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(54) **Title**: BIOFINISHING SYSTEM

(57) **Abstract**: The present disclosure relates to a biofinishing system comprising a combination of cellulases, in particular a biofinishing system comprising a combination of GH45 cellulases. The present disclosure further relates to a process for treating a cellulose-containing textile comprising biofinishing the cellulose-containing textile with a combination of GH45 cellulases.

BIOFINISHING SYSTEM

Reference to a Sequence Listing

This application contains a Sequence Listing in computer readable form, which is incorporated herein by reference.

Background of the Invention

Field of the Invention

The present invention relates to a biofinishing system comprising a combination of celluases, in particular to a combination of cellulases for use in treating a cellulose-containing textile.

Description of the Related Art

Cellulase enzymes are widely used to improve the appearance and softness of cellulose-containing fabrics. A widespread application of cellulase enzymes is to remove cotton fuzz and loose surface fibers in or on the fabric. This process is referred to as "biopolishing" and smoothes the surface of the fabric, which in turn improves its softness and appearance. Cellulase treatment also aids in the prevention of subsequent formation of fiber pills that make the garments appear worn. During depilling it is desirable to minimize strength loss of the fabric due to the hydrolytic action of the cellulases.

Another industrial application of cellulase enzymes is for treating denim fabrics so as to impart to them a "stone-washed" appearance. Such a process is known in the industry as "biostoning". The term biostoning was adopted as pumice stones were traditionally used to treat the fabric. However, cellulases have largely replaced pumice stones in recent years. Biostoning is quite different from depilling in that biostoning aims to remove colour from denim and control its re-deposition on the fabric while depilling aims to solely improve softness and appearance as in depilling.

Cellulase enzymes are a group of glycoside hydrolase enzymes that catalyze the hydrolysis of beta-1,4-glycosidic linkages in the cellulose polymer and often comprise a cellulose binding domain (CBD) and a catalytic domain. A region between these two domains known as a "linker" or "linker peptide" serves as a flexible spacer between the CBD and the catalytic domain. The catalytic domains of individual cellulase components are classified by both the Enzyme Commission (EC) and the Glycoside Hydrolase (GH) family systems. The Enzyme Commission distinguishes two classes of cellulases based on their preference for cleavage of internal beta- 1,4 linkages (endoglucanase or "EG", EC 3.2.1.4) or the release of

cellobiose from the reducing or non-reducing end of the cellulose polymer (cellobiohydrolases or "CBH", EC 3.2.1. 91, sometimes also referred to as exoglucanases). In contrast, the GH family system distinguishes the catalytic domains of cellulase components based on the conservation of primary and secondary structure, as well as the stereochemistry of the catalytic reaction. The GH family designations for all known cellulase catalytic and binding domains is provided and continually updated through the Carbohydrate-Active EnZymes (CAZy) database (Cantarel et al, 2009, Nucleic Acids Res 37:D233-238) available at the URL: cazy.org. Cellulase enzymes may be found in a number of GH Families including, but not limited to, Families 5, 6, 7, 8, 9, 10, 12, 16, 18, 19, 26, 44, 45, 48, 51, 61 and 74. Further, cellulase in some of the larger GH Families may be grouped into subfamilies.

A number of groups have contemplated the use of GH45 cellulases in depilling. WO 97/14804 discloses a neutral Cel45A cellulase (20 K cellulase) from *Melanocarpus* origin for use in the textile and detergent industry. WO2010/076388 discloses the production and use of *Geomyces or Fusarium* CelA5 endoglucanases in denim washing and depilling. U.S. Publication No. 2007/0111278 discloses the use of STCE1, a Cel45 endoglucanase, derived from *Staphylotrichum*, in washing or depilling of cellulose-containing fabrics. U.S. Patent No. 7,741,093 discloses fusion of the *Melanocarpus* CelA5 endoglucanase to a linker peptide of *Trichoderma reesei* CBH I and a cellulose binding domain for biostoning and biofinishing. The purpose of constructing such fusion proteins was to increase the size of the *Melanocarpus* Cel45A enzyme, thereby decreasing the ability of the enzyme to penetrate the fabric, which in turn reduces strength loss. Similar approaches with Cel45 endoglucansaes and other cellulase enzymes are disclosed in WO 2007/118935 and U.S. Patent No. 7,256,032.

Other groups have focused on elucidating whether or not cellulase enzyme components synergize with one another. The identification of synergistic combinations of enzyme components that provide for enhanced depilling could be a step forward with respect to improving process economics. Such improvements may be achieved since less enzyme protein, which is costly, would be needed to impart the desired depilling effect.

Heikinheimo and Buchert (Textile Research Journal, 2001, 71 (8):672-677) investigated the depilling properties of *Trichoderma reesei* EG I and II and CBH I and II cellulase components alone and in combination. Treatment of cotton interlock fabric with EG II-based combinations with CBH I or CBH II resulted in favourable depilling properties. However, the investigators also reported decreased depilling activity for combinations of the two endoglucanases, EG I and EG II. That is, no endo-endo synergy between the cellulase components was observed.

Cavaco-Paulo and Almeida (Textile Chemist and Colorist, 1996, 28(6):28-32) observed a high activity of EGI and II- deleted *Trichoderma reesei* cellulase mixtures on cotton cellulose.

The authors state therein that the effect may possibly be due to synergy between the two CBH components or the CBH components and residual EG III or EG V. In Cavaco-Paulo, Carbohydrate Polymers, 1998, 37:273-277, it was stated that minor EG components seem to cooperate with the CBHs, in a synergistic fashion, to fully hydrolyse cotton. However, no testing was carried out to examine which particular components exhibited synergism with one another.

U.S. Patent No. 5,958,083 discloses binary cellulase enzyme mixtures for use in biostoning. The first component is a Family 5 endoglucanase derived from *Bacillus* or *Clostridium*, or Family 7 endoglucanase derived from *Humicola insolens*. The second component is a mechanical abrading agent, and/or an abrading cellulase (to form localized variation in color density), which may be a Family 12 or a Family 45 cellulase with a cellulose binding domain. Although improved biostoning with low backstaining was obtained, the properties of these compositions in depilling assays were not investigated.

Miettinen-Oinonen et al. (Enzyme and Microbial Technology, 2004, 34:332-341) examined the effect of Family 45 enzymes in biostoning, alone or in combination with other cellulase components, including endoglucanases. However, the depilling properties of these enzyme compositions were not tested in these studies.

WO13167613 discloses the use of combinations of GH45 cellulases and auxiliary care enzymes comprising Family 5 cellulase and/or Family 7 cellulase for biopolishing and general fabric care at low temperatures.

WO12106824 discloses a cellulase enzyme mixtures for depilling, comprising a Family 45 cellulase enzyme component and one or more additional cellulase enzyme components selected from a Family 5 cellulase, a Family 6 celluase or a bomination thereof, wherein said enzyme mixture is secreted by a genetically modified microbe overexpressing (i) a Family 45 gene encoding said Family 45 cellulase enzyme, and (ii) a gene or genes encoding the one ore more additional cellulase enzyme components selected from a Family 5 cellulase, a Family 6 celluase or a bomination thereof.

Despite these efforts, there is still a need for improved combinations of cellulase enzymes and compositions thereof that are more effective in biofinishing a cellulose-containing textile. In particular, there is a continuous need for more efficient cellulase enzyme composition to improve the process economics. The present invention aims to meet these needs.

Summary of the Invention

The present invention provides an enzyme composition comprising, a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity.

The present invention also relates to a nucleic acid construct or expression vector comprising a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity.

The present invention also relates to a recombinant host cell comprising a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity.

The present invention also relates to a process for biofinishing a cellulose-containing textile comprising contacting the cellulose-containing textile with the enzyme composition or the recombinant host cell of the present invention.

The present invention also relates to a process for biofinishing a cellulose-containing textile, comprising

(a) treating the cellulose-containing textile with a first polypeptide having GH45 cellulase activity and biofinishing activity; and

(b) treating the cellulose-containing textile with a second polypeptide having GH45 cellulase activity and biofinishing activity.

The present invention also relates to a process for treating a cellulose-containing textile, comprising

(a) desizing;

(b) color modification;

wherein a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity are added before, during or after step (a) and step (b).

The present invention also relates to a process for treating a cellulose-containing textile, comprising

(a) desizing;

(b) scouring;

(c) bleaching;

(d) dyeing;

wherein a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity are added before, during or after step (a), (b), (c) or (d).

The present invention further relates to use of a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity for biofinishing a cellulose-containing textile.

Disclosed herein are combinations of a first GH45 cellulase and a second GH45 cellulase that are particularly effective in the biofinishing a cellulose-containing textile. The

combinations of GH45 cellulase enzyme components of the present invention provide for enhanced biofinishing of cotton-containing textile relative to the biofinishing effect of the individual enzyme components. For example, the combination of a first GH45 cellulase and a second GH45 cellulase at different protein ratio delivers about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biopolishing effect than the individual GH45 cellulases. The combination of a first GH45 cellulase and a second GH45 cellulase at different protein ratio delivers about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biostoning effect than the individual GH45 cellulases in denim abrasion. In a pilling notes test, the combination of a first GH45 cellulase and a second GH45 cellulase at different protein ratio delivers about 0.1, about 0.2, about 0.5, about 0.8, about 1.0 pilling note more than that of the individual GH45 cellulases. The utilization of such enzyme combinations could be a step forward with respect to improving process economics. For example, the combination of a first GH45 cellulase and a second GH45 cellulase delivers an equivalent biofinishing effect with about 1/2, about 1/3, about 1/4, about 1/5, about 1/6, about 1/8 or about 1/10 shorter time than the individual GH45 cellulases. For a high-temperature tolerant GH45 cellulase and low-temperature adapted GH45 cellulase, the combination of the GH45 cellulases delivers a stable biofinishing performance at a broad range from the high temperature to the low temperature.

Detailed Description of the Invention

GH45 cellulases

Glycoside hydrolases (GHs) are a large group of enzymes that cleave glycosidic bonds between individual carbohydrate monomers in large polysaccharide molecules. Cellulases cleave the beta 1-4 bond between glucose monomers in the cellulose polymer. GH enzymes all share one of two common mechanisms, called inverting and retaining, for introducing a water molecule at a glycosidic bond thus cleaving the polysaccharide.

The GH Family 45 cellulase enzymes (formerly Family K) act with inversion of anomeric configuration to generate the alpha-D anomer of the oligosaccharide as a product. It has been elucidated that, in the active site, one aspartic acid amino acid acts as a general acid and another as a general base.

The three dimensional structure of Family 45 enzymes has been elucidated (see, for example, the structure of *Humicola insolens* in Davies et al, 1996, ActaCrystallographica Section D-Biological Crystallography 52:7-17 Part 1). The enzymes contain a six-stranded beta-barrel to which a seventh strand is appended. The structure contains both parallel and anti-parallel beta-strands. The active center is located in an open substrate- binding groove.

As used herein, the term "GH45 cellulase", "Family 45 cellulase" or "Cel45" means a carbohydrate active cellulase enzyme that contains a glycoside hydrolase Family 45 catalytic domain that is classified under EC 3.2.1.4. The term encompasses a carbohydrate active enzyme that hydrolyzes cellulose and cello-oligosaccharides using an inverting mechanism, and has either of the following two signature sequences in the vicinity of the catalytic aspartic acid amino acids: (i) both a first conserved signature sequence of A/S/T - T - R/N/T - Y/F/T - X - D - X - X - X - X - X - C/A - A/G/S - W/C and a second conserved signature sequence of H/Q/D/N - F/L - D - I/L/F; or (ii) has the second conserved signature sequence of H/Q/D/N - F/L - D - I/L/F but lacks said first conserved sequence. In one embodiment, the second conserved signature sequence is H-F-D-I.

Family 45 cellulase enzymes have been divided into at least two subfamilies referred to as "A" and "B" (Igarashi et al, Applied and Environmental Microbiology, 2008, 74(18):5628-5634). According to one embodiment of the invention, the Family 45 cellulase enzyme is a subfamily A member. According to another embodiment of the invention, the Family 45 cellulase enzyme is a subfamily B member. This includes, but is not limited to, those subfamily A and subfamily B enzymes listed in the tables below.

Family 45 cellulase subfamily B members:

Organism	Abreviated Name	GenBank Accession Noumber	SEQ ID NO:
Trichoderma reesei	TrCel45A	CAA83846.1	9
Trichomderma viride	TvEGV	AAQ21385.1	10
Penicillium decumbens	PdCel 45A	ACF33814.1	11
Aspergillus nidulans	AnAN6786.2	EAA58604.1	12
Hadiotis discus discus	HddEG1	ABO26608.1	13
Ampullaria crossean	AcEG27I	ABR92637.1	14
Ampullaria crossean	AcEG27II	ABR92638.1	15
Mytilus edulis	MeEG	CAC59695.1	16
Phanerochaete chrysosporium	PcCel45A	BAG68300.1	17

Family 45 cellulase subfamily A members:

Organism	Abreviated Name	GenBank Accession Number and WO publication Number	SEQ ID NO: herein
Humicola insolens	HiCel45A	AAE16508.1	18
Humicola grisea var. thermoidea	HgEgl3	BAA74956.1	19
Humicola nigrescens	HnCel45A	CAB42308.1	20

Geomyces pannorum	Gp RF6293 Cel45A	SEQ ID NO: 13 in WO2010/076388	21
Geomyces pannorum	Gp RF6293 Cel45B	SEQ ID NO: 15 in WO2010/076388	22
Fusarium cf. equiseti	Fe RF6318 Cel45B	SEQ ID NO: 17 in WO2010/076388	23
Geomyces pannorum	Gp RF6546 Cel45A	SEQ ID NO: 19 in WO2010/076388	24
Geomyces pannorum	Gp RF6608 Cel45A	SEQ ID NO: 21 in WO2010/076388	25
Geomyces pannorum	Gp RF6608 Cel45B	SEQ ID NO: 23 in WO2010/076388	26
Staphylotrichumcoccosporum	ScSTCE1	BAG69187.1	27
Staphylotrichum coccosporum	ScSTCE1	SEQ ID NO: 3 in WO2005/054475	2
Sordaria fimicola	Sfcel45	SEQ ID NO: 2 in WO 2014/026630	6
Melanocarpus albomyces	MaCel45A	CAD56665.1	28
Podospora anserina	PaCel45A	CAP61565.1	29
Acremonium thermophilum	AtSEQ6	ACE10216.1	30
Thielavia terrestris	TtCel45A	SEQ ID NO: 4 in WO 2012/089024	4
Trichothecium roseum	TroCel45A	CAB42312.1	31
Acremonium thermophilum	AtSEQ2	ABW41463.1	32
Fusarium anguioides	FaCel45A	CAB42310.1	33
Clonostachys rosea f. catenulata	CrCel45A	CAB42311.1	34
Neurospora crassa	NcCEl45A	CAD70529.1	35
Volutella colletotrichoides	VcSEQ22	AAY00854.1	36
Gibberella zeae	GzCel45A	AAR02399.1	37
Fusarium oxysporum	FoCel45A	AAA65589.1	38
Acremonium SP.	AsSEQ10	AAY00848.1	39

Acremonium SP.	AsSEQ8	AAY00847.1	40
Chrysosporium lucknowense	ClCel45A	AAQ38150.1	41
Thielavia heterothallica	ThSEQ2	AAY00844.1	42
Mucor circinelloides	McMce1	BAD95808.1	43
Reticulitermes speratus	RshpCel45A	BAA98037.1	44
Bursaphelenchus xylophilus	BxEng1	BAD34546.1	45
Botryotinia fuckeliana	BfCel45A	XP_JX11547700.1	46
Acremonium thermophilum	AtSEQ4	ABW41464.1	47
Scopulariopsis brevicaulis	SbEgl	Q7M4T4*	48
Syncephalastrum racemosum	SrCBHI	ABU49185.2	49
Rhizopus oryzae	RoRce1	BAC53956.1	50
Crinipellis scabella	CsSEQ16	AAY00851.1	51
Macrophomina phaseolina	MpSEQ14	AAY00850.1	52
Podospora anserina	PaCel45B	CAP69443.1	53
Rhizopus oryzae	RoRce3	BAC53988.1	54
Bursaphelenchus xylophilus	BxEng2	BAD34544.1	55
Bursaphelenchus xylophilus	BxEng3	BAD34548.1	56
Humicolagrisea var. thermoidea	HgEgl4	BAA74957.1	57
Phycomyces nitens	PnPcel	BAD77808.1	58
Rhizopus oryzae	RoRce2	BAC53987.1	59
Mastotermes darwiniensis hindgut symbiont sp.	MdhsFm4	CAD39200.1	60
Magnaporthe grisea	MgCel45A	XP_363402.1	61
Mastotermes darwiniensis hindgut symbiont sp.	MdhsFm3	CAD39199.1	62
Mastotermes darwiniensis hindgut symbiont sp.	MdhsFmI	CAD39197.1	63
Mastotermes darwiniensis hindgut symbiont sp.	MdhsFm2	CAD39198.1	64
Neurospora tetrasperma	Ntcel45	SEQ ID NO: 2 in WO 2015/058700	8
Pichia pastoris GS115	PpCel45A	CAY71902.1	65
Piromyces equi	PeCel45A	CAB92325.1	66
Apriona germari	AgCelI	AAN78326.1	67
Apriona germari	AgCelII	AAR22385.1	68
Alternaria alternata	AaKI	AAF05700.1	69
Phaedon cochleariae	PcEg	CAA76931.1	70

Talaromyces emersonii	TeCel45A	CAJ75963.1	71
Ustilago maydis	UmEgII	AAB36147.1	72

*Uniprot entry

GH5 Cellulases

The amino acid sequences for over 3,000 naturally occurring Family 5 cellulases of fungal and bacterial origin have been elucidated. Regions of Family 5 cellulases are well conserved in most Family 5 cellulase enzymes and this has allowed the alignment of parts of the catalytic domains of family members.

Enzymes of Family 5 or GH5 cellulase share a common (beta/alpha)s-barrel fold and a catalytic mechanism resulting in a net retention of the anomeric sugar conformation. Glycoside hydrolase catalysis is driven by two carboxylic acids found on the side chain of aspartic acid and/or glutamic acid. These two amino acids are highly conserved among family members.

GH6 Cellulase

Family 6 cellulases comprise two aspartic acid (D) amino acids which may serve as catalytic amino acids. These aspartic acid amino acids are found at positions 175 and 221, as determined by alignment with a wild-type *Trichoderma reesei* enzyme. Most of the Family 6 cellulases identified thus far are mesophilic; however, this family also includes thermostable cellulases from *Thermobifida fusca* (TfCel6A and TfCel6B) and the alkalophilic cellulases from *Humicola insolens* (HiCel6A and HiCel6B). Family 6 cellulases also share a similar three dimensional structure: an alpha/beta-barrel with a central beta-barrel containing seven parallel beta-strands connected by five alpha-helices. The three dimensional structures of several Family 6 cellulases are known, such as TrCel6A, *Thermobifida fusca* endo-beta-l,4-glucanase Cel6A, *Humicola insolens* cellobiohydrolase Cel6A, *Humicola insolens* endo-beta-l,A-glucanase Cel6B and *Mycobacterium tuberculosis* H37Rv Cel6A.

As used herein, the term “GH6 cellulase”, “Family 6 cellulase” or “Cel6” encompasses a carbohydrate active cellulase enzyme that contains aglycohydrolase (GH) Family 6 catalytic domain that is classified under EC 3.2.1.91 or EC 3.2.1.4.

GH 7 celllulase

Family 7 cellulase or GH7 cellulase includes endo-beta-1,4-glucanase (EC 3.2.1.4); reducing end-acting cellobiohydrolase (EC 3.2.1.176); chitosanase (EC 3.2.1.132); endo-beta-1,3-1,4-glucanase (EC 3.2.1.73). These enzymes were formerly known as cellulase family C.

Endoglucanase

The term "endoglucanase" means a 4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase (E.C. 3.2.1.4) that catalyzes endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenin, beta-1,4 bonds in mixed beta-1,3-1,4 glucans such as cereal beta-D-glucans or xyloglucans, and other plant material containing cellulosic components. Endoglucanase activity can be determined by measuring reduction in substrate viscosity or increase in reducing ends determined by a reducing sugar assay (Zhang *et al.*, 2006, *Biotechnology Advances* 24: 452-481). Endoglucanase activity can also be determined using carboxymethyl cellulose (CMC) as substrate according to the procedure of Ghose, 1987, *Pure and Appl. Chem.* 59: 257-268, at pH 5, 40°C.

Sources of Cellulase

A polypeptide having cellulase activity, including GH45 cellulase activity, GH5 cellulase activity, GH6 cellulase, or GH7 cellulase activity, of the present invention may be obtained from microorganisms or plants or animals of any genus. For purposes of the present invention, the term "obtained from" as used herein in connection with a given source shall mean that the polypeptide encoded by a polynucleotide is produced by the source or by a strain in which the polynucleotide from the source has been inserted. In one aspect, the polypeptide obtained from a given source is secreted extracellularly.

The polypeptide may be a bacterial polypeptide. For example, the polypeptide may be a Gram-positive bacterial polypeptide such as a *Bacillus*, *Clostridium*, *Enterococcus*, *Geobacillus*, *Lactobacillus*, *Lactococcus*, *Oceanobacillus*, *Staphylococcus*, *Streptococcus*, or *Streptomyces* polypeptide having cellulase activity, or a Gram-negative bacterial polypeptide such as a *Campylobacter*, *E. coli*, *Flavobacterium*, *Fusobacterium*, *Helicobacter*, *Ilyobacter*, *Neisseria*, *Pseudomonas*, *Salmonella*, or *Ureaplasma* polypeptide.

In one aspect, the polypeptide is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* polypeptide.

In another aspect, the polypeptide is a *Streptococcus equisimilis*, *Streptococcus pyogenes*, *Streptococcus uberis*, or *Streptococcus equi* subsp. *Zooepidemicus* polypeptide.

In another aspect, the polypeptide is a *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, or *Streptomyces lividans* polypeptide.

The polypeptide may be a fungal polypeptide. For example, the polypeptide may be a yeast polypeptide such as a *Candida*, *Kluyveromyces*, *Pichia*, *Saccharomyces*,

Schizosaccharomyces, or *Yarrowia* polypeptide; or a filamentous fungal polypeptide such as an *Acremonium*, *Agaricus*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botryospaeria*, *Ceriporiopsis*, *Chaetomidium*, *Chrysosporium*, *Claviceps*, *Cochliobolus*, *Coprinopsis*, *Coptotermes*, *Corynascus*, *Cryphonectria*, *Cryptococcus*, *Diplodia*, *Exidia*, *Filibasidium*, *Fusarium*, *Gibberella*, *Holomastigotoides*, *Humicola*, *Irpex*, *Lentinula*, *Leptospaeria*, *Magnaporthe*, *Melanocarpus*, *Meripilus*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Piromyces*, *Poitrasia*, *Pseudoplectania*, *Pseudotrichonympha*, *Rhizomucor*, *Schizophyllum*, *Scytalidium*, *Sordaria*, *Staphylotrichum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trichoderma*, *Trichophaea*, *Verticillium*, *Volvariella*, or *Xylaria* polypeptide.

In another aspect, the polypeptide is a *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis*, or *Saccharomyces oviformis* polypeptide.

In another aspect, the polypeptide is an *Acremonium cellulolyticus*, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Chrysosporium inops*, *Chrysosporium keratinophilum*, *Chrysosporium lucknowense*, *Chrysosporium merdarium*, *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium tropicum*, *Chrysosporium zonatum*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum*, *Humicola grisea*, *Humicola insolens*, *Humicola lanuginosa*, *Irpex lacteus*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Neurospora tetrasperma*, *Penicillium funiculosum*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Sordaria fimicola*, *Staphylotrichum coccosporum*, *Thielavia achromatica*, *Thielavia albomyces*, *Thielavia albopilosa*, *Thielavia australeinsis*, *Thielavia fimeti*, *Thielavia microspora*, *Thielavia ovispora*, *Thielavia peruviana*, *Thielavia setosa*, *Thielavia spededonium*, *Thielavia subthermophila*, *Thielavia terrestris*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride* polypeptide.

It will be understood that for the aforementioned species, the invention encompasses both the perfect and imperfect states, and other taxonomic equivalents, *e.g.*, anamorphs, regardless of the species name by which they are known. Those skilled in the art will readily recognize the identity of appropriate equivalents.

Strains of these species are readily accessible to the public in a number of culture collections, such as the American Type Culture Collection (ATCC), Deutsche Sammlung von

Mikroorganismen und Zellkulturen GmbH (DSMZ), Centraalbureau Voor Schimmelcultures (CBS), and Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL).

The polypeptide may be identified and obtained from other sources including microorganisms isolated from nature (*e.g.*, soil, composts, water, etc.) or DNA samples obtained directly from natural materials (*e.g.*, soil, composts, water, etc.) using the above-mentioned probes. Techniques for isolating microorganisms and DNA directly from natural habitats are well known in the art. A polynucleotide encoding the polypeptide may then be obtained by similarly screening a genomic DNA or cDNA library of another microorganism or mixed DNA sample. Once a polynucleotide encoding a polypeptide has been detected with the probe(s), the polynucleotide can be isolated or cloned by utilizing techniques that are known to those of ordinary skill in the art (see, *e.g.*, Sambrook *et al.*, 1989, *supra*).

For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the --nobrief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the --nobrief option) is used as the percent identity and is calculated as follows:

(Identical Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

In an preferable embodiment, the polypeptide having GH45 cellulase activity and biofinishing activity has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 2, the mature polypeptide of SEQ ID

NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8 of the present invention.

In one aspect, the mature polypeptide of SEQ ID NO: 4 is amino acids 22-299 of SEQ ID NO: 4; the mature polypeptide of SEQ ID NO: 6 is amino acids 22 to 294 of SEQ ID NO: 6; and the mature polypeptide of SEQ ID NO: 8 is amino acids 22-293 of SEQ ID NO: 8 of the present invention.

In a further preferable embodiment, a polynucleotide encoding a polypeptide having GH45 cellulase activity and biofinishing activity has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to nucleotides 64-948 of SEQ ID NO: 1 (*i.e.*, nucleotides 64-948 of SEQ ID NO: 2 in WO2005/054475), the mature polypeptide coding sequence of SEQ ID NO: 3 (*i.e.*, the mature polypeptide coding sequence of SEQ ID NO: 3 in WO2012/089024), the mature polypeptide coding sequence of SEQ ID NO: 5 (*i.e.*, the mature polypeptide coding sequence of SEQ ID NO: 1 in WO2014/026630) or the mature polypeptide coding sequence of SEQ ID NO: 7 (*i.e.*, the mature polypeptide coding sequence of SEQ ID NO: 1 in WO2015/058700) of the present invention.

In the present invention, the polypeptide having the cellulase activity, including GH45 cellulase activity, GH5 cellulase activity, GH6 cellulase activity, or GH7 cellulase activity can a naturally- occurring or wild-type cellulase or a modified cellulase. Preferably, amino acid modifications (*i.e.* substitution, deletion, and/or insertion of one or more (or several) amino acids) are of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of one to about 30 amino acids; small amino or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to about 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

Examples of conservative substitutions are within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In*, *The Proteins*, Academic Press, New York. The most commonly occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

Essential amino acids in a parent polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for endoglucanse activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identities of essential amino acids can also be inferred from analysis of identities with polypeptides that are related to the parent polypeptide.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer, 1988, *Science* 241: 53-57; Bowie and Sauer, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2152-2156; WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (*e.g.*, Lowman *et al.*, 1991, *Biochemistry* 30: 10832-10837; U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis (Derbyshire *et al.*, 1986, *Gene* 46: 145; Ner *et al.*, 1988, *DNA* 7: 127).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides expressed by host cells (Ness *et al.*, 1999, *Nature Biotechnology* 17: 893-896). Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using standard methods in the art. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide.

Preferably, the total number of amino acid substitutions, deletions and/or insertions of the polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8, is not more than 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8 or 9.

The polypeptide may be hybrid polypeptide in which a portion of one polypeptide is fused at the N-terminus or the C-terminus of a portion of another polypeptide.

The polypeptide may be a fused polypeptide or cleavable fusion polypeptide in which another polypeptide is fused at the N-terminus or the C-terminus of the polypeptide of the present invention. A fused polypeptide is produced by fusing a polynucleotide encoding another

polypeptide to a polynucleotide of the present invention. Techniques for producing fusion polypeptides are known in the art, and include ligating the coding sequences encoding the polypeptides so that they are in frame and that expression of the fused polypeptide is under control of the same promoter(s) and terminator. Fusion proteins may also be constructed using intein technology in which fusions are created post-translationally (Cooper *et al.*, 1993, *EMBO J.* 12: 2575-2583; Dawson *et al.*, 1994, *Science* 266: 776-779).

A fusion polypeptide can further comprise a cleavage site between the two polypeptides. Upon secretion of the fusion protein, the site is cleaved releasing the two polypeptides. Examples of cleavage sites include, but are not limited to, the sites disclosed in Martin *et al.*, 2003, *J. Ind. Microbiol. Biotechnol.* 3: 568-576; Svetina *et al.*, 2000, *J. Biotechnol.* 76: 245-251; Rasmussen-Wilson *et al.*, 1997, *Appl. Environ. Microbiol.* 63: 3488-3493; Ward *et al.*, 1995, *Biotechnology* 13: 498-503; and Contreras *et al.*, 1991, *Biotechnology* 9: 378-381; Eaton *et al.*, 1986, *Biochemistry* 25: 505-512; Collins-Racie *et al.*, 1995, *Biotechnology* 13: 982-987; Carter *et al.*, 1989, *Proteins: Structure, Function, and Genetics* 6: 240-248; and *Stevens, 2003, Drug Discovery World 4: 35-48.*

Measurement of the biofinishing activity of the enzyme composition

In order to determine the specific biofinishing activity of the cellulase components in isolation and in combination, they are typically purified using known techniques.

The term "biofinishing" as used herein refers to the treatment of a textile using cellulases and includes, but not limited to, biopolishing and biostoning.

The "biofinishing activity", especially "biopolishing activity", as used herein, is determined as set forth in Examples. The biopolishing effectiveness of the first GH45 cellulase in combination with the second GH45 cellulase and the respective individual cellulase components can be measured by the activity in removing fuzz, or small balls of fuzz (referred to as pills), from fabric. The depilling can be expressed as the depilling activity per unit of protein (*i.e.*, specific depilling activity).

According to one embodiment of the invention, the first GH45 cellulase enzyme component and the second GH45 cellulase component are present in an enzyme composition that exhibits synergy in an assay that measures biofinishing activity. Preferably, the assay is a pilling note test for biopolishing activity. The combinations of GH45 cellulase enzyme components of the present invention provide for enhanced biofinishing of a cotton-containing textile relative to the biofinishing effect of the individual enzyme components. For example, the combination of a first GH45 cellulase and a second GH45 cellulase delivers about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biopolishing effect than the individual GH45 cellulases. The combination of a first GH45 cellulase and a second

GH45 cellulase delivers about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biostoning effect than the individual GH45 cellulases in denim abrasion. In a pilling notes test, the combination of a first GH45 cellulase and a second GH45 cellulase delivers about 0.1, about 0.2, about 0.5, about 0.8, about 1.0 pilling note more than that of the individual GH45 cellulases. The utilization of such enzyme combinations could be a step forward with respect to improving process economics. For example, the combination of a first GH45 cellulase and a second GH45 cellulase delivers an equivalent biofinishing effect with about 1/2, about 1/3, about 1/4, about 1/5, about 1/6, about 1/8 or about 1/10 shorter time than the individual GH45 cellulases. For a high-temperature tolerant GH45 cellulase and low-temperature adapted GH45 cellulase, the combination of the GH45 cellulases delivers a stable biofinishing performance, including but not limited to biopolishing and biostoning performance, at a broad range from the high temperature to the low temperature.

Nucleic Acid Constructs

The present invention also relates to nucleic acid constructs comprising a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, and a polynucleotide encoding a second polypeptide having GH45 cellulase activity and biofinishing activity, operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

In an embodiment, the present invention relates to nucleic acid constructs comprising a first nucleic acid construct and a second nucleic acid construct, wherein the first nucleic acid construct comprises a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences; and the second nucleic acid construct comprises a polynucleotide encoding a second polypeptide having GH45 cellulase activity and biofinishing activity, operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

The polynucleotide may be manipulated in a variety of ways to provide for expression of the GH45 cellulases. Manipulation of the polynucleotide prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides utilizing recombinant DNA methods are well known in the art.

The control sequence may be a promoter, a polynucleotide that is recognized by a host cell for expression of a polynucleotide encoding a polypeptide. The promoter contains transcriptional control sequences that mediate the expression of the polypeptide. The promoter may be any polynucleotide that shows transcriptional activity in the host cell including variant,

truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a bacterial host cell are the promoters obtained from the *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus licheniformis* penicillinase gene (*penP*), *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus subtilis xylA* and *xylB* genes, *Bacillus thuringiensis cryIIIA* gene (Agaisse and Lereclus, 1994, *Molecular Microbiology* 13: 97-107), *E. coli lac* operon, *E. coli trc* promoter (Egon *et al.*, 1988, *Gene* 69: 301-315), *Streptomyces coelicolor* agarase gene (*dagA*), and prokaryotic beta-lactamase gene (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* 75: 3727-3731), as well as the *tac* promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in Gilbert *et al.*, 1980, *Scientific American* 242: 74-94; and in Sambrook *et al.*, 1989, *supra*. Examples of tandem promoters are disclosed in WO 99/43835.

Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*), *Aspergillus oryzae* TAKA amylase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Fusarium oxysporum* trypsin-like protease (WO 96/00787), *Fusarium venenatum* amyloglucosidase (WO 00/56900), *Fusarium venenatum* Daria (WO 00/56900), *Fusarium venenatum* Quinn (WO 00/56900), *Rhizomucor miehei* lipase, *Rhizomucor miehei* aspartic proteinase, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei* cellobiohydrolase I, *Trichoderma reesei* cellobiohydrolase II, *Trichoderma reesei* endoglucanase I, *Trichoderma reesei* endoglucanase II, *Trichoderma reesei* endoglucanase III, *Trichoderma reesei* endoglucanase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* xylanase III, *Trichoderma reesei* beta-xylosidase, and *Trichoderma reesei* translation elongation factor, as well as the NA2-tpi promoter (a modified promoter from an *Aspergillus* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus* triose phosphate isomerase gene; non-limiting examples include modified promoters from an *Aspergillus niger* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus nidulans* or *Aspergillus oryzae* triose phosphate isomerase gene); and variant, truncated, and hybrid promoters thereof. Other promoters are described in U.S. Patent No. 6,011,147.

In a yeast host, useful promoters are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* galactokinase (GAL1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH1, ADH2/GAP), *Saccharomyces cerevisiae* triose phosphate isomerase (TPI), *Saccharomyces cerevisiae* metallothionein (CUP1), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are described by Romanos *et al.*, 1992, *Yeast* 8: 423-488.

The control sequence may also be a transcription terminator, which is recognized by a host cell to terminate transcription. The terminator is operably linked to the 3'-terminus of the polynucleotide encoding the polypeptide. Any terminator that is functional in the host cell may be used in the present invention.

Preferred terminators for bacterial host cells are obtained from the genes for *Bacillus clausii* alkaline protease (*aprH*), *Bacillus licheniformis* alpha-amylase (*amyL*), and *Escherichia coli* ribosomal RNA (*rrnB*).

Preferred terminators for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase, *Aspergillus oryzae* TAKA amylase, *Fusarium oxysporum* trypsin-like protease, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei* cellobiohydrolase I, *Trichoderma reesei* cellobiohydrolase II, *Trichoderma reesei* endoglucanase I, *Trichoderma reesei* endoglucanase II, *Trichoderma reesei* endoglucanase III, *Trichoderma reesei* endoglucanase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* xylanase III, *Trichoderma reesei* beta-xylosidase, and *Trichoderma reesei* translation elongation factor.

Preferred terminators for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be an mRNA stabilizer region downstream of a promoter and upstream of the coding sequence of a gene which increases expression of the gene.

Examples of suitable mRNA stabilizer regions are obtained from a *Bacillus thuringiensis cryIIIA* gene (WO 94/25612) and a *Bacillus subtilis* SP82 gene (Hue *et al.*, 1995, *Journal of Bacteriology* 177: 3465-3471).

The control sequence may also be a leader, a nontranslated region of an mRNA that is important for translation by the host cell. The leader is operably linked to the 5'-terminus of the

polynucleotide encoding the polypeptide. Any leader that is functional in the host cell may be used.

Preferred leaders for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase and *Aspergillus nidulans* triose phosphate isomerase.

Suitable leaders for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP).

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3'-terminus of the polynucleotide and, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence that is functional in the host cell may be used.

Preferred polyadenylation sequences for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase *Aspergillus oryzae* TAKA amylase, and *Fusarium oxysporum* trypsin-like protease.

Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, *Mol. Cellular Biol.* 15: 5983-5990.

The control sequence may also be a signal peptide coding region that encodes a signal peptide linked to the N-terminus of a polypeptide and directs the polypeptide into the cell's secretory pathway. The 5'-end of the coding sequence of the polynucleotide may inherently contain a signal peptide coding sequence naturally linked in translation reading frame with the segment of the coding sequence that encodes the polypeptide. Alternatively, the 5'-end of the coding sequence may contain a signal peptide coding sequence that is foreign to the coding sequence. A foreign signal peptide coding sequence may be required where the coding sequence does not naturally contain a signal peptide coding sequence. Alternatively, a foreign signal peptide coding sequence may simply replace the natural signal peptide coding sequence in order to enhance secretion of the polypeptide. However, any signal peptide coding sequence that directs the expressed polypeptide into the secretory pathway of a host cell may be used.

Effective signal peptide coding sequences for bacterial host cells are the signal peptide coding sequences obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* beta-lactamase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus stearothermophilus* neutral proteases (*nprT*, *nprS*, *nprM*), and *Bacillus subtilis prsA*. Further signal peptides are described by Simonen and Palva, 1993, *Microbiological Reviews* 57: 109-137.

Effective signal peptide coding sequences for filamentous fungal host cells are the signal peptide coding sequences obtained from the genes for *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Aspergillus oryzae* TAKA amylase, *Humicola insolens* cellulase, *Humicola insolens* endoglucanase V, *Humicola lanuginosa* lipase, and *Rhizomucor miehei* aspartic proteinase.

Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase. Other useful signal peptide coding sequences are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be a propeptide coding sequence that encodes a propeptide positioned at the N-terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to an active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding sequence may be obtained from the genes for *Bacillus subtilis* alkaline protease (*aprE*), *Bacillus subtilis* neutral protease (*nprT*), *Myceliophthora thermophila* laccase (WO 95/33836), *Rhizomucor miehei* aspartic proteinase, and *Saccharomyces cerevisiae* alpha-factor.

Where both signal peptide and propeptide sequences are present, the propeptide sequence is positioned next to the N-terminus of a polypeptide and the signal peptide sequence is positioned next to the N-terminus of the propeptide sequence.

It may also be desirable to add regulatory sequences that regulate expression of the polypeptide relative to the growth of the host cell. Examples of regulatory sequences are those that cause expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory sequences in prokaryotic systems include the *lac*, *tac*, and *trp* operator systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the *Aspergillus niger* glucoamylase promoter, *Aspergillus oryzae* TAKA alpha-amylase promoter, and *Aspergillus oryzae* glucoamylase promoter, *Trichoderma reesei* cellobiohydrolase I promoter, and *Trichoderma reesei* cellobiohydrolase II promoter may be used. Other examples of regulatory sequences are those that allow for gene amplification. In eukaryotic systems, these regulatory sequences include the dihydrofolate reductase gene that is amplified in the presence of methotrexate, and the metallothionein genes that are amplified with heavy metals. In these cases, the polynucleotide encoding the polypeptide would be operably linked to the regulatory sequence.

Expression Vectors

The present invention also relates to recombinant expression vectors comprising a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing

activity, and a polynucleotide encoding a second polypeptide having GH45 cellulase activity and biofinishing activity, a promoter, and transcriptional and translational stop signals.

In an embodiment, the present invention relates to recombinant host cells, comprising a first nucleic acid construct or expression vector encoding a first polypeptide having GH45 cellulase activity and biofinishing activity; and a second nucleic acid construct or expression vector encoding a second polypeptide having GH45 cellulase activity and biofinishing activity. In a further embodiment, the present invention relates to recombinant expression vectors comprising a first recombinant expression vector and a second recombinant expression vector, wherein the first recombinant expression vector comprises a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, a promoter, and transcriptional and translational stop signals; and the second recombinant expression vector comprising a polynucleotide encoding a second polypeptide having GH45 cellulase activity and biofinishing activity, a promoter, and transcriptional and translational stop signals.

The various nucleotide and control sequences may be joined together to produce a recombinant expression vector that may include one or more convenient restriction sites to allow for insertion or substitution of the polynucleotide encoding the GH45 cellulases at such sites. Alternatively, the polynucleotide may be expressed by inserting the polynucleotide or a nucleic acid construct comprising the polynucleotide into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

The recombinant expression vector may be any vector (*e.g.*, a plasmid or virus) that can be conveniently subjected to recombinant DNA procedures and can bring about expression of the polynucleotide. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may be a linear or closed circular plasmid.

The vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one that, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids that together contain the total DNA to be introduced into the genome of the host cell, or a transposon, may be used.

The vector preferably contains one or more selectable markers that permit easy selection of transformed, transfected, transduced, or the like cells. A selectable marker is a

gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are *Bacillus licheniformis* or *Bacillus subtilis dal* genes, or markers that confer antibiotic resistance such as ampicillin, chloramphenicol, kanamycin, neomycin, spectinomycin, or tetracycline resistance. Suitable markers for yeast host cells include, but are not limited to, ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *adeA* (phosphoribosylaminoimidazole-succinocarboxamide synthase), *adeB* (phosphoribosyl-aminoimidazole synthase), *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus* cell are *Aspergillus nidulans* or *Aspergillus oryzae amdS* and *pyrG* genes and a *Streptomyces hygroscopicus bar* gene. Preferred for use in a *Trichoderma* cell are *adeA*, *adeB*, *amdS*, *hph*, and *pyrG* genes.

The selectable marker may be a dual selectable marker system as described in WO 2010/039889. In one aspect, the dual selectable marker is an *hph-tk* dual selectable marker system.

The vector preferably contains an element(s) that permits integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the polynucleotide's sequence encoding the polypeptide or any other element of the vector for integration into the genome by homologous or non-homologous recombination. Alternatively, the vector may contain additional polynucleotides for directing integration by homologous recombination into the genome of the host cell at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should contain a sufficient number of nucleic acids, such as 100 to 10,000 base pairs, 400 to 10,000 base pairs, and 800 to 10,000 base pairs, which have a high degree of sequence identity to the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding polynucleotides. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. The origin of

replication may be any plasmid replicator mediating autonomous replication that functions in a cell. The term "origin of replication" or "plasmid replicator" means a polynucleotide that enables a plasmid or vector to replicate *in vivo.*

Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in *E. coli*, and pUB110, pE194, pTA1060, and pAMß1 permitting replication in *Bacillus*.

Examples of origins of replication for use in a yeast host cell are the 2 micron origin of replication, ARS1, ARS4, the combination of ARS1 and CEN3, and the combination of ARS4 and CEN6.

Examples of origins of replication useful in a filamentous fungal cell are AMA1 and ANS1 (Gems *et al.*, 1991, *Gene* 98: 61-67; Cullen *et al.*, 1987, *Nucleic Acids Res.* 15: 9163-9175; WO 00/24883). Isolation of the AMA1 gene and construction of plasmids or vectors comprising the gene can be accomplished according to the methods disclosed in WO 00/24883.

More than one copy of a polynucleotide of the present invention may be inserted into a host cell to increase production of a polypeptide. An increase in the copy number of the polynucleotide can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the polynucleotide where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the polynucleotide, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, *e.g.*, Sambrook *et al.*, 1989, *supra*).

Host Cells

The present invention also relates to recombinant host cells, comprising a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity of the present invention, operably linked to one or more control sequences that direct the production of polypeptides of the present invention.

In an embodiment, the present invention relates to recombinant host cells comprising a first recombinant host cell and a second recombinant host cell, wherein the first recombinant host cell comprises a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, operably linked to one or more control sequences that direct the production of the polypeptide; and the second recombinant host cell comprises a second

polypeptide having GH45 cellulase activity and biofinishing activity, operably linked to one or more control sequences that direct the production of the polypeptide.

In an embodiment, the polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity and the second polypeptide having GH45 cellulase activity and biofinishing activity are heterologous to the host cell. A construct or vector comprising a polynucleotide is introduced into a host cell so that the construct or vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector as described earlier. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication. The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source.

The host cell may be any cell useful in the recombinant production of a polypeptide of the present invention, *e.g.*, a prokaryote or a eukaryote.

The prokaryotic host cell may be any Gram-positive or Gram-negative bacterium. Gram-positive bacteria include, but are not limited to, *Bacillus*, *Clostridium*, *Enterococcus*, *Geobacillus*, *Lactobacillus*, *Lactococcus*, *Oceanobacillus*, *Staphylococcus*, *Streptococcus*, and *Streptomyces*. Gram-negative bacteria include, but are not limited to, *Campylobacter*, *E. coli*, *Flavobacterium*, *Fusobacterium*, *Helicobacter*, *Ilyobacter*, *Neisseria*, *Pseudomonas*, *Salmonella*, and *Ureaplasma*.

The bacterial host cell may be any Bacillus cell including, but not limited to, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, and Bacillus thuringiensis cells.

The bacterial host cell may also be any Streptococcus cell including, but not limited to, Streptococcus equisimilis, Streptococcus pyogenes, Streptococcus uberis, and Streptococcus equi subsp. Zooepidemicus cells.

The bacterial host cell may also be any Streptomyces cell including, but not limited to, Streptomyces achromogenes, Streptomyces avermitilis, Streptomyces coelicolor, Streptomyces griseus, and Streptomyces lividans cells.

The introduction of DNA into a *Bacillus* cell may be effected by protoplast transformation (see, *e.g.*, Chang and Cohen, 1979, *Mol. Gen. Genet.* 168: 111-115), competent cell transformation (see, *e.g.*, Young and Spizizen, 1961, *J. Bacteriol.* 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, *J. Mol. Biol.* 56: 209-221), electroporation (see, *e.g.*, Shigekawa and Dower, 1988, *Biotechniques* 6: 742-751), or conjugation (see, *e.g.*, Koehler and Thorne, 1987, *J. Bacteriol.* 169: 5271-5278). The introduction of DNA into an *E. coli* cell may be effected by protoplast transformation (see, *e.g.*, Hanahan, 1983, *J. Mol. Biol.* 166: 557-580) or

electroporation (see, *e.g.*, Dower *et al.*, 1988, *Nucleic Acids Res.* 16: 6127-6145). The introduction of DNA into a *Streptomyces* cell may be effected by protoplast transformation, electroporation (see, *e.g.*, Gong *et al.*, 2004, *Folia Microbiol.* (*Praha*) 49: 399-405), conjugation (see, *e.g.*, Mazodier *et al.*, 1989, *J. Bacteriol.* 171: 3583-3585), or transduction (see, *e.g.*, Burke *et al.*, 2001, *Proc. Natl. Acad. Sci. USA* 98: 6289-6294). The introduction of DNA into a *Pseudomonas* cell may be effected by electroporation (see, *e.g.*, Choi *et al.*, 2006, *J. Microbiol. Methods* 64: 391-397) or conjugation (see, *e.g.*, Pinedo and Smets, 2005, *Appl. Environ. Microbiol.* 71: 51-57). The introduction of DNA into a *Streptococcus* cell may be effected by natural competence (see, *e.g.*, Perry and Kuramitsu, 1981, *Infect. Immun.* 32: 1295-1297), protoplast transformation (see, *e.g.*, Catt and Jollick, 1991, *Microbios* 68: 189-207), electroporation (see, *e.g.*, Buckley *et al.*, 1999, *Appl. Environ. Microbiol.* 65: 3800-3804), or conjugation (see, *e.g.*, Clewell, 1981, *Microbiol. Rev.* 45: 409-436). However, any method known in the art for introducing DNA into a host cell can be used.

The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell.

The host cell may be a fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota as well as the Oomycota and all mitosporic fungi (as defined by Hawksworth *et al.*, *In, Ainsworth and Bisby's Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK).

The fungal host cell may be a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (*Endomycetales*), basidiosporogenous yeast, and yeast belonging to the *Fungi Imperfecti* (*Blastomycetes*). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in *Biology and Activities of Yeast* (Skinner, Passmore, and Davenport, editors, *Soc. App. Bacteriol. Symposium Series* No. 9, 1980).

The yeast host cell may be a *Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces,* or *Yarrowia* cell, such as a *Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis, or Yarrowia lipolytica* cell.

The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth *et al.*, 1995, *supra*). The filamentous fungi are generally characterized by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In

contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative.

The filamentous fungal host cell may be an *Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes,* or *Trichoderma* cell.

For example, the filamentous fungal host cell may be an *Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei,* or *Trichoderma viride* cell.

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known *per se*. Suitable procedures for transformation of *Aspergillus* and *Trichoderma* host cells are described in EP 238023, Yelton *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474, and Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Suitable methods for transforming *Fusarium* species are described by Malardier *et al.*, 1989, *Gene* 78: 147-156, and WO 96/00787. Yeast may be transformed using the procedures described by Becker and Guarente, *In* Abelson, J.N. and Simon, M.I., editors, *Guide to Yeast Genetics and Molecular Biology*, *Methods in Enzymology*, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito *et al.*, 1983, *J. Bacteriol.* 153: 163; and Hinnen *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* 75: 1920.

Methods of Production

The present invention also relates to methods of producing a composition comprising a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity, comprising (a) cultivating a recombinant host cell comprising a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity under conditions conducive for production of the polypeptides; and optionally, (b) recovering the composition.

The present invention further relates to methods of producing a composition comprising a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity, comprising (a) cultivating a recombinant host cell comprising a first recombinant host cell and a second recombinant host cell of the present invention under conditions conducive for production of the polypeptides; and optionally, (b) recovering the polypeptides and combining the first polypeptide and the second polypeptide.

The present invention further relates to methods of producing a composition comprising a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity, comprising (a) cultivating a first recombinant host cell of the present invention under conditions conducive for production of the polypeptides and cultivating a second recombinant host cell of the present invention under conditions conducive for production of the polypeptides; and optionally, (b) recovering the polypeptides and combining the first polypeptide and the second polypeptide.

The present invention further relates to methods of producing a composition comprising a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity, comprising (a) cultivating a recombinant host cell comprising a polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, and a polynucleotide encoding a second polypeptide having GH45 cellulase activity and biofinishing activity under conditions conducive for production of the polypeptides; and optionally, (b) recovering the composition.

The host cells are cultivated in a nutrient medium suitable for production of the polypeptides using methods known in the art. For example, the cells may be cultivated by shake flask cultivation, or small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors in a suitable medium and under conditions allowing the polypeptides to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from

commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

The polypeptide may be detected using methods known in the art that are specific for a polypeptide. These detection methods include, but are not limited to, use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the composition.

The polypeptide may be recovered using methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures including, but not limited to, collection, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation. In one aspect, a fermentation broth comprising the composition is recovered.

The polypeptide may be purified by a variety of procedures known in the art including, but not limited to, chromatography (*e.g.*, ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (*e.g.*, preparative isoelectric focusing), differential solubility (*e.g.*, ammonium sulfate precipitation), SDS-PAGE, or extraction (see, *e.g.*, *Protein Purification*, Janson and Ryden, editors, VCH Publishers, New York, 1989) to obtain substantially pure polypeptides.

In an alternative aspect, the polypeptides are not recovered, but rather a combination of a first host cell of the present invention expressing the first polypeptide having GH45 cellulase activity and biofinishing activity and a second host cell of the present invention expressing the second polypeptide having GH45 cellulase activity and biofinishing activity used as a source of the composition. In another embodiment, a host cell of the present invention expressing the first polypeptide and the second polypeptide is used as a source of the composition.

In an alternative aspect, the polypeptides are not recovered, but rather a combination of a first fermentation broth formulation of the present invention comprising the first polypeptide having GH45 cellulase activity and biofinishing activity and a second fermentation broth formulation of the present invention comprising the second polypeptide having GH45 cellulase activity and biofinishing activity used as a source of the composition. In another embodiment, a fermentation broth formulation of the present invention comprising the first polypeptide having GH45 cellulase activity and biofinishing activity and the second polypeptide having GH45 cellulase activity and biofinishing activity is used as a source of the composition.

Fermentation Broth Formulations or Cell Compositions

The present invention also relates to a fermentation broth formulation or a cell composition comprising a first polypeptide having GH45 cellulase activity and a second polypeptide having GH45 cellulase activity of the present invention.

The present invention further relates to a fermentation broth formulation or a cell composition comprising a first fermentation broth formulation or a cell composition and a second fermentation broth formulation or a cell composition, wherein the first fermentation broth formulation or a cell composition comprises a first polypeptide having GH45 cellulase activity and biofinishing activity, and the second fermentation broth formulation or a cell composition comprises a second polypeptide having GH45 cellulase activity and biofinishing activity.

The fermentation broth product further comprises additional ingredients used in the fermentation process, such as, for example, cells (including, the host cells containing the gene encoding the polypeptide of the present invention which are used to produce the polypeptide of interest), cell debris, biomass, fermentation media and/or fermentation products. In some embodiments, the composition is a cell-killed whole broth containing organic acid(s), killed cells and/or cell debris, and culture medium.

The term "fermentation broth" as used herein refers to a preparation produced by cellular fermentation that undergoes no or minimal recovery and/or purification. For example, fermentation broths are produced when microbial cultures are grown to saturation, incubated under carbon-limiting conditions to allow protein synthesis (*e.g.*, expression of enzymes by host cells) and secretion into cell culture medium. The fermentation broth can contain unfractionated or fractionated contents of the fermentation materials derived at the end of the fermentation. Typically, the fermentation broth is unfractionated and comprises the spent culture medium and cell debris present after the microbial cells (*e.g.*, filamentous fungal cells) are removed, *e.g.*, by centrifugation. In some embodiments, the fermentation broth contains spent cell culture medium, extracellular enzymes, and viable and/or nonviable microbial cells.

In an embodiment, the fermentation broth formulation and cell compositions comprise a first organic acid component comprising at least one 1-5 carbon organic acid and/or a salt thereof and a second organic acid component comprising at least one 6 or more carbon organic acid and/or a salt thereof. In a specific embodiment, the first organic acid component is acetic acid, formic acid, propionic acid, a salt thereof, or a mixture of two or more of the foregoing and the second organic acid component is benzoic acid, cyclohexanecarboxylic acid, 4-methylvaleric acid, phenylacetic acid, a salt thereof, or a mixture of two or more of the foregoing.

In one aspect, the composition contains an organic acid(s), and optionally further contains killed cells and/or cell debris. In one embodiment, the killed cells and/or cell debris are

removed from a cell-killed whole broth to provide a composition that is free of these components.

The fermentation broth formulations or cell compositions may further comprise a preservative and/or anti-microbial (*e.g.*, bacteriostatic) agent, including, but not limited to, sorbitol, sodium chloride, potassium sorbate, and others known in the art.

The cell-killed whole broth or composition may contain the unfractionated contents of the fermentation materials derived at the end of the fermentation. Typically, the cell-killed whole broth or composition contains the spent culture medium and cell debris present after the microbial cells (*e.g.*, filamentous fungal cells) are grown to saturation, incubated under carbon-limiting conditions to allow protein synthesis. In some embodiments, the cell-killed whole broth or composition contains the spent cell culture medium, extracellular enzymes, and killed filamentous fungal cells. In some embodiments, the microbial cells present in the cell-killed whole broth or composition can be permeabilized and/or lysed using methods known in the art.

A whole broth or cell composition as described herein is typically a liquid, but may contain insoluble components, such as killed cells, cell debris, culture media components, and/or insoluble enzyme(s). In some embodiments, insoluble components may be removed to provide a clarified liquid composition.

The whole broth formulations and cell compositions of the present invention may be produced by a method described in WO 90/15861 or WO 2010/096673.

Enzyme composition

In the present invention, the enzyme composition comprises or consists of a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity. In one embodiment of the present invention, the first polypeptide and the second polypeptide exhibit synergy in an assay that measures biofinishing activity.

In a preferable embodiment, the assay that measures biofinishing activity is a pilling note test for biopolishing activity. In another preferable embodiment, the first GH45 cellulase and the second GH45 cellulase deliver about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biopolishing effect than the individual GH45 cellulases.

In a preferable embodiment, the biofinishing activity is a biostoning activity. In another preferable embodiment, the first GH45 cellulase and the second GH45 cellulase deliver about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biostoning effect than the individual GH45 cellulases in denim abrasion.

In the enzyme composition of the present invention, the first polypeptide having GH45 cellulase activity and biofinishing activity and the second polypeptide having GH45 cellulase

activity and biofinishing activity are different polypeptides. In another embodiment of the present invention, the first GH45 cellulase is a high-temperature tolerant GH45 cellulase, and the second GH45 cellulase is a low-temperature adapted GH45 cellulase.

In another embodiment of the present invention, the enzyme composition further comprises a polypeptide having GH5 cellulase activity, a polypeptide having a GH6 cellulase activity and/or a polypeptide having GH7 cellulase activity.

Preferably, the enzyme compositions are enriched in such polypeptides having GH45 cellulases activity and biofinishing activity. The term "enriched" indicates that the polypeptides having GH45 cellulase activity and biofinishing activity of the composition has been increased, *e.g.*, with an enrichment factor of at least 1.1.

In an embodiment, the first polypeptide is in an amount of from about 5% to 1000%, preferably, from about 20% to about 500%, more preferably, from about 30% to about 330%, even more preferably from about 40 to about 250%, by weight of the second polypeptide.

The enzyme compositions may be prepared in accordance with the methods of the presention and may be in the form of a liquid or a dry composition. The compositions may be stabilized in accordance with methods known in the art.

Examples are given below of preferred uses of the enzyme compositions of the present invention. The dosage of the enzyme composition and other conditions under which the enzyme composition is used may be determined on the basis of methods known in the art.

Textile

As used herein, the term "textile" refers to fibers, yarns, fabrics, garments, and non-wovens. The term encompasses textiles made from natural, synthetic (e.g., manufactured), and various natural and synthetic blends. Textiles may be unprocessed or processed fibers, yarns, woven or knit fabrics, non-wovens, and garments and may be made using a variety of materials, some of which are mentioned, herein.

The process of the invention is most beneficially applied to a cellulose-containing textile, such as cotton, viscose, rayon, ramie, linen, Tencel, or mixtures thereof, or mixtures of any of these fibres, or mixtures of any of these fibres together with synthetic fibres such as mixtures of cotton and spandex (stretch-denim). In particular, the fabric is dyed fabric. In an embodiment, the fabric is denim. The denim fabric may be dyed with vat dyes such as indigo, or indigo-related dyes such as thioindigo.

In an embodiment of the process of the invention, a cellulose-containing textile is a cotton-containing textile or a man-made cellulose-containing textile.

Textile manufacturing process

The processing of a fabric, such as of a cellulosic material, into material ready for garment manufacture involves several steps: spinning of the fiber into a yarn; construction of woven or knit fabric from the yarn; and subsequent preparation processes, dyeing/printing and finishing operations. Preparation processes are necessary for removing natural and man-induced impurities from fibers and for improving their aesthetic appearance and processability prior to for instance dyeing/printing and finishing. Common preparation processes comprise desizing (for woven goods), scouring, and bleaching, which produce a fabric suitable for dyeing or finishing.

Woven fabric is constructed by weaving "filling" or "weft" yarns between warp yarns stretched in the longitudinal direction on the loom. The warp yarns must be sized before weaving in order to lubricate and protect them from abrasion at the high speed insertion of the filling yarns during weaving. Common size agents are starches (or starch derivatives and modified starches), poly(vinyl alcohol), carboxyl methyl cellulose (i.e. CMC) where starches are dominant. Paraffin, acrylic binders and variety of lubricants are often included in the size mix. The filling yarn can be woven through the warp yarns in a "over one - under the next" fashion (plain weave) or by "over one - under two" (twill) or any other myriad of permutations. Generally, dresses, shirts, pants, sheeting's, towels, draperies, etc. are produced from woven fabric. After the fabric is made, size on the fabric must be removed again (i.e. desizing).

Knitting is forming a fabric by joining together interlocking loops of yarn. As opposed to weaving, which is constructed from two types of yarn and has many "ends", knitted fabric is produced from a single continuous strand of yarn. As with weaving, there are many different ways to loop yarn together and the final fabric properties are dependent both upon the yarn and the type of knit. Underwear, sweaters, socks, sport shirts, sweat shirts, etc. are derived from knit fabrics.

Desizing

Desizing is the degradation and/or removal of sizing compounds from warp yarns in a woven fabric. Starch is usually removed by an enzymatic desizing procedure. In addition, oxidative desizing and chemical desizing with acids or bases are sometimes used.

In some embodiments, the desizing enzyme is an amylolytic enzyme, such as an alpha-amylase, a beta-amylase, a mannanase, a glucoamylase, or a combination thereof.

Suitable alpha and beta-amylases include those of bacterial or fungal origin, as well as chemically or genetically modified mutants and variants of such amylases. Suitable alpha-amylases include alpha-amylases obtainable from *Bacillus* species. Suitable commercial amylases include but are not limited to OPTISIZE® NEXT, OPTISIZE® FLEX and OPTISIZE® COOL (all from Genencor International Inc.), and DURAMYL™, ERMAMYL™, FUNGAMYL™ TERMAMYL™, AQUAZYME™ and BAN™ (all available from Novozymes A/S, Bagsvaerd, Denmark).

Other suitable amylolytic enzymes include the CGTases (cyclodextrin glucanotransferases, EC 2.4.1.19), e.g., those obtained from species of *Bacillus, Thermoanaerobactor* or *Thermoanaero-bacterium*.

Scouring

Scouring is used to remove impurities from the fibers, to swell the fibers and to remove seed coat. It is one of the most critical steps. The main purposes of scouring is to a) uniformly clean the fabric, b) soften the motes and other trashes, c) improve fabric absorbency, d) saponify and solubilize fats, oils, and waxes, and e) minimize immature cotton. Sodium hydroxide scouring at about boiling temperature is the accepted treatment for 100% cotton, while calcium hydroxide and sodium carbonate are less frequently used. Synthetic fibers are scoured at much milder conditions. Surfactant and chelating agents are essential for alkaline scouring. Enzymatic scouring has been introduced, wherein cellulase, hemicellulase, pectinase, lipase, and protease are all reported to have scouring effects.

Bleaching

Bleaching is the destruction of pigmented color and/or colored impurities as well as seed coat fragment removal. Bleaching is performed by the use of oxidizing or reducing chemistry. Oxidizing agents can be further subdivided into those that employ or generate: a) hypochlorite (OCl^-), b) chloride dioxide (ClO_2), c) permanganate (MnO_4-), d) ozone, and hydroperoxide species (OOH^- and/or OOH). Reducing agents are typical sulfur dioxide, hydrosulfite salts, etc. Enzymatic bleaching using glucose oxidase or peroxidase (for example, see WO 2013/040991) has been reported. Traditionally, hydrogen peroxide is used in this process.

Printing and dyeing

Printing and dyeing of textiles is carried out by applying dyes to the textile by any appropriate method for binding the dyestuff to the fibres in the textiles. The dyeing of textiles may for example be carried out by passing the fabric through a concentrated solution of dye, followed by storage of the wet fabric in a vapour tight enclosure to permit time for diffusion and reaction of the dye with the fabric substrate prior to rinsing off un-reacted dye. Alternatively, the dye may be fixed by subsequent steaming of the textile prior to rinsing. The dyes include synthetic and natural dyes. Typical dyes are those with anionic functional groups (e.g. acid dyes, direct dyes, Mordant dyes and reactive dyes), those with cationic groups (e.g. basic dyes), those requiring chemical reaction before application (e.g. vat dyes, sulphur dyes and azoic dyes), disperse dyes and solvent dyes.

Excess soluble dyestuff not bound to the fibres must be removed after dyeing to ensure fastness of the dyed textiles and to prevent unwanted dye transfer during laundering of the textiles by the consumer. Generally, a large amount of water is required for complete removal of excess dye. In a conventional process, the printed or dyed textile is first rinsed with cold

water, then washed at high temperature with the addition of a suitable additive to decrease back-staining, like poly(vinylpyrrolidone) (PVP).

An enzymatic process for removal of excess dye from dyed fabric with a rinse liquor comprising at least one peroxidase, an oxidase agent and at least one mediator, such as liquor comprising a peroxidase, hydrogen peroxidise and a mediator like 1-hydroxy-benzotriazole is disclosed in WO99/34054.

Biopolishing

Most cotton fabrics and cotton blend fabrics have a hand-feeling problem that is rather hard and stiff without the application of finishing components. The fabric surface also is not smooth because small fuzzy microfibrils protrude from it. In addition, after a relatively short period of wear, pilling appears on the fabric surface thereby giving it an unappealing, worn look.

Biopolishing is a method to treat cellulosic fabrics during their manufacture by enzymes such as cellulases, which improves fabric quality with respect to "reduced pilling formation". The most important effects of biopolishing can be characterized by less fuzz and pilling, increased gloss/luster, improved fabric handle, increased durable softness and/or improved water absorbency. Biopolishing usually takes place in the wet processing of the manufacture of knitted and woven fabrics or garments. Wet processing comprises such steps as *e.g.*, desizing, scouring, bleaching, washing, dying/printing and finishing. Biopolishing could be performed as a separate step after any of the wetting steps or in combination with any of those wetting steps.

In the present invention, the step of biofinishing is carried out before, during or after step of desizing, bleaching, or printing and dyeing.

Manufacturing of Denim Fabric

Some dyed fabric such as denim fabric, requires that the yarns are dyed before weaving. For denim fabric, the warp yarns are dyed for example with indigo, and sized, before weaving. Preferably the dyeing of the denim yarn is a ring-dyeing. A preferred embodiment of the invention is ring-dyeing of the yarn with a vat dye such as indigo, or an indigo-related dye such as thioindigo, or a sulfur dye, or a direct dye, or a reactive dye, or a naphthol. The yarn may also be dyed with more than one dye, e.g., first with a sulphur dye and then with a vat dye, or vice versa.

Preferably, the yarns undergo scouring and/or bleaching before they are dyed, in order to achieve higher quality of denim fabric. In general, after woven into dyed fabric, such as denim, the dyed fabric or garment proceeds to a desizing stage, preferably followed by a stoning or abrasion step and/or a color modification step.

The desizing process as used herein is the same process as mentioned above in the text.

After desizing, the dyed fabric undergoes a biostoning step. The biostoning step can be performed with enzymes or pumice stones or both. As used herein, the term "biostoning", "stone washing" and "abrasion" are interchangeable, which means agitating the denim in an aqueous medium containing a mechanical abrasion agent such as pumice, an abrading cellulase or a combination of these, to provide a "stone-washed" look. In all cases, mechanical action is needed to remove the dye, and the treatment is usually carried out in washing machines, like drum washers, belly washers. As a result of uneven dye removal there are contrasts between dyed areas and areas from which dye has been removed. Treatment with cellulase can completely replace treatment with pumice stones. However, cellulase treatment can also be combined with pumice stone treatment, when it is desired to produce a heavily abraded finish. For denim manufacture, "biofinishing" includes "biostoning".

Preferably, the abrasion is followed by a color modification step. As used herein, the terms "color modification" or "color adjustment" are used without distinction to refer to any change to the color of a textile resulting from the destruction, modification, or removal of a dyestuff associated with the textile. Without being limited to a theory, it is proposed that color modification results from the modification of chromaphores associated with a textile material, thereby changing its visual appearance. The chromophores may be naturally-associated with the material used to manufacture a textile (e.g., the white color of cotton) or associated with special finishes, such as dying or printing. Color modification encompasses chemical modification to a chromophore as well as chemical modification to the material to which a chromophore is attached.

Getting faded or bleached look in certain areas on textile especially denim, is an important part in textile manufacturing. This is normally achieved by applying $KMnO_4$ (or $KMnO_4$/ H_3PO_4) solution (via brushing, rubbing or spray) onto dried denim after abrasion step. The stained area would get bleached after drying and washing with $Na_2S_2O_5$ solution. During this process indigo/sulphur dyes are destroyed by $KMnO_4$ through oxidation, and then $Na_2S_2O_5$ washing is applied to get rid of the brown colour caused by products of the oxidation. Such treatment will form a local color modification, *i.e.* a specific bleached pattern on denim to meet the customers' needs. In the present invention, the step of biofinishing is carried out before, during or after step of desizing, or color modification.

The invention is further defined in the following paragraphs:

[1]. An enzyme composition comprising, a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity.

[2]. The enzyme composition of paragraph 1, wherein the first polypeptide and the second polypeptide exhibits synergy in an assay that measures biofinishing activity.

[3]. The enzyme composition of paragraph 1 or 2, wherein the assay is a pilling note test for biopolishing activity.

[4]. The enzyme composition of paragraph 3, wherein the first GH45 cellulase and the second GH45 cellulase deliver about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biopolishing effect than the individual GH45 cellulases.

[5]. The enzyme compositon of paragraph 1 or 2, whererin the first GH45 cellulase and the second GH45 cellulase deliver about 1%, about 5%, about 10%, about 15%, about 20%, about 30%, about 50% stronger biostoning effect than the individual GH45 cellulases in denim abrasion.

[6]. The enzyme compositon of paragraph 1 or 2, wherein the first GH45 cellulase is a high-temperature tolerant GH45 cellulase, and the second GH45 cellulase is a low-temperature adapted GH45 cellulase.

[7]. The enzyme composition of any of paragraphs 1-6, wherein the first polypeptide and the second polypeptide are endoglucanases.

[8]. The enzyme compositon of any of paragraphs 1-7, wherein the first polypeptide and the second polypetides are GH45 cellulase subfamily A members.

[9]. The enzyme composition of any of paragraphs 1-8, wherein the first polypeptide is in an amount of from about 5% to 1000%, preferably, from about 20% to about 500%, more preferably, from about 30% to about 330%, even more preferably from about 40 to about 250%, by weight of the second polypeptide.

[10]. The enzyme composition of any of paragraphs 1-9, further comprising a polypeptide having GH5 cellulase activity, a polypeptide having a GH6 cellulase activity and/or a polypeptide having GH7 cellulase activity.

[11]. The enzyme composition of any of paragraphs 1-10, wherein the first polypeptide or the second polypeptide is derived from the group consisting of *Staphylotrichum*, *Thielavia*, *Sordaria* or *Neurospora.*

[12]. The enzyme composition of any of paragraphs 1-11, wherein the first polypeptide or the second polypeptide is derived from the group consisting of *Staphylotrichum coccosporum*, *Thielavia terrestris, Sordaria fimicola or Neurospora tetrasperma.*

[13]. The enzyme composition of any paragraphs 1-12, wherein the first polypeptide or the second polypeptide has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 2, the mature polypeptide of

SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

[14]. The enzyme composition of paragraph 13 wherein the first polypeptide or the second polypeptide comprises or consists of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

[15]. The enzyme composition of any of paragraphs 1-14, wherein the first polypeptide or the second polypeptide is encoded by a polynucleotide sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7.

[16]. A nucleic acid construct or expression vector comprising a first polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polynucleotide encoding a second polypeptide having GH45 cellulase activity and biofinishing activity, operably linked to one or more control sequences that direct the production of the polypeptides in an expression host.

[17]. The nucleic acid construct or expression vector of paragraph 16, wherein the first polypeptide or the second polypeptide is derived from the group consisting of *Staphylotrichum*, *Thielavia*, *Sordaria* or *Neurospora*.

[18]. The nucleic acid construct or expression vector of paragraph 17, wherein the first polypeptide or the second polypeptide is derived from the group consisting of *Staphylotrichum coccosporum*, *Thielavia terrestris, Sordaria fimicola or Neurospora tetrasperma.*

[19]. The nucleic acid construct or expression vector any of paragraph 16-18, wherein the first polypeptide or the second polypeptide has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

[20]. The nucleic acid construct or expression vector of paragraph 19, wherein the first polypeptide or the second polypeptide comprises or consists of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

[21]. The nucleic acid construct or expression vector of any of paragraph 16-20, wherein the first polynucleotide or the second polynucleotide has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7.

[22]. A recombinant host cell comprising the nucleic acid construct or expression vector of any of paragraphs 16-21.

[23]. The recombinant host cell of paragraph 22, comprising a first nucleic acid construct or expression vector encoding a first polypeptide having GH45 cellulase activity and biofinishing activity; and a second nucleic acid construct or expression vector encoding a second polypeptide having GH45 cellulase activity and biofinishing activity.

[24] A method of producing a composition comprising a first polypeptide having GH45 cellulase activity and a second polypeptide having GH45 cellulase activity, comprising (a) cultivating a recombinant host cell of paragraph 22 under conditions conducive for production of the polypeptides; and optionally, (b) recovering the composition.

[25]. A method of producing a composition comprising a first polypeptide having GH45 cellulase activity and a second polypeptide having GH45 cellulase activity, comprising comprising (a) cultivating a first recombinant host cell comprising the first polypeptide under conditions conducive for production of the polypeptide and cultivating a second recombinant host cell comprising the second polypeptide under conditions conducive for production of the polypeptide; and optionally, (b) recovering the polypeptides and combining the first polypeptide and the second polypeptide.

[26]. A process for biofinishing a cellulose-containing textile, comprising contacting the cellulose-containing textile with the composition of any of paragraphs 1-15 or the recombinant host cell of paragraph 22.

[27]. The process of paragraph 26, wherein the cellulose-containing textile is cotton-containing textile or man-made cellulose-containing textile.

[28]. A process for biofinishing a cellulose-containing textile, comprising.

(a) treating the cellulose-containing textile with a first polypeptide having GH45 cellulase activity and biofinishing activity; and

(b) treating the cellulose-containing textile with a second polypeptide having GH45 cellulase activity and biofinishing activity.

[29]. The process of paragraph 28, wherein the first polypeptide and the second polypeptide exhibits synergy in an assay that measures biofinishing activity.

[30]. The process of paragraph 28 or 29, wherein the first polypeptide and the second polypeptide are endoglucanases or GH45 cellulase subfamily A members.

[31]. The process of any of paragraphs 28-30, wherein the first polypeptide is in an amount of from about 5% to 1000%, preferably, from about 20% to about 500%, more preferably, from about 30% to about 330%, even more preferably from about 40 to about 250%, by weight of the second polypeptide.

[32]. The process of any of paragraphs 28-31, further comprising a GH5 cellulase, GH6 cellulase and/or GH7 cellulase.

[33]. The process of any of paragraphs 28-32, wherein the first polypeptide or the second polypeptide is derived from the group consisting of *Staphylotrichum*, *Thielavia*, *Sordaria* or *Neurospora*.

[34]. The process of paragraph 33 wherein the first polypeptide or the second polypeptide is derived from the group consisting of *Staphylotrichum coccosporum*, *Thielavia terrestris, Sordaria fimicola or Neurospora tetrasperma.*

[35]. The process of any paragraphs 28-34, wherein the first polypeptide or the second polypeptide has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

[36]. The process of paragraphs 35, wherein the first polypeptide or the second polypeptide comprises or consists of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

[37]. The process of any of paragraphs 28-36, wherein the first polypeptide or the second polypeptide is encoded by a polynucleotide sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7.

[38]. A process for treating a cellulose-containing textile, comprising

(a) desizing;

(b) color modification;

wherein a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity are added before, during or after step (a) and step (b).

[39]. The process of paragraph 38, wherein the cellulose-containing textile is denim.

[40]. A process for treating a cellulose-containing textile, comprising

(a) desizing;

(b) scouring;

(c) bleaching;

(d) dyeing;

wherein a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity are added before, during or after step (a), (b), (c) or (d).

[41] The process of any paragraphs 20-34, wherein the biofinishing is biopolishing or biostoning.

[42]. Use of a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity for biofinishing a cellulose-containing textile.

[43]. A fermentation broth formulation or a cell composition comprising a first polypeptide having GH45 cellulase and biofinishing activity, and a second polypeptide having GH45 cellulase and biofinishing activity.

The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

Examples

Materials

Chemicals used as buffers and substrates were commercial products of at least reagent grade.

Media

1) pH 5.0 buffer with 50 mM acetate: 2.873 g sodium acetate and 0.901 g acetic acid dissolved in 1 L de-ionized water;

2) pH 6.5 buffer with 50 mM phosphate: 5.642 g disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and 5.344 g sodium dihydrogen phosphate dehydrate($NaH_2PO_4 \cdot 2H_2O$) dissolved in 1 L de-ionized water;

3) pH 7.5 buffer with 50 mM phosphate:15.045 g disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and 1.248 g sodium dihydrogen phosphate dehydrate($NaH_2PO_4 \cdot 2H2O$) dissolved in 1 L de-ionized water;

4) pH 8.5 buffer with 50 mM phosphate: 17.607 g disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and 0.116 g potassium dihydrogen phosphate (KH_2PO_4) dissolved in 1 L de-ionized water.

Enzymes

GH45-1: a *Staphylotrichum coccosporum* GH45 cellulase disclosed as mature polypeptide of SEQ ID NO: 3 in WO2005/054475, and as SEQ ID NO: 2 herein.

GH45-2: a *Thielavia terrestris* GH45 cellulase disclosed as the mature polypeptide of SEQ ID NO: 4 in WO 2012/089024 and as the mature polypeptide of SEQ ID NO: 4 herein .

GH45-3: a *Sordaria fimicola* GH45 cellulase disclosed as the mature polypeptide of SEQ ID NO: 2 in WO 2014/026630 and as the mature polypeptide of SEQ ID NO: 6 herein..

GH45-4: *Neurospora tetrasperma* GH45 cellulase disclosed in the mature polypeptide of SEQ ID NO: 2 in WO 2015/058700 and as the mature polypeptide of SEQ ID NO: 8 herein.

Fabrics

Cotton interlock: 40S, bleached, HM-A0008, available from HM Cotton, Guangzhou, China.

Method

Weight loss determination

The swatches were placed in a conditioned room (65%+/-5% humidity, 20+/-1°C) for 24 hours before they were numbered, weighed by an analytical balance (for samples below 100 g) or a precision balance (for samples over 100 g) and recorded. After treatment, all samples were tumbled dried for 1 hour and conditioned for 24 hours in the conditioned room mentioned as above. For each sample, the weight loss was defined as below:

$$Weight\ loss\ \% = \frac{(\text{weight before treatment} - \text{weight after treatment}) * 100}{\text{weight before treatment}}$$

Pilling notes test

Fabrics including treated and untreated were pre-conditioned in norm climate (65% humidity, 21°C) for at least 24 hours and then tested for the pilling notes with Nu-Martindale Tester (James H. Heal Co. Ltd, England), with untreated fabrics of the same type as the abraded fabrics on the bottom. A standard pilling test (Swiss Norm (SN) 198525) was carried out after 2000 Revolutions by marking from 1-5, with the meaning defined as below, where 1 shows poor anti-pilling and 5 shows excellent anti-pilling property. Thus the higher the Martindale pilling notes score the more effective the cellulase biopolishing treatment.

Note 5: No pilling
Note 4: Slight Pilling
Note 3: Moderate Pilling
Note 2: Distinct Pilling
Note 1: Heavy Pilling

1/2, 1/4 notes are allowed

To make the test result more reliable, 3 separate readings were carried out by different persons for each sample, and the average of the 3 readings was adopted as the final result of pilling notes.

Protein Content

The enzyme protein in an enzyme product can be measured with BCA™ Protein Assay Kit (product number 23225, commercial available from Thermo Fisher Scientific Inc.) according to the product manual.

Example 1: Biopolishing with GH45-1 and GH45-2 in Launder-O-meter

Cellulases GH45-1 and GH45-2 were tested on their own and in blending for biopolishing performance in Launder-O-meter (LOM).

Cotton fabric swatches were cut into about 16 cm * 16 cm (about 5 grams each). The swatches were placed in a conditioned room (65% humidity, 21°C) for 24 hours before they were numbered, weighed by an analytical balance and recorded. The biopolishing was conducted with a LOM. Two conditioned swatches were put into each beaker. 20 big steel balls (total eight of 220 grams) or 4 rubber balls (total weight of 5 grams) were placed in each beaker to supply high and low level of mechanical actions, respectively. The beaker was filled with enzymes according to Table 1 and buffers prepared as described in media part to a total volume of around 100 ml, which could get a liquid to fabric ratio of about 10:1 (v/w).

The LOM machine was started after the required program was chosen, and it would hold when the temperature reached the pre-set temperature, e.g. 55°C. Each beaker was fitted with a lid lined with 2 neoprin gaskets and closed tightly with a metal clamping device. The beakers were loaded into the preheated LOM. Metal racks were used to accommodate and secure 5 beakers, in the vertical position, in each of the 4 drum positions. The LOM lid was closed and the washing program was continued and the timing was initiated. 1 hour later, all beakers were removed from LOM and the denim samples were transferred to an inactivation solution (2g/L sodium carbonate) at 80°C for 10 minutes. Then the swatches were rinsed in hot water for 2 times and in cold water for 2 times and they were tumble-dried (AEG, LAVATHERM 37700, Germany) for 1 hour, conditioned for 24 hours at 65% relative humidity, 21°C prior to evaluation in weight loss and pilling notes.

As summarized in Table 1, it is evident that the blendings of GH45-1 and GH45-2 can deliver stronger biopolishing performance than any one of these two on their own, *i.e.* the two GH45 can work in a synergetic way: at high mechanical aids with 20 steel balls, when applied alone, 0.049 mg/g GH45-1 and 0.038 mg/g GH45-2 as 100% on their own delivered 3.8 and 3.6 in pilling notes, respectively; while when these two products were applied together at the ratio 50:50 or 25:75, the blendings can delivered pilling notes 4.5, which was 0.7-0.9 higher than they were applied on their own; at low mechanical aids with 4 rubber balls, when applied alone, 0.049 mg/g GH45-1 and 0.076 mg/g GH45-2 as 100% on their own delivered 1.5 and 3.5 in pilling notes, respectively, while when these two products were applied together at the ratio 95:5, 86:14, 67:33 and 40:60, the blendings can delivered pilling notes 3.3, 3.6, 3.5, 3.9, which

were much higher than GH45-1 alone and similar or stronger than GH45-2 alone. To obtain a same benefits in pilling notes, the risk in weight loss was also obviously reduced with the blendings with different ratios.

Table 1 Biopolishing in LOM at pH 6.5, 55 °C, 1 h

Enzyme dosage(mg protein/g fabric)		Ratio	Mechanical aids	Weight loss(%)	Pilling notes
GH45-2	GH45-1				
0.038		100% GH45-2	20 steel balls in each beaker	2.9	3.6
	0.049	100% GH45-1		1.7	3.8
0.019	0.025	50% GH45-1 50% GH45-2		2.8	4.5
0.029	0.012	25% GH45-1 75% GH45-2		2.9	4.5
0.076		100% GH45-2	4 rubber balls in each beaker	2.5	3.5
	0.049	100% GH45-1		1.3	1.5
0.004	0.047	95% GH45-1 5% GH45-2		1.4	3.3
0.011	0.042	86% GH45-1 14% GH45-2		1.7	3.6
0.025	0.033	67% GH45-1 33% GH45-2		2.0	3.5
0.046	0.020	40% GH45-1 60% GH45-2		2.3	3.9

Example 2: Biopolishing with GH45-2 and GH45-3 in Launder-O-meter

Cellulases GH45-2 and GH45-3 were tested on their own and in blending for biopolishing performance in LOM with the same protocol and procedure as in Example 1 except that the biopolishing trials were conducted at 45 °C and 55 °C, respectively.

From the results as summarized in Table 2, it can be seen that GH45-2 and GH45-3 also had synergetic effects when they were applied together. For example, at 45 °C, when applied alone, 0.146 mg/g GH45-2 and 0.115 mg/g GH45-3 as 100% on their own delivered 4.0 in pilling notes, respectively; while when these two products were applied together at the ratio 80:20 or 60:40, the blendings can delivered pilling notes 4.4, which was 0.4 higher than they were applied on their own. At 55 °C similar synergetic effects between these two GH45s were observed. Meanwhile the blendings of GH45-2 and GH45-3 delivered more consistent performance when temperature changed from 45 °C to 55 °C.

Table 2 LOM biopolishing at pH 6.5, 45/55 °C, 1 h, 20 steel balls in each beaker

Enzyme dosage (mg protein/g fabric)		Ratio	45 °C		55 °C	
GH45-2	GH45-3		Weight loss(%)	Pilling notes	Weight loss(%)	Pilling notes
	0.115	100% GH45-3	2.3	4.0	1.2	3.5
0.0432	0.023	80% GH45-2 20% GH45-3	2.3	4.4	2.2	4.5
0.0324	0.046	60% GH45-2 40% GH45-3	2.3	4.4	2.4	4.3
0.0540		100% GH45-2	1.9	4.0	2.2	4.3

Example 3: Biopolishing with GH45-1 and GH45-3 in Launder-O-meter

Cellulase GH45-1 and GH45-3 were tested on their own and in blending for biopolishing performance in LOM with the same protocol and procedure as in Example 1 except that the biopolishing trials were conducted at 35 °C.

As summarized in Table 3, at 35 °C, 0.114 mg/g GH45-1 and 0.0805 mg/g GH45-3, were set as 100% base for blending, respectively. GH45-1/GH45-3 blending with all the blending ratios (90:10, 75:26, 50:50, 25:76, 10:90 for GH45-1/GH45-3) delivered better anti-pilling performance than GH45-1 alone or GH45-3 alone.

Table 3 LOM biopolishing at pH 6.5, 35 °C, 1 h

Enzyme dosage (mg protein/g fabric)		Ratio	Mechanical aids	Weight loss(%)	Pilling notes
GH45-3	GH45-1				
0.0805		100% GH45-3	20 steel balls in each beaker	1.3	3.5
	0.114	100% GH45-1		1.0	3.1
0.00805	0.103	90% GH45-1 10% GH45-3		1.0	3.8
0.0207	0.0855	75% GH45-1 26% GH45-3		1.3	3.7
0.0403	0.0570	50% GH45-1 50% GH45-3		1.3	3.9
0.0610	0.0285	25% GH45-1 76% GH45-3		1.5	3.7
0.0725	0.0114	10% GH45-1 90% GH45-3		1.4	4.2

Example 4: Biopolishing with GH45-2 and GH45-4 in Launder-O-meter

Similar to Example 3, biopolishing trials were conducted to compare cellulases GH45-2 and GH45-4 on their own and in blending.

As summarized in Table 4, at 55 °C, 0.0394 mg/g GH45-2 and 0.0378 mg/g GH45-4 as 100% on their own delivered pilling notes at 3.8 and 2.9, respectively, while the blendings of GH45-2 and GH45-4 at different ratios (30:70, 52:50, 70:30 for GH45-2/GH45-4) can deliver similar performance to GH45-2 and much better performance than GH45-4 alone, which also suggested that these two GH45s can work in a synergetic way.

Table 4 LOM biopolishing at pH 6.5, 55 °C,1 h, 20 steel balls in each beaker

Enzyme dosage (mg protein/g fabric)		Ratio	Weight loss(%)	Pilling notes
GH45-2	GH45-4			
0.0394		100% GH45-2	2.3	3.8
	0.0378	100% GH45-4	1.7	2.9
0.0117	0.0265	30% GH45-2 70% GH45-4	2.2	3.6
0.0204	0.0189	52% GH45-2 50% GH45-4	2.5	3.9
0.0277	0.0113	70% GH45-2 30% GH45-4	2.0	3.5

Example 5: Biopolishing with GH45-1 and GH45-4 in Launder-O-meter

Similar to Example 4, biopolishing trials were conducted to compare cellulases GH45-1 and GH45-4 on their own and in blending.

As summarized in Table 5, at 35 °C, 0.114 mg/g GH45-1 and 0.0945 mg/g GH45-4 as 100% on their own delivered pilling notes at 3.1 and 4.3, respectively. When 25%, 50%, 75% and 90% GH45-1 was replaced with the corresponding percentages of GH45-4, the resulting blendings delivered pilling notes in the range of 4.1 to 4.3, which indicated the synergetic effects between these two cellulases.

Table 5 LOM biopolishing at pH 6.5, 35 °C, 1 h, 20 steel balls in each beaker

Enzyme dosage (mg protein/g fabric)		Ratio	Weight loss(%)	Pilling notes
GH45-1	GH45-4			
0.114		100% GH45-1	1.0	3.1
	0.0945	100% GH45-4	2.2	4.3
0.0855	0.0236	75% GH45-1 25% GH45-4	2.0	4.2
0.0570	0.0473	50% GH45-1 50% GH45-4	1.9	4.3
0.0285	0.0709	25% GH45-1 75% GH45-4	2.4	4.3

0.0114	0.0851	10% GH45-1 90% GH45-4	2.3	4.1

Example 6: Biopolishing with GH45-2 and GH45-3 in a jet dyer

Celluases GH45-2 and GH45-3 which had been tested in Example 2, were also tested in a production machine in a textile mill.

In each trial, about 20 kg 100% cotton fabrics which had been scoured and bleached by the mill were loaded into a dyeing machine (Shuangxi ECO Jet). The winch speed and pump pressure were adjusted to make sure the fabric circulate once every 55 seconds and ran smoothly in the machine; 200 L water was loaded in each step to keep the liquor ratio of about 10: 1 (water volume/fabric weight). In each trial, the fabric was treated as followed:

1) Pre-washed with water at room temperature for 10 min;

2) Drained;

3) Main-washed with a cellulase bath at 35 °C or 55 °C, pH 6-6.5 adjusted with acetic acid, initiated timing when cellulase was loaded into the bath and collected the fabrics after 45, 60, 75, 90 and 105 min;

4) Drained;

5) Rinsed with water twice;

6) Centrifuged and dried.

The fabrics collected in the process were sent for further evaluation for fuzz level and pilling notes (after Nu-Martindale treatment).

From Table 6 it can be seen that the two cellulases works in a synergetic way: 0.244 mg/g GH45-2 at 55 °C delivered similar biopolishing performance as 0.380 mg/g GH45-3 at 35 °C, while the blending of these two cellulases at 50:50 or 70: 30 for GH45-2/GH45-3 at 55 °C delivered a stronger and faster biopolishing than both on their own. In 60 min treatment, GH45-2 and GH45-3 delivered pilling notes 2.0 and 2.5, respectively; while the blendings at two ratios delivered 3.5 and 2.8, respectively. It can also been seen that a similar trend in fuzz level: the blendings delivered an equivalent fuzz level with 15-30 min shorter time than GH45-2 or GH45-3 on their own. It can further been seen that the blending with 50% GH45-2 and 50% GH45-3 delivered a stable biopolishing performance when the main washes were conducted at 35 °C or 55 °C.

Table 6 Biopolishing at pH 6-6.5, 35 °C or 55 °C in a jet dyer

Enzyme dosage (mg protein/g fabric)		**Ratio**	**Temperature (°C)**	**Pilling notes**				
GH45-2	GH45-3			45'	60'	75'	90'	105'
0.244		100% GH45-2	55	1.9	2.0	3.3	3.5	4.0
	0.380	100% GH45-3	35	2.0	2.5	2.8	3.4	3.9
0.121	0.192	50% GH45-2	55	2.3	3.5	3.4	3.6	3.9

		50% GH45-3						
0.121	0.192	50% GH45-2 50% GH45-3	35	2.1	3.5	3.3	3.4	3.9
0.171	0.115	70% GH45-2 30% GH45-3	55	2.1	2.8	3.0	3.9	4.1

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

CLAIMS

What is claimed is:

1. An enzyme composition comprising, a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polypeptide having GH45 cellulase activity and biofinishing activity.

2. The enzyme composition of claim 1, wherein the first polypeptide and the second polypeptide exhibits synergy in an assay that measures biofinishing activity.

3. The enzyme composition of claim 1 or 2, wherein the first polypeptide and the second polypeptide are endoglucanases.

4. The enzyme composition of any of claims 1-3, wherein the first polypeptide is in an amount of from about 5% to 1000%, preferably, from about 20% to about 500%, more preferably, from about 30% to about 330%, even more preferably from about 40 to about 250%, by weight of the second polypeptide.

5. The enzyme composition of any claims 1-4, wherein the first polypeptide or the second polypeptide has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

6. The enzyme composition of claim 5, wherein the first polypeptide or the second polypeptide comprises or consists of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

7. A nucleic acid construct or expression vector comprising a first polynucleotide encoding a first polypeptide having GH45 cellulase activity and biofinishing activity, and a second polynucleotide encoding a second polypeptide having GH45 cellulase activity and biofinishing activity, operably linked to one or more control sequences that direct the production of the polypeptides in an expression host.

8. A recombinant host cell comprising the nucleic acid construct or expression vector of claim 7.

9. A process for biofinishing a cellulose-containing textile, comprising contacting the cellulose-containing textile with the composition of any of claims 1-7 or the recombinant host cell of claim 8.

10. The process of claim 9, wherein the cellulose-containing textile is a cotton-containing textile or man-made cellulose-containing textile.

11. A process for biofinishing a cellulose-containing textile, comprising

(a) treating the cellulose-containing textile with a first polypeptide having GH45 cellulase activity and biofinishing activity; and

(b) treating the cellulose-containing textile with a second polypeptide having GH45 cellulase activity and biofinishing activity.

12. The process of claim 11, wherein the first polypeptide or the second polypeptide has at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6 or the mature polypeptide of SEQ ID NO: 8.

13. A process for treating a cellulose-containing textile, comprising

(a) desizing;

(b) color modification;

Wherein a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity are added before, during or after step (a) and step (b).

14. A process for treating a cellulose-containing textile, comprising

(a) desizing;

(b) scouring;

(c) bleaching;

(d) dyeing;

wherein a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity are added before, during or after step (a), (b), (c) or (d).

15. Use of a first polypeptide having GH45 cellulase activity and biofinishing activity and a second polypeptide having GH45 cellulase activity and biofinishing activity for biofinishing a cellulose-containing textile.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/086993

A. CLASSIFICATION OF SUBJECT MATTER

C12N 9/42(2006.01)i; C12N 15/56(2006.01)i; D06M 16/00(2006.01)i

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)

C12N 9/-; C12N 15/-; D06M 16/-

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

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

CNPAT, WPI, EPODOC, CNKI, PubMed, ISI Web of Knowledge, GenBank, GH45, glycoside hydrolase 45, family 45, cel45, cellulase, synergy, biofinishing, textile, fabric, cellulose, SEQ ID NOs:2,4,6,8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/010444 A2 (NOVOZYMES A/S ET AL.) 22 January 2009 (2009-01-22) specification, page 1, lines 18-27, page 4, lines 20-30, 34-35, example 1	1, 3, 15
X	WO 2012/106824 A1 (IOGEN BIO-PRODUCTS CORPORATION ET AL.) 16 August 2012 (2012-08-16) claims 1-23, paragraphs 0011-0017, tables 1-2	1-15
X	ANDERSEN, N. et al. "Hydrolysis of cellulose using mono-component enzymes shows synergy during hydrolysis of phosphoric acid swollen cellulose (PASC), but competition on Avicel." *ENZYME AND MICROBIAL TECHNOLOGY,* Vol. 42, 31 December 2008 (2008-12-31), pages 362-370	1-15
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A	WO 2012/089024 A1 (NOVOZYMES A/S ET AL.) 05 July 2012 (2012-07-05) the whole document	1-15

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2016	**22 September 2016**
Name and mailing address of the ISA/CN **STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China** Facsimile No. **(86-10)62019451**	Authorized officer **LI,Zidong** Telephone No. **(86-10)62414280**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 2007/057418 A1 (NOVOZYMES A/S ET AL.) 24 May 2007 (2007-05-24) the whole document	1-15
A	WO 96/17994 A1 (NOVONORDISK A/S ET AL.) 13 June 1996 (1996-06-13) the whole document	1-15

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2016/086993

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