanase polypeptide or an enzyme preparation thereof.

The term "effective amount" of an endoglucanase polypeptide variant refers to the quantity of the enzyme necessary to perform sufficiently in the specific detergent application. The amount of enzyme preparation in a detergent composition may vary depending on type and concentration of the detergent. Preferably the detergent composition comprises from about 0.00001 % to about 10% by weight of the detergent composition of an endoglucanase polypeptide variant of the invention, more preferably from 0.0001 % to about 1%, still more preferably from 0.0001 % to 0.5%, even more preferably from 0.0005% to 0.1 %.

A person skilled in art is capable in defining suitable dosages.

An endoglucanase polypeptide variant or an enzyme preparation thereof may be added directly into a detergent or it can be applied separately on top of the detergent during or before wash, or, for example, in liquid/liquid or liquid/powder sachets or multicompartent sachets or bottles, in which it may be separated from some of the detergent components or other enzymes, like protease, to maximize the storage stability. An endoglucanase polypeptide variant or an enzyme preparation thereof can also be used in cleaning agents or boosters that are added on top of the detergent during or before the wash and that are for example in the form of liquid, gel, powder, granules or tablets. Enzyme preparation and detergent components may also be soaked in a carrier like textiles.

The variant endoglucanase polypeptides and the enzyme preparations thereof may be used for treating any cellulosic material. In the present context, "cellulosic material" refers to any material comprising cellulose or derivatives thereof as a significant component. Such a material may be textile material,
plants or material of plant origin used in food or animal feed, plant material for oil extraction, or wood-derived mechanical or chemical pulp or secondary fiber.

The variant endoglucanase polypeptides are especially useful in the treatment of textile materials. The textile material to be treated may be any textile material including yarns, yarn intermediates, fibers, non-woven materials, natural materials, synthetic materials, and any other textile material, fabrics made of these materials and products made from fabrics (e.g., garments and other articles). The textile or fabric may be in the form of knits, wovens, non-wovens, felts, yarns, and towelling. The textile may be cellulose based such as natural cellulosics, including cotton, flax/linen, jute, ramie, sisal or coir or manmade cellulosics (e.g. originating from wood pulp) including viscose/rayon, modal, cellulose acetate fibers (tricell), lyocell, cupro or blends thereof. Fabric may be conventional washable laundry, for example, stained household laundry. When the term fabric or garment is used it is intended to include the broader term textiles as well. By "denim" is meant, in connection of this invention, denim fabric, usually denim garments, particularly jeans. Advantageously, the denim is Indigo dyed denim. Denim can also be treated with derivatives of Indigo or with Indigo together with some other dye, for example, Indigo-dyed denim with sulphur bottom.

The cellulosic material is reacted with the variant endoglucanase polypeptides of the invention or the enzyme preparation comprising said variant endoglucanase polypeptides under suitable conditions, such as appropriate pH, and temperature, and the reaction is allowed to continue for a time sufficient for the enzymatic reaction to take place, whereby at least partially hydrolyzed cellulosic material is obtained. The enzymes are added in an enzymatically effective amount either simultaneously e.g. in the form of an enzyme mixture, or sequentially.

The variant endoglucanase polypeptides may also be added into detergent compositions to improve fiber and color care properties by prevention and removal of fuzz and pills resulting in brightening or freshening of colors and softening, and to improve textile cleaning, for instance by removal of pigment dirt and by antiredeposition and antigraying. Terms depilling (removal of pilling) and color revival are typically used to describe the cellulase effects on old, used cotton textiles. Terms antipilling (prevention of pilling), color maintenance or color care are typically used to describe cellulase effects on new garments.
As used in the present context the expression "cellulase performance" in detergent application refers to the effect of cellulase on the fiber and color care properties of detergent, that can be measured as a visible and measureable decrease of lightness (i.e. increase of darkness) or change in color of colored cotton textiles. When the surface fibers and fibrils protruding from the yarn forming pills and giving the fabric a greyish look are removed by cellulase, the lightness of the fabric decreases, and the surface of the fabric appears darker and colors get brighter. Lightness or change in color values can be measured, for example by measuring the color as reflectance values with a spectrophotometer using L*a*b* color space coordinates as described in Examples 4 and 6. Cellulase performance is for example calculated as ΔL* (delta L*), which means lightness value L* of enzyme treated fabric minus lightness value L* of fabric treated with washing liquor without enzyme (enzyme blank, control). When the test material is consisting of textiles with different colors (e.g. commercially available pilling monitors containing 4 stripes), the total cellulase performance is calculated as a sum of ΔL* of each color after several washing cycles and the final results are shown as increase of darkness (sum of -ΔL*).

The variant endoglucanase polypeptides and the enzyme preparations containing them are especially useful in finishing processes of the textile industry, such as biofinishing of fabrics, garments or yarn. As used in the present context, the expression "biofinishing" (also called depilling, defuzzing, dehairing or biopolishing) refers to the use of the variant enzymes in a controlled hydrolysis of cellulosic fibers in order to modify the fabric or yarn surface in a manner that permanently prevents the tendency for pilling, improves fabric handle like softness and smoothness, clears the surface structure by reducing fuzzing. Biofinishing results in clarification of colors, improves the drapability of the fabric and improves moisture absorbability, which may further improve also the dyeability. Biofinishing may be performed before, after or at the same time as dyeing.

Enzymatic depilling can be carried out at any stage during textile wet processing, preferably after optional desizing and/or bleaching, and similar conditions as in biostoning can be used. Also textiles in garment form can be treated.

The variant endoglucanase polypeptides and enzyme preparations containing them may be used in biostoning of denim. As used in the present context, the expression "biostoning" of fabric or garment means the use of enzymes in place of, or in addition to, pumice stones for the treatment of fabric or
garment, especially denim to obtain an aged or worn look. The term "aged or worn look" means that as a result of uneven dye removal, there are contrasts between dyed areas and areas from which dye has been removed.

The liquor ratio (the ratio of the volume of liquid per weight of fabric) in both biostoning and biofinishing may range from about 3:1 to 20:1, preferably 5:1 to 10:1. The treatment time can vary between 15 min to 90 min and preferably between 30 min to 60 min. It should be emphasized that the enzyme dosage greatly depends on the type of the fabrics, machinery, process conditions (pH, temperature, liquor ratio, treatment time, denim load, process scale) and type of the enzyme preparation or composition. Typical process parameters for e.g. industrial biofinishing are pH 4.5 - 8 at temperature of 40 - 65°C. The variant endoglucanase polypeptides of the invention show performance at a wide range of pH and temperature conditions. A person skilled in art is capable in defining suitable dosages and conditions.

The variant endoglucanase polypeptides of the invention and enzyme preparations or detergent compositions containing them provide unexpected advantages when used in detergent and textile industries. The novel variants are considerably more efficient than the cellulases of the prior art. In detergent applications, the novel variant endoglucanase polypeptides have considerably better color revival (pilling removal) and color care (antipilling) performance at lower dosing range. In biofinishing, high performance is achieved by using the variant endoglucanase polypeptides of the invention.

It will be obvious to a person skilled in the art that, as the technology advances, the inventive concept can be implemented in various ways. The invention and its embodiments are not limited to the examples described but may vary within the scope of the claims.

EXAMPLES

Example 1. Production of Acremonium thermophilum ACM0 cellulase variants in Trichoderma reesei

Standard molecular biology methods were used in isolation and enzyme treatments of DNA (plasmids, DNA fragments), in E. coli transformations, etc. The basic methods used are described in molecular biology handbooks, e.g. Sambrook J. and Russell, D.W., 2001.

Cellulase variants were derived from a parental molecule, designated here as ce/45A-ACM0 (nucleic acid sequence SEQ ID NO: 3, corresponding to
amino acid sequence SEQ ID NO: 12), containing the catalytic core of *A. thermophilaum* Cel45A attached to the linker and CBM region of *T. reesei* Cel7A. Expression plasmids were constructed for production of recombinant ACMO variants. The constructs contain *T. reesei* celUA promoter and terminator and the *amdS* marker gene as described in Paloheimo *et al.*, 2003. Synthetic genes (Table 1), including mutations introduced in the core region of the parental molecule, were exactly fused as SacI-BamHI fragments to the *T. reesei* cel7A promoter by ligation. For construction of the expression plasmid for ACM88 variant a 222 bp SgrA1 fragment of pALK3923 was isolated and ligated into an 8859 bp SgrA1 fragment of pALK3928. Expression plasmids are listed in Table 1.

**Table 1. The synthetic genes used in construction of the expression cassettes for production of ACMO cellulase variants in *T. reesei***

<table>
<thead>
<tr>
<th>Gene designation</th>
<th>Mutation</th>
<th>Expression plasmid</th>
<th>SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>cel45A-ACM30</td>
<td>S109N, S116Q, Q118H</td>
<td>pALK3675</td>
<td>4</td>
</tr>
<tr>
<td>cel45A-ACM44</td>
<td>S51N, S82Q, A135Q, S150Q, S205N</td>
<td>pALK3779</td>
<td>5</td>
</tr>
<tr>
<td>cel45A-ACM86</td>
<td>S51N, A75S, A77S, S82Q, S109N, Q118H, A135Q, S150Q, S205N</td>
<td>pALK3942</td>
<td>7</td>
</tr>
<tr>
<td>cel45A-ACM88</td>
<td>S51N, S82Q, S116E, Q118H, A135Q, S150Q, S205N</td>
<td>pALK3967</td>
<td>8</td>
</tr>
</tbody>
</table>

The linear expression cassette (Figure 1) was isolated from the vector backbone by *EcoR*I digestion, transformed into *T. reesei* A152, and transformants were selected with acetamide as sole nitrogen source. The host strain lacks four major endogenous cellulases: CBHII/Cel7A, CBHII/Cel6A, EGI/Cel7B and EGII/Cel5A. The transformations were performed according to Penttila *et al.*, 1987, with the modifications described in Karhunen *et al.*, 1993. The transformants were purified on selection plates through single conidia prior to sporulating them on potato dextrose agar.

The endoglucanase production of the transformants was analyzed from the culture supernatants of shake flask cultivation (50 ml). The transformants were grown for 7 days in a complex cellulase-inducing medium (Joutsjoki *et al.*, 1993) buffered with 5% KH2PO4 at pH 5.5. The enzyme activity of the recombinant protein was measured from the culture supernatant as the release of reducing sugars from carboxymethylcellulose (3% CMC) at 50°C in 50 mM Citrate buffer pH 4.8 essentially as described by Bailey, M. J. and Nevalainen, K.M.H., 1981; Haakana, H., *et al.*, 2004. Production of the recombinant
protein was also detected from the culture supernatant by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The chosen transformants (Table 2) and the reference strain producing ACMO cellulase were cultivated in laboratory scale bioreactors in complex cellulase-inducing medium to obtain material for the application tests (Examples 4 to 6).

Table 2. Transformants chosen for cultivation in laboratory scale bioreactors

<table>
<thead>
<tr>
<th>Cellulase variant</th>
<th>Transformant</th>
</tr>
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<tbody>
<tr>
<td>ACM30</td>
<td>RF10612</td>
</tr>
<tr>
<td>ACM44</td>
<td>RF10834</td>
</tr>
<tr>
<td>ACM72</td>
<td>RF11193</td>
</tr>
<tr>
<td>ACM86</td>
<td>RF11081</td>
</tr>
<tr>
<td>ACM88</td>
<td>RF11217</td>
</tr>
</tbody>
</table>

Example 2. Production of *A. thermophilum* Cel45A cellulase variants in *T. reesei*

Standard molecular biology methods were used as described in Example 1. Two cellulase variants, derived from a parental molecule SEQ ID NO: 9 (corresponding to amino acid sequence SEQ ID NO: 13), were constructed. Expression plasmids (Table 3) were constructed by cloning synthetic genes and recombinant proteins were produced as described in Example 1.

Table 3. The synthetic genes used in construction of the expression cassettes for production of At_Cel45A cellulase variants in *T. reesei*

<table>
<thead>
<tr>
<th>Gene designation</th>
<th>Mutation</th>
<th>Expression plasmid</th>
<th>SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>cel45A-ACM91</td>
<td>S51N, S82Q, S116E, Q118H, A135Q, S150Q, S205N</td>
<td>pALK3973</td>
<td>11</td>
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</table>

The endoglucanase production of the transformants was analyzed from the culture supernatants of shake flask cultivations (50 ml) as described in Example 1. The chosen transformants (Table 4) and the corresponding reference strain producing At_Cel45A cellulase were cultivated in laboratory scale
bioreactors in complex cellulase-inducing medium to obtain material for the application tests (Examples 4 to 6).

Table 4. Transformants cultivated in laboratory scale bioreactors

<table>
<thead>
<tr>
<th>Cellulase variant</th>
<th>Transformant</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACM90</td>
<td>RF11351</td>
</tr>
<tr>
<td>ACM91</td>
<td>RF11352</td>
</tr>
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</table>

Example 3. Purification of variant endoglucanases

Cells and solids were removed from the fermentation culture medium by centrifugation for 10 min, 4000 g at 4°C. The supernatant of 15 ml was used for protein purification. After centrifugation, solid ammonium sulfate was added to the sample to obtain a final salt concentration of 1 M. The sample was then filtered through 0.44 μm filter before applying to phenyl Sepharose HiPrep 16/10 FF column (GE Healthcare) equilibrated with 20 mM MES, 1 M ammonium sulfate pH 6. The proteins were eluted with a linear decreasing ammonium sulfate gradient (from 1 to 0 M). Fractions of 10 ml were collected and analyzed on SDS-PAGE. The fractions containing endoglucanase activity were combined and concentrated to 2 ml using Vivaspin 20, 10 kDa MWCO ultrafiltration devices (GE Healthcare). The concentrated sample was further fractionated using Superdex 75 26/60 gel-filtration column equilibrated with 20 mM MES, 200 mM NaCl pH 6. Fractions of 2 ml were collected and analyzed by SDS-PAGE. Fractions containing pure endoglucanase were combined. Enzyme content of the purified sample was determined using UV absorbance 280 nm measurements. Excitation coefficients for the purified endoglucanases were calculated on the bases of amino acid sequence of the enzyme by using ExPASy Server http://web.expasy.org/protparam/.

The enzyme activity of purified samples was measured as the release of reducing sugars from carboxymethylcellulose (3% CMC) at 50°C in 50 mM Hepes buffer pH 7.0 in 10 min (NCU activity), Bailey and Nevalainen, 1981; Haakana et ai, 2004). The specific activity (NCU/mg) of enzyme variants was calculated by dividing NCU activity with the amount of purified enzyme. Obtained values were used for calculating enzyme dosages used in Examples 4 to 6.
Example 4. Launder-Ometer tests of A. thermophilum ACMO cellulase variants with liquid detergent application

A. thermophilum ACMO cellulase variants (ACM30, ACM44, ACM72, ACM86 and ACM88) produced in Trichoderma, as described in Example 1, were tested for their performance with Commercial liquid color detergent at 40°C and compared to ACMO cellulase and commercial cellulase preparation of Carezyme® (Cellulase of Aspergillus sp., Sigma-Aldrich C-2605-50 ml). The following pilling monitors (multicolor printed Jersey, 94% Cotton, 6% Dorlastan) supplied from Center For Testmaterials BV (The Netherlands) were used: E-252 (original fabric, unpilled) and E-253 (prepilled/predamaged material). Monitors E-253 were used for the demonstration of the removal of pilling (depilling) from material representing used cotton textiles. The same predamaged monitors were also used for demonstration of the color revival effect of used colored textiles. Monitors of original fabric (E-252) were used for the demonstration of the color maintenance/color care and/or prevention of pilling (antipilling) effect of new fabrics. Both test fabrics were cut into swatches (approx. 29 cm x 15 - 16.5 cm, total weight of two swatches approx. 24 g) containing full width stripes of each color (black, red, green, blue) and the edges were neated.

Cellulase treatments were performed in Atlas LP-2 Launder-Ometer as follows. Launder-Ometer was first preheated to 40°C. 60 g of steel balls (diameter 0.6 cm), 240 ml of wash liquor and diluted enzyme (<1.0 ml) were added into 1.2 liter containers. After that, one swatch of E-253 and E-252 were placed in containers (reverse side on reverse side) and the Launder-Ometer was run at 40°C for 60 min with a rotation speed of 42 rpm.

Enzymes were dosed as mg of active enzyme protein (AEP). AEP content of each preparation was calculated on the basis of specific activities defined as described in Example 3. Dosage of the enzyme preparations was 0.2 or 0.1 mg of active enzyme protein per liter of wash liquor and control sample contained no enzyme. The wash liquor contained 5 g of Commercial liquid color detergent per litre of synthetic tap water (16°dH) and its pH was approx. 8.5.

For synthetic tap water with hardness of 16°dH the following stock solutions were prepared in deionized water (Milli-Q or equivalent):

- Stock solution with 1000°d Calcium-hardness: CaCl₂ x 2 H₂O (1.02382.1 000, Merck KGaA, Germany) 26.22 g/l
- Stock solution with 200°d Magnesium-hardness: MgSO₄ x 7 H₂O (1.05886.1 000, Merck KGaA, Germany) 8.79 g/l H₂O
NaHCO₃ stock solution: NaHCO₃ (1.06329.1000 Merck KGaA, Germany) 29.6 g/l
13.3 ml CaCl₂ solution, 13.3 ml MgSO₄ solution and 10.0 ml of freshly
made NaHCO₃ solution were added in volumetric flask in the given order, made
up to 1 liter with deionized water and mixed. The hardness of water was deter-
mimed by complexometric titration and found correct.

After the cellulase treatment in Lauder-Ometer, the swatches were
first rinsed separately under running water (ca. 20°C) and then in a washing
machine (Whirlpool) using rinsing program with extraction. Swatches were dried
in a tumbler. Washing and tumbling cycles were repeated 10 times.

The cellulase performance in detergent application was evaluated by
measuring the color of as reflectance values with Konica Minolta CM-3610A
spectrophotometer using L’a’b’ color space coordinates (illuminant D65/1 0°,
420 nm cut). The color of each 4 stripes of test monitors was measured after 10
washing cycles. Decrease of lightness (L’), i.e. increase of darkness compared
to treatment without cellulase, was used as an indication of cellulase effect.
When the surface fibers and fibrils protruding from the yarn forming pills and
giving the fabric a greyish look are removed by cellulase, the lightness of the
fabric decreases, and the surface of the fabric appears darker and colors get
brighter.

Cellulase performance was calculated as ΔL’ (delta L’), which means
lightness value L’ of enzyme treated fabric minus lightness value L’ of fabric
treated with washing liquor without enzyme (enzyme blank, control). Sum of ΔL’
for each 4 stripes was calculated and the final results were shown as increase
of darkness ( ΔL’).

The results of the tests for increase of darkness using dosage 0.2 mg
of active enzyme protein per liter of wash liquor are shown in Figs 2A (prepilled
monitors E-253) and 2B (unpilled monitors E-252). Pilling removal /color revival
effect (Fig 2A) of variants ACM30, ACM44, ACM72, ACM86, and especially
ACM88 was considerably improved as compared to ACM0. Also, the antipill-
ing/color care effect of variants ACM30, ACM44, ACM72, ACM86, and espe-
cially ACM88 was better compared to ACM0. Variants ACM30, ACM44, ACM72,
ACM86, and especially ACM88 had also better performance in detergent applica-
tion than commercial reference Carezyme®. Furthermore, according to the
tests performed with smaller dosage (0.1 mg of active enzyme protein per liter
of wash liquor), as shown in Figure 3, ACM72 and ACM88 had considerably
better performance in detergent application with both pre-pilled (Fig 3A) and unpilled (Fig 3B) test monitors compared to ACMO and commercial reference.

The spectrophotometrical results were also confirmed by visual evaluation. Prepilled/predamaged fabric, which appeared worn and uninteresting, regained its original look considerably better with ACM72 and especially with ACM88 compared to ACMO and the commercial reference in both tests. Also the maintenance of colors of new fabric was visually clearly better with ACM72 and especially with ACM88 than with the references.

Example 5. Testing the performance of A. thermophilum ACMO variants in biofinishing application

The performance of A. thermophilum ACMO variant ACM88 and ACMO cellulase, produced in Trichoderma, as described in Example 1, was tested in biofinishing (depilling/defuzzing) of cotton knitwear. The cellulase treatments were performed with Electrolux's Wascator FOM 71 CLS washer extractor using 1 kg fabric and 15 liter of tap water. Rugged three yarn college knitwear made of 100% cotton (Type 9761, Orneule, Finland) was used as test material with filling material. The fabric was first prewashed for 10 min at 50°C and rinsed 3 times. After that, the cotton knit fabric was treated with cellulase at 50°C for 60 minutes. The enzymes were dosed as 0.025 or 0.05 mg of active enzyme protein (AEP) per the weight (g) of the fabric, as described in Example 4. pH of the washing liquid was adjusted to 6 with acetic acid or tests were performed without pH adjustment, when pH of the washing liquid was approx. 7.6 at start and 8 at the end of cellulase treatment. After draining, the enzyme was inactivated (for 10 min at 50°C) by raising the pH above 11 with sodium hydroxide. The fabric was then rinsed three times and dried in a tumbler.

The fabric samples were evaluated visually according to how much surface fibrils and fuzz was detected. The result of each evaluation was quantified by indicating the result relative to a scale consisting of standards. These standards were pieces of the same fabric washed with different amounts of cellulase and they had a range of intensity of surface fibrils/fuzz from number 1 to 5 with half unit's intervals. Number 1 was a control sample treated without enzyme. The higher the number, the better the defuzzing effect. The results are shown in Fig. 4. The biofinishing (defuzzing) performance of ACM88 variant was better than that of ACMO at 50°C, both at pH 6 and without pH adjustment (pH approx. 8).
Example 6. Launder-Ometer tests of *A. thermophilum* Cel45A cellulase variants with liquid detergent application

*A. thermophilum* Cel45A cellulase variants ACM90 and ACM91 produced in *Trichoderma*, were tested for their performance with Commercial liquid color detergent at 40°C and were compared to the At_Cel45A cellulase (SEQ ID NO: 13) and commercial cellulase preparation of Carezyme® (Sigma-Aldrich C-2605-50 ml).

The tests were performed and results evaluated as described in Example 3, except for that the dosages of At_Cel45A cellulase were 0, 0.1, 0.2 or 0.4 mg of active enzyme protein (AEP) per liter of wash liquor. The corresponding dosages of variant ACM90 or ACM91 were 0, 0.05, 0.1 or 0.2 mg of AEP/liter.

The results are shown in Figs 5A and B. The pilling removal/color revival effect measured with prepilled test monitor E-253 and antipilling/color care effect measured with unpilled monitor E-252 of variants ACM90 and ACM91 were considerably improved as compared to At_Cel45A cellulase.

As a conclusion, the results described in Examples 3 and 6 display that the performance of the endoglucanase variants is improved, as compared to the parental enzyme, regardless of the origin of the linker and the CBM region.

REFERENCES


CLAIMS

1. A variant endoglucanase polypeptide comprising an amino acid sequence having at least 95% sequence identity with amino acids 1 to 214 of SEQ ID NO: 1 and an amino acid substitution at one or more positions selected from the group consisting of 51, 75, 77, 82, 109, 116, 118, 135, 150 and 205, the positions being numbered with reference to SEQ ID NO: 1, wherein the variant polypeptide has endoglucanase activity.

2. A variant endoglucanase polypeptide of claim 1 attached to a carbohydrate binding module and optionally to a linker region.

3. A variant endoglucanase polypeptide of claim 1, wherein the variant endoglucanase polypeptide is attached to a carbohydrate binding module and a linker region, which are heterologous or homologous.

4. A variant endoglucanase polypeptide of claim 3, wherein at least one of the carbohydrate binding module and the linker region is heterologous.

5. A variant endoglucanase polypeptide of any one of the previous claims, wherein the polypeptide variant comprises a substitution at a position corresponding to one or more of S51N, A75S, A77S, S82Q, S109N, S116Q, S116E, Q118H, A135Q, S150Q and S205N.

6. A variant endoglucanase polypeptide of any one of the previous claims, wherein the polypeptide variant has a substitution at a position corresponding to S51N, S82Q, S109N, S116E, Q118H, A135Q, S150Q and S205N.

7. A variant endoglucanase polypeptide of any one of the claims 1 to 5, wherein the polypeptide variant has a substitution at a position corresponding to S51N, S82Q, S116E, Q118H, A135Q, S150Q and S205N.

8. A variant endoglucanase polypeptide of claim 1, wherein the polypeptide has an amino acid sequence of SEQ ID NO: 12 and an amino acid substitution at one or more positions selected from the group consisting of 51, 75, 77, 82, 109, 116, 118, 135, 150 and 205, the positions being numbered with reference to SEQ ID NO: 1.

9. A variant endoglucanase polypeptide of claim 1, wherein the polypeptide has an amino acid sequence of SEQ ID NO: 12 and an amino acid substitution at a position corresponding to S51N, S82Q, S109N, S116E, Q118H, A135Q, S150Q and S205N.
10. A variant endoglucanase polypeptide of claim 1, wherein the polypeptide has an amino acid sequence of SEQ ID NO: 12 and an amino acid substitution at a position corresponding to S51 N, S82Q, S116E, Q118H, A135Q, S150Q and S205N.

11. An isolated polynucleotide selected from the group consisting of:
   a) a polynucleotide or complementary DNA encoding an endoglucanase polypeptide variant of claims 1 to 10;
   b) a polynucleotide encoding a fragment of a polypeptide encoded by a polynucleotide of a) wherein said fragment is having endoglucanase activity
   c) a polynucleotide comprising a nucleotide sequence which is degenerate to the nucleotide sequence of a polynucleotide sequence of a) or b), or the complementary strand of such a polynucleotide.

12. A vector, which comprises a polynucleotide of claim 11 operably linked to regulatory sequences capable of directing expression of the endoglucanase polypeptide variant of claim 1.

13. A host cell comprising the vector of claim 12.

14. A method of producing the endoglucanase polypeptide variant of claim 1, said method comprising the steps of transforming a host cell with an expression vector encoding said polypeptide, and culturing said host cell under conditions enabling expression of said polypeptide, and optionally recovering and purifying said polypeptide variant.

15. An enzyme preparation comprising the endoglucanase polypeptide variant according to any one of claims 1 to 10.

16. The enzyme preparation of claim 15 further comprising at least one enzyme selected from a group consisting of other cellulases, amylases, lipases, proteases, hemicellulases, ligninases, pectinolytic enzymes and/or oxidative enzymes.

17. A detergent composition comprising an endoglucanase polypeptide variant of any one of claims 1 to 10 or an enzyme preparation of claim 15 or 16, a surfactant and optionally one or more additives selected from a group consisting of stabilizers, buffers, surface active agents, builders, cobuilders, bleaching agents, bleach activators, other detergent enzymes, mediators of oxidative enzymes, anti-corrosion agents, antiredeposition agents and soil release polymers, caustics, abrasives, optical brighteners, dyes, pigments, perfumes and preservatives.
18. A method for treating cellulosic material, wherein the method comprises reacting the cellulosic material with the endoglucanase polypeptide variant of any one of claims 1 to 10 or the enzyme preparation of claim 15 or 16.

19. The method of claim 18, wherein the cellulosic material is textile material, plants used in animal feed, or wood-derived pulp or secondary fiber.

20. The method of claim 19, wherein the method comprises contacting the textile material with the detergent composition of claim 17.

21. The method of claim 18, which is laundry.

22. The method of claim 18, which is biostoning or biofinishing.

23. Use of the endoglucanase polypeptide variant of any one of claims 1 to 10, or the enzyme preparation according to claim 15 or 16 in textile and detergent industry, in biomass processing, in biofuel, starch, pulp and paper, food, baking, feed or beverage industry.

24. Use according to claim 23 for biofinishing or biostoning textile materials like fabrics or garments or yarn.
Figure 2

Figure 2A

E-253 (prepillled)

Increase of darkness (sum of 4 stripes)

ACM0  ACM30  ACM44  ACM72  ACM86  ACM88  Carezyme (Sigma)

0.2 mg AEP/l

Figure 2B

E-252 (unpillled)

Increase of darkness (sum of 4 stripes)

ACM0  ACM30  ACM44  ACM72  ACM86  ACM88  Carezyme (Sigma)

0.2 mg AEP/l
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

FI, SE, NO, DK

Electronic database consulted during the international search (name of database, and, where practicable, search terms used)

EPO-Internal, WPI, BIOSIS, EMBASE, MEDLINE, XPESP, sequence searches

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>A</td>
<td>WO 2007071 818 A1 (ROAL OY [FI]) 28 June 2007 (28.06.2007) page 15, lines 13-17; page 20, lines 6-11; claims 24, 35; SEQ ID NO: 12</td>
<td>1-24</td>
</tr>
<tr>
<td>A</td>
<td>WO 2007071 820 A1 (AB ENZYMES OY [FI]) 28 June 2007 (28.06.2007) abstract; example 5; the claims; SEQ ID NO 36, 40</td>
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<tr>
<td>A</td>
<td>WO 20081 51999 A1 (NOVOZYMES AS [DK]) 18 December 2008 (18.12.2008) abstract; page 7, lines 6-12; claims 1, 8-10; SEQ ID NO. 1</td>
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<tr>
<td>A</td>
<td>WO 2012 106824 A1 (IOGEN BIO PRODUCTS CORP [CA]) 16 August 2012 (16.08.2012) abstract; paragraph [0019], SEQ ID NO: 18</td>
<td>1-24</td>
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</table>

[X] Further documents are listed in the continuation of Box C.  
[×] See patent family annex.

* Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search  
02 February 2016 (02.02.2016)

Date of mailing of the international search report  
04 February 2016 (04.02.2016)

Name and mailing address of the ISA/FI  
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