(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 699 249 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
13.06.2001 Bulletin 2001/24

(21) Application number: **95913675.5**

(22) Date of filing: **14.03.1995**

(51) Int Cl.[7]: **D06M 16/00**, C11D 3/386, C11D 11/00

(86) International application number:
PCT/US95/03146

(87) International publication number:
WO 95/25841 (28.09.1995 Gazette 1995/41)

(54) **METHODS FOR TREATING NON-COTTON-CONTAINING FABRICS WITH CELLULASE**

VERFAHREN ZUR BEHANDLUNG VON BAUMWOLLEFREIEN GEWEBEN MIT CELLULASEN

PROCEDES POUR TRAITER AVEC DE LA CELLULASE DES TISSUS NE CONTENANT PAS DE COTON

(84) Designated Contracting States:
DE DK FR GB NL

(30) Priority: **18.03.1994 US 210520**

(43) Date of publication of application:
06.03.1996 Bulletin 1996/10

(73) Proprietor: **GENENCOR INTERNATIONAL, INC.**
Rochester, New York 14618 (US)

(72) Inventors:
- **CLARKSON, Kathleen A.,**
  Genencor International, Inc
  South San Francisco, CA 94080 (US)
- **LARENAS, Edmund,**
  Genencor International, Inc.
  South San Francisco, CA 94080 (US)
- **WEISS, Geoffrey, L., Genencor International, Inc.**
  South San Francisco, CA 94080 (US)

(74) Representative: **Kiddle, Simon John et al**
Mewburn Ellis,
York House,
23 Kingsway
London WC2B 6HP (GB)

(56) References cited:
WO-A-91/17243
WO-A-92/06183
WO-A-92/17574
WO-A-94/23113

- **TEXTILE RESEARCH JOURNAL, vol. 64, no. 5, May 1994 US, pages 270-279, XP 000441840 G. BUSCHLE-DILLER ET AL. 'Enzymatic Hydrolysis of Cotton, Linen, Ramie, and Viscose Rayon Fabrics'**
- **THE BIOCHEMICAL JOURNAL, vol. 260, no. 1, 15 May 1989 GREAT BRITAIN, pages 37-43, WOOD ET AL. 'The mechanism of fungal cellulase action'**
- **TEXTILE CHEMIST AND COLORIST, vol. 24, no. 6, June 1992 NORT CAROLINA US, pages 23-26, XP 000278729 R. MICHAEL TYNDALL 'Improving the Softness and Surface Appearance of Cotton Fabrics and Garments by Treatment with Cellulase Enzymes'**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 0 699 249 B1

Description

1. Field of the Invention.

[0001] The present invention is directed to methods for treating non-cotton containing cellulosic fabrics with cellulase as well as to the fabrics produced from these methods. In particular, the improved methods of the present invention are directed to contacting and non-cotton containing fabrics with an aqueous solution containing a fungal cellulase composition which comprises one or more EG type components and which contains low concentrations of CBH I type components. When the fabric and non-cotton containing cellulosic fabric are treated with such solutions, the resulting fabrics possess the expected enhancements in, for example, feel, appearance, and/or softening, etc., as compared to the fabric prior to treatment.

2. State of the Art.

[0002] During or shortly after their manufacture, cotton-containing fabrics can be treated with cellulase in order to impart desirable properties to the fabric. For example, in the textile industry, cellulase has been used to improve the feel and/or appearance of cotton-containing fabrics, to remove surface fibers from cotton-containing knits, for imparting a stone washed appearance to cotton-containing denims and the like.

[0003] In particular, Japanese Patent Application Nos. 58-36217 and 58-54032 as well as Ohishi et al., "Reformation of Cotton Fabric by Cellulase" and JTN December 1988 journal article "What's New -- Weight Loss Treatment to Soften the Touch of Cotton Fabric" each disclose that treatment of cotton-containing fabrics with cellulase results in an improved feel for the fabric. It is generally believed that this cellulase treatment removes cotton fuzzing and/or surface fibers which reduces the weight of the fabric. The combination of these effects imparts improved feel to the fabric, i. e., the fabric feels more like silk.

[0004] Additionally, it was heretofore known in the art to treat cotton-containing knitted fabrics with a cellulase solution under agitation and cascading conditions, for example, by use of a jet, for the purpose of removing broken fibers and threads common to these knitted fabrics. When so treated, buffers are generally not employed because they are believed to adversely affect dye shading with selected dyes.

[0005] It was still further heretofore known in the art to treat cotton-containing woven fabrics with a cellulase solution under agitation and cascading conditions. When so treated, the cotton-containing woven fabric possesses improved feel and appearance as compared to the fabric prior to treatment.

[0006] Lastly, it was also heretofore known that the treatment of cotton-containing dyed denim with cellulase solutions under agitating and cascading conditions, i.e., in a rotary drum washing machine, would impart a "stone washed" appearance to the denim.

[0007] A common problem associated with the treatment of such cotton-containing fabrics with a cellulase solution is that the treated fabrics exhibit significant strength loss as compared to the untreated fabric. Strength loss arises because the cellulase hydrolyzes cellulose (β-1,4-glucan linkages) which, in turn, can result in a breakdown of a portion of the cotton polymer. As more and more cotton polymers are disrupted (brokendown), the tensile strength of the fabric is reduced.

[0008] Because methods involving agitation and cascading of cellulase solutions over cotton woven fabrics require shorter reaction times, these methods are believed to provide cotton-containing woven fabrics of reduced strength loss as compared to cellulase treatment methods not involving agitation and cascading. In any event, such methods still nevertheless result in significant strength loss.

[0009] Accordingly, it would be particularly desirable to modify such cellulase treatment methods so as to provide reduced strength loss while still achieving the desired enhancements in the treated non-cotton-containing fabric arising from treatment with cellulase.

[0010] Additionally, because fungal sources of cellulase are known to secrete very large quantities of cellulase and further because fermentation procedures for such fungal sources as well as isolation and purification procedures for isolating the cellulase are well known in the art, it would be particularly advantageous to use such fungal cellulases in the methods for improving feel and/or appearance.

SUMMARY OF THE INVENTION

[0011] The present invention is directed to the discovery that heretofore known methods for treating non-cotton containing cellulosic fabrics with fungal cellulases can be improved by employing a fungal cellulase composition which comprises one or more EG type components and which contains sufficiently low concentrations of CBH I. Surprisingly, it has been found that EG type components are capable of imparting enhancements to the treated fabrics with regard to feel, appearance, softness, color enhancement, and/or a stone washed appearance as compared to fabrics before

treatment with such a cellulase composition. Additionally, it has been found that it is the CBH I type components in combination with the EG type components which account for a sizable portion of the strength loss in the treated fabric. Accordingly, in the present invention, the cellulase composition employed to treat non-cotton containing cellulosic fabrics is tailored so as to contain sufficiently low concentrations of CBH I type components so as to be strength loss resistant.

[0012] In view of the above, in one of its method aspects, the present invention is directed to a method for enhancing the feel and/or appearance and/or for providing color enhancement and/or a stone washed appearance to non-cotton containing cellulosic fabrics during manufacture of the fabric by treatment of the fabric with a composition comprising a complete fungal cellulase composition which comprises exo-cellobiohydrolase I type component(s) and endoglucanase type component(s), wherein the method comprises employing a composition comprising a fungal cellulase composition comprising one or more EG type components and one or more CBH I type components wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH I type components of greater than 5:1, and wherein said non-cotton containing cellulosic fabric comprises jute, flax, ramie, acetate derivatized cellulose or solvent-spun cellulosic fibres.

In a preferred embodiment, the fungal cellulase composition employed herein comprises one or more EG type components and one or more CBH type components wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH type components of greater than 5:1. In still another preferred embodiment, the fungal cellulase composition comprises at leat about 10 weight percent and preferably at least about 20 weight percent of EG components based on the total weight of protein in the cellulase composition.

[0013] In a preferred embodiment, the method is conducted with agitation of the cellulase composition under conditions so as to produce a cascading effect of the cellulase composition over the fabric.

[0014] Non-cotton containing cellulosic fabrics treated by the methods of this invention'have been found to exhibit the imparted enhancements with regard to feel, appearance, softness, color enhancement and/or stone washed appearance as compared to - untreated non-cotton containing cellulosic fabric.

[0015] In its composition aspects, the present invention is directed to non-cotton containing cellulosic fabrics treated in the methods of this invention as defined above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 is an outline of the construction of pΔCBHIpyr4.

[0017] FIG. 2 illustrates deletion of the Trichoderma longibrachiatum cbhI gene by integration of the larger EcoRI fragment from p△CBHIpyr4 at the cbhI locus on one of the Trichoderma longibrachiatum chromosomes.

[0018] FIG. 3 is an autoradiograph of DNA from a Trichoderma longibrachiatum strain GC69 transformed with EcoRI digested p△CBHIpyr4 after Southern blot analysis using a ^{32}P labelled p△CBHIpyr4 as the probe.

[0019] FIG. 4 is an autoradiograph of DNA from a Trichoderma longibrachiatum strain GC69 transformed with EcoRI digested pΔCBHIpyr4 after Southern blot analysis using a ^{32}P labelled pIntCBHI as the probe.

[0020] FIG. 5 is an isoelectro focusing gel displaying the proteins secreted by the wild type and by transformed strains of Trichoderma longibrachiatum. Specifically, in FIG. 5, Lane A of the isoelectrofocusing gel employs partially purified CBH I from Trichoderma longibrachiatum; Lane B employs protein from a wild type Trichoderma longibrachiatum; Lane C employs protein from a Trichoderma lonaibrachiatum strain with the cbhI gene deleted; and Lane D employs protein from a Trichoderma longibrachiatum strain with the cbhI and cbh2 genes deleted.

[0021] In FIG. 5, the right hand side of the figure is marked to indicate the location of the single proteins found in one or more of the secreted proteins. Specifically, BG refers to β -glucosidase; E1 refers to endoglucanase I; E2 refers to endoglucanase II; E3 refers to endoglucanase III; C1 refers to exo-cellobiohydrolase I; and C2 refers to exo-cellobiohydrolase II.

[0022] FIG. 6A is a representation of the Trichoderma longibrachiatum cbh2 locus cloned as a 4.1 kB EcoRI fragment of genomic DNA and FIG. 6B is a representation of the cbh2 gene deletion vector, pPΔCBHII.

[0023] FIG. 7 is an autoradiograph of DNA from a Trichoderma longibrachiatum strain P37PΔCBHI transformed with EcoRI digested pPΔCBHII after Southern blot analysis using a ^{32}P labelled pPΔCBHII as the probe.

[0024] FIG. 8 is a diagram of the plasmid pEGI pyr4.

[0025] FIG. 9 illustrates the RBB-CMC activity profile of an acidic EG enriched fungal cellulase composition (CBH I and II deleted) derived from Trichoderma longibrachiatum over a pH range at 40°C; as well as the activity profile of an enriched EG III cellulase composition derived from Trichoderma longibrachiatum over a pH range at 40°C.

[0026] FIG. 10 illustrates the softness panel test results for a non-cotton containing cellulosic fabric treated with an EG enriched cellulase composition derived from a strain of Trichoderma longibrachiatum genetically modified so as to be incapable of producing CBHI&II.

[0027] FIG. 11 illustrates an appearance panel test results for a non-cotton containing cellulosic fabric treated with an EG enriched cellulase composition derived from a strain of Trichoderma lonaibrachiatum genetically modified so

as to be incapable of producing CBHI&II.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0028] As noted above, the methods of this invention relate to methods for treating non-cotton containing cellulosic fabrics with cellulase. the fabric. However, prior to discussing this invention in detail, the following terms will first be defined.

[0029] The term "cotton-containing fabric" refers to sewn or unsewn fabrics made of pure cotton or cotton blends including cotton woven fabrics, cotton knits, cotton denims, cotton yarns and the like. When cotton blends are employed, the amount of cotton in the fabric should be at least about 40 percent by weight cotton; preferably, more than about 60 percent by weight cotton; and most preferably, more than about 75 percent by weight cotton. When employed as blends, the companion material employed in the fabric can include one or more non-cotton fibers including synthetic fibers such as polyamide fibers (for example, nylon 6 and nylon 66), acrylic fibers (for example, polyacrylonitrile fibers), and polyester fibers (for example, polyethylene terephthalate), polyvinyl alcohol fibers (for example, Vinylon), polyvinyl chloride fibers, polyvinylidene chloride fibers, polyurethane fibers, polyurea fibers and aramid fibers. It is contemplated that regenerated cellulose, such as rayon, could be used as a substitute for cotton in the methods of this invention.

[0030] The term "cellulosic-containing fabric" refers to any non-cotton containing cellulosic fabric or non-cotton containing cellulosic blend including natural cellulosics (such as jute, flax, ramie and the like) and manmade cellulosics. Other manmade cellulosics include chemical modification of cellulose fibers (e.g, cellulose derivatized by acetate and the like) and solvent-spun cellulose fibers (e.g.lyocell).

[0031] The above non-cotton containing cellulosics can also be employed as blends that include lyocell-rayon, lyocell-linen, viscose rayon acetate, rayon-wool, silk-acetate, and the like.

[0032] The term "finishing" as employed herein means the application of a sufficient amount of finish to a non-cotton containing cellulosic fabric so as to substantially prevent cellulolytic activity of the cellulase on the fabric. Finishes are generally applied at or near the end of the manufacturing process of the fabric for the purpose of enhancing the properties of the fabric, for example, softness, drapability, etc., which additionally protects the fabric from reaction with cellulases. Finishes useful for finishing a non-cotton-containing fabric are well known in the art and include resinous materials, such as melamine, glyoxal, or ureaformaldehyde, as well as waxes, silicons, fluorochemicals and quaternaries. When so finished, the cotton-containing fabric is substantially less reactive to cellulase.

[0033] The term "fungal cellulase" refers to the enzyme composition derived from fungal sources or microorganisms genetically modified so as to incorporate and express all or part of the cellulase genes obtained from a fungal source. Fungal cellulases act on cellulose and its derivatives to hydrolyze cellulose and give primary products, glucose and cellobiose. Fungal cellulases are distinguished from cellulases produced from non-fungal sources including microorganisms such as actinomycetes, gliding bacteria (myxobacteria) and true bacteria. Fungi capable of producing cellulases useful in preparing cellulase compositions described herein are disclosed in British Patent No. 2 094 826A.

[0034] Most fungal cellulases generally have their optimum activity in the acidic or neutral pH range although some fungal cellulases are known to possess significant activity under neutral and slightly alkaline conditions, i.e., for example, cellulase derived from Humicola insolens is known to have activity in neutral to slightly alkaline conditions.

[0035] Fungal cellulases are known to be comprised of several enzyme classifications having different substrate specificity, enzymatic action patterns, and the like. Additionally, enzyme components within each classification can exhibit different molecular weights, different degrees of glycosylation, different isoelectric points, different substrate specificity etc. For example, fungal cellulases can contain cellulase classifications which include endoglucanases (EGs), exo-cellobiohydrolases (CBHs), β-glucosidases (BGs), etc. On the other hand, while bacterial cellulases are reported in the literature as containing little or no CBH components, there are a few cases where CBH-like components derived from bacterial cellulases have been reported to possess exo-cellobiohydrolase activity.

[0036] A fungal cellulase composition produced by a naturally occurring fungal source and which comprises one or more CBH and EG components wherein each of these components is found at the ratio produced by the fungal source is sometimes referred to herein as a "complete fungal cellulase system" or a "complete fungal cellulase composition" to distinguish it from the classifications and components of cellulase isolated therefrom, from incomplete cellulase compositions produced by bacteria and some fungi, or from a cellulase composition obtained from a microorganism genetically modified so as to overproduce, underproduce, or not produce one or more of the CBH and/or EG components of cellulase.

[0037] The fermentation procedures for culturing fungi for production of cellulase are known per se in the art. For example, cellulase systems can be produced either by solid or submerged culture, including batch, fed-batch and continuous-flow processes. The collection and purification of the cellulase systems from the fermentation broth can also be effected by procedures known per se in the art.

[0038] "Endoglucanase ("EG") type components" refer to all of those fungal cellulase components or combination of components which exhibit textile activity properties similar to the endoglucanase components of Trichoderma Longi-

brachiatum. In this regard, the endoglucanase components of Trichoderma longibrachiatum (specifically, EG I, EG II, EG III, and the like either alone or in combination) impart improved feel, improved appearance, softening, color enhancement, and/or a stone washed appearance to cotton-containing fabrics (as compared to the fabric prior to treatment) when these components are incorporated into a textile treatment medium and the fabric is treated with this medium. Additionally, treatment of cotton-containing fabrics with endoglucanase components of Trichoderma longibrachiatum results in less strength loss as compared to the strength loss arising from treatment with a similar composition but which additionally contains CBH I type components.

[0039] Accordingly, endoglucanase type components are those fungal cellulase components which impart improved feel, improved appearance, softening, color enhancement, and/or a stone washed appearance to non-cotton-containing fabrics (as compared to the fabric before treatment) when these components are incorporated into a medium used to treat the fabrics and which impart reduced strength loss to non-cotton-containing fabrics as compared to the strength loss arising from treatment with a similar cellulase composition but which additionally contains CBH I type components.

[0040] Such endoglucanase type components may not include components traditionally classified as endoglucanases using activity tests such as the ability of the component (a) to hydrolyze soluble cellulose derivatives such as carboxymethylcellulose (CMC), thereby reducing the viscosity of CMC containing solutions, (b) to readily hydrolyze hydrated forms of cellulose such as phosphoric acid swollen cellulose (e.g., Walseth cellulose) and hydrolyze less readily the more highly crystalline forms of cellulose (e.g., Avicel, Solkafloc, etc.). On the other hand, it is believed that not all endoglucanase components, as defined by such activity tests, will impart one or more of the enhancements to non-cotton-containing fabrics as well as reduced strength loss to non-cotton-containing fabrics. Accordingly, it is more accurate for the purposes herein to define endoglucanase type components as those components of fungal cellulase which possess similar textile activity properties as possessed by the endoglucanase components of Trichoderma longibrachiatum.

[0041] Fungal cellulases can contain more than one EG type component. The different components generally have different isoelectric points, different molecular weights, different degrees of glycosylation, different substrate specificity, different enzymatic action patterns, etc. The different isoelectric points of the components allow for their separation via ion exchange chromatography and the like. In fact, the isolation of components from different fungal sources is known in the art. See, for example, WO93/22414, Schulein et al., International Application WO 89/09259, Wood et al., Biochemistry and Genetics of Cellulose Degradation, pp. 31 to 52 (1988); Wood et al., Carbohydrate Research, Vol. 190, pp. 279 to 297 (1989); Schulein, Methods in Enzymology, Vol. 160, pp. 234 to 242 (1988); and the like.

[0042] In general, it is contemplated that combinations of EG type components may give a synergistic response in imparting enhancements to the non-cotton containing fabrics as well as imparting reduced strength loss as compared to a single EG component. On the other hand, a single EG type component may be more stable or have a broader spectrum of activity over a range of pHs. Accordingly, the EG type components employed in this invention can be either a single EG type component or a combination of two or more EG type components. When a combination of components is employed, the EG type component may be derived from the same or different fungal sources.

[0043] It is contemplated that EG type components can be derived from bacterially derived cellulases.

[0044] "Exo-cellobiohydrolase type ("CBH type") components" refer to those fungal cellulase components which exhibit textile activity properties similar to CBH I and/or CBH II cellulase components of Trichoderma longibrachiatum. In this regard, when used in the absence of EG type cellulase components (as defined above), the CBH I and CBH II components of Trichoderma longibrachiatum alone do not impart any significant enhancements in feel, appearance, color enhancement and/or stone washed appearance to the so treated non-cotton-containing fabrics. Additionally, when used in combination with EG type components, the CBH I component of Trichoderma longibrachiatum imparts enhanced strength loss to the non-cotton-containing fabrics.

[0045] Accordingly, CBH I type components and CBH II type components refer to those fungal cellulase components which exhibit textile activity properties similar to CBH I and CBH II components of Trichoderma longibrachiatum, respectively. As noted above, for CBH I type components, this includes the property of enhancing strength loss of cotton-containing fabrics when used in the presence of EG type components. In a preferred embodiment and when used in combination with EG type components, the CBH I type components of Trichoderma longibrachiatum can impart an incremental cleaning benefit. Additionally, it is contemplated that the CBH I components of Trichoderma longibrachiatum, when used alone in or in combination with EG type components, can impart an incremental softening benefit.

[0046] Such exo-cellobiohydrolase type components could possibly not include components traditionally classed as exo-cellobiohydrolases using activity tests such as those used to characterize CBH I and CBH II from Trichoderma longibrachiatum. For example, such components (a) are competitively inhibited by cellobiose (K_i approximately 1mM); (b) are unable to hydrolyze to any significant degree substituted celluloses, such as carboxymethylcellulose, etc., and (c) hydrolyze phosphoric acid swollen cellulose and to a lesser degree highly crystalline cellulose. On the other hand, it is believed that some fungal cellulase components which are characterized as CBH components by such activity tests, will impart improved feel, appearance, softening, color enhancement, and/or a stone washed appearance to non-cotton-containing fabrics with minimal strength loss when used alone in the cellulase composition. Accordingly, it is

believed to be more accurate for the purposes herein to define such exo-cellobiohydrolases as EG type components because these components possess similar functional properties in textile uses as possessed by the endoglucanase components of Trichoderma longibrachiatum.

[0047] Fungal cellulase compositions having one or more EG type components and one or more CBH I type components wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH I type components of greater than 5:1 can be obtained by purification techniques. Specifically, the complete cellulase system can be purified into substantially pure components by recognized separation techniques well published in the literature, including ion exchange chromatography at a suitable pH, affinity chromatography, size exclusion and the like. For example, in ion exchange chromatography (usually anion exchange chromatography), it is possible to separate the cellulase components by eluting with a pH gradient, or a salt gradient, or both a pH and a salt gradient. After purification, the requisite amount of the desired components could be recombined.

[0048] It is also contemplated that mixtures of cellulase components having the requisite ratio of EG type components to CBH I type cellulase components could be prepared by means other than isolation and recombination of the components. In this regard, it may be possible to modify the fermentation conditions for a natural microorganism in order to give relatively high ratios of EG to CBH components. Likewise, recombinant techniques can alter the relative ratio of EG type components to CBH type components so as to produce a mixture of cellulase components having a relatively high ratio of EG type components to CBH type components.

[0049] In regard to the above, a preferred method for the preparation of cellulase compositions described herein is by genetically modifying a microorganism so as to overproduce one or more acidic EG type components. Likewise, it is also possible to genetically modify a microorganism so as to be incapable of producing one or more CBH type components which methods do not produce any heterologous protein. In such a case, a requisite amount of the cellulase produced by such modified microorganism could be combined with the cellulase produced by the natural microorganism (i.e., containing CBH I type components) so as to provide for a cellulase composition containing one or more EG type components and one or more CBH I type components wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH I type components of greater than 5:1.

[0050] In regard to the above, EP 0 551 394 A discloses methods for genetically engineering Trichoderma longibrachiatum so as to be incapable of producing one or more CBH components and/or overproducing one or more EG components. Moreover, the methods of that application create Trichoderma longibrachiatum strains which do not produce any heterologous proteins. Likewise, Miller et al., "Direct and Indirect Gene Replacement in Aspergillus nidulans", Molecular and Cellular Biology, p. 1714-1721 (1985) discloses methods for deleting genes in Aspergillus nidulans by DNA mediated transformation using a linear fragment of homologous DNA. The methods of Miller et al., would achieve gene deletion without producing any heterologous proteins.

[0051] In view of the above, the deletion of the genes responsible for producing CBH I type and/or CBH II type cellulase components would have the effect of enriching the amount of EG components present in the cellulase composition.

[0052] It is still further contemplated that fungal cellulase compositions can be used herein from fungal sources which produce low concentrations of CBH I type components.

[0053] Additionally, a requisite amount of one or more CBH I type components purified by conventional procedures can be added to a cellulase composition produced from a microorganism genetically engineered so as to be incapable of producing CBH I type components so as to achieve a specified ratio of EG type components to CBH I type components, i.e., a cellulase composition free of all CBH type components so as to be enriched in EG type components can be formulated to contain 2 weight percent of a CBH I type component (or CBH II type component) merely by adding this amount of a purified CBH I type component (or CBH II type component) to the cellulase composition.

[0054] "β-Glucosidase (BG) components" refer to those components of cellulase which exhibit BG activity; that is to say that such components will act from the non-reducing end of cellobiose and other soluble cellooligosaccharides ("cellobiose") and give glucose as the sole product. BG components do not adsorb onto or react with cellulose polymers. Furthermore, such BG components are competitively inhibited by glucose (K_i approximately 1mM). While in a strict sense, BG components are not literally cellulases because they cannot degrade cellulose, such BG components are included within the definition of the cellulase system because these enzymes facilitate the overall degradation of cellulose by further degrading the inhibitory cellulose degradation products (particularly cellobiose) produced by the combined action of CBH components and EG components. Without the presence of BG components, moderate or little hydrolysis of crystalline cellulose will occur. BG components are often characterized on aryl substrates such as p-nitrophenol B-D-glucoside (PNPG) and thus are often called aryl-glucosidases. It should be noted that not all aryl glucosidases are BG components, in that some do not hydrolyze cellobiose.

[0055] It is contemplated that the presence or absence of BG components in the cellulase composition can be used to regulate the activity of any CBH components in the composition. Specifically, because cellobiose is produced during cellulose degradation by CBH components, and because high concentrations of cellobiose are known to inhibit CBH activity, and further because such cellobiose is hydrolyzed to glucose by BG components, the absence of BG compo-

nents in the cellulase composition will "turn-off" CBH activity when the concentration of cellobiose reaches inhibitory levels. It is also contemplated that one or more additives (e.g., cellobiose, glucose, etc.) can be added to the cellulase composition to effectively "turn-off", directly or indirectly, some or all of the CBH I type activity as well as other CBH activity. When such additives are employed, the resulting composition is considered to be a composition suitable for use in this invention if the amount of additive employed is sufficient to lower the CBH I type activity to levels equal to or less than the CBH I type activity levels achieved by using the cellulase compositions described herein.

[0056] On the other hand, a cellulase composition containing added amounts of BG components may increase overall hydrolysis of cellulose if the level of cellobiose generated by the CBH components becomes restrictive of such overall hydrolysis in the absence of added BG components.

[0057] Methods to either increase or decrease the amount of BG components in the cellulase composition are disclosed in EP 0 562 003 A

[0058] Fungal cellulases can contain more than one BG component. The different components generally have different isoelectric points which allow for their separation via ion exchange chromatography and the like. Either a single BG component or a combination of BG components can be employed.

[0059] When employed in textile treatment solutions, the BG component is generally added in an amount sufficient to prevent inhibition by cellobiose of any CBH and EG components found in the cellulase composition. The amount of BG component added depends upon the amount of cellobiose produced in the textile composition which can be readily determined by the skilled artisan. However, when employed, the weight percent of BG component relative to any CBH type components present in the cellulase composition is preferably from about 0.2 to about 10 weight percent and more preferably, from about 0.5 to about 5 weight percent.

[0060] Preferred fungal cellulases for use in preparing the fungal cellulase compositions used in this invention are those obtained from Trichoderma longibrachiatum. Trichoderma koningii, Pencillum sp., Humicola insolens, and the like. Certain fungal cellulases are commercially available, i.e., CELLUCAST (available from Novo Industry, Copenhagen, Denmark), RAPIDASE (available from Gist Brocades, N.V., Delft, Holland), CYTOLASE 123 (available from Genencor International, South San Francisco, California) and the like. Other fungal cellulases can be readily isolated by art recognized fermentation and isolation procedures.

[0061] The term "buffer" refers to art recognized acid/base reagents which stabilize the cellulase solution against undesired pH shifts during the cellulase treatment of the cotton-containing fabric. In this regard, it is art recognized that cellulase activity is pH dependent. That is to say that a specific cellulase composition will exhibit cellulolytic activity within a defined pH range with optimal cellulolytic activity generally being found within a small portion of this defined range. The specific pH range for cellulolytic activity will vary with each cellulase composition. As noted above, while most cellulases will exhibit cellulolytic activity within an acidic to neutral pH profile, there are some cellulase compositions which exhibit cellulolytic activity in an alkaline pH profile.

[0062] During cellulase treatment of the non-cotton containing cellulosic fabric, it is possible that the pH of the initial cellulase solution could be outside the range required for cellulase activity. It is further possible for the pH to change during treatment of the non-cotton containing cellulosic fabric, for example, by the generation of a reaction product which alters the pH of the solution. In either event, the pH of an unbuffered cellulase solution could be outside the range required for cellulolytic activity. When this occurs, undesired reduction or cessation of cellulolytic activity in the cellulase solution occurs. For example, if a cellulase having an acidic activity profile is employed in a neutral unbuffered aqueous solution, then the pH of the solution will result in lower cellulolytic activity and possibly in the cessation of cellulolytic activity. On the other hand, the use of a cellulase having a neutral or alkaline pH profile in a neutral unbuffered aqueous solution should initially provide significant cellulolytic activity.

[0063] In view of the above, the pH of the cellulase solution should be maintained within the range required for cellulolytic activity. One means of accomplishing this is by simply monitoring the pH of the system and adjusting the pH as required by the addition of either an acid or a base. However, in a preferred embodiment, the pH of the system is preferably maintained within the desired pH range by the use of a buffer in the cellulase solution. In general, a sufficient amount of buffer is employed so as to maintain the pH of the solution within the range wherein the employed cellulase exhibits activity. Insofar as different cellulase compositions have different pH ranges for exhibiting cellulase activity, the specific buffer employed is selected in relationship to the specific cellulase composition employed. The buffer(s) selected for use with the cellulase composition employed can be readily determined by the skilled artisan taking into account the pH range and optimum for the cellulase composition employed as well as the pH of the cellulase solution. Preferably, the buffer employed is one which is compatible with the cellulase composition and which will maintain the pH of the cellulase solution within the pH range required for optimal activity. Suitable buffers include sodium citrate, ammonium acetate, sodium acetate, disodium phosphate, and any other art recognized buffers.

[0064] The tensile strength of non-cotton-containing fabrics can be measured in a warp and fill direction which are at right angles to each other. Accordingly, the term "warp tensile strength" as used herein refers to the tensile strength of the non-cotton-containing fabric as measured along the length of the non-cotton-containing fabric whereas the term "fill tensile strength" refers to the tensile strength of the non-cotton-containing fabric as measured across the width of

the non-cotton-containing fabric. The tensile strength of the resulting non-cotton-containing fabric treated with a cellulase solution is compared to its tensile strength prior to treatment with the cellulase solution so as to determine the strength reducing effect of the treatment. If the tensile strength is reduced too much, the resulting non-cotton-containing fabric will easily tear and/or form holes. Accordingly, it is desirable to maintain a tensile strength (both warp and fill) after treatment which is at least about 50% of the tensile strength before treatment.

[0065] The tensile strength of non-cotton-containing fabrics is readily conducted following ASTM D1682 test methodology. Equipment suitable for testing the tensile strength of such fabrics include a Scott tester or an Instron tester, both of which are commercially available. In testing the tensile strength of non-cotton-containing fabrics which have been treated with cellulase solutions, care should be taken to prevent fabric shrinkage after treatment and before testing. Such shrinkage would'result in erroneous tensile strength data.

[0066] Enhancements to the non-cotton containing cellulosic fabric are achieved by those methods heretofore used. For example, cotton-containing fabrics having improved feel can be achieved as per Japanese Patent Application Nos. 58-36217 and 58-54032 as well as Ohishi et al., "Reformation of Cotton Fabric by Cellulase" and JTN December 1988 journal article "What's New -- Weight Loss Treatment to Soften the Touch of Cotton Fabric".

[0067] Similarly, methods for improving both the feel and appearance of non-cotton containing cellulosic fabric include contacting the fabric with an aqueous solution containing cellulase under conditions so that the solution is agitated and so that a cascading effect of the cellulase solution over the non-cotton containing cellulosic fabric is achieved. Such methods result in improved feel and appearance of the so treated non-cotton containing cellulosic fabric and are described in WO92/07134

[0068] Methods for the enhancement of cotton-containing knits are described in International Textile Bulletin, Dyeing/Printing/Finishing, pages 5 et seq., 2nd Quarter, 1990.

[0069] Likewise, methods for imparting a stone washed appearance to cotton-containing denims are described in U.S. Patent No. 4,832,864.

[0070] Other methods for enhancing cotton-containing fabrics by treatment with a cellulase composition are known in the art. Preferably, in such methods, the treatment of the cotton-containing fabric with cellulase is conducted prior to finishing the cotton-containing fabric.

[0071] The use of the cellulase compositions described herein result in fabric/color enhancement of stressed non-cotton containing cellulosic fabrics. Specifically, during the manufacture of non-cotton containing fabrics, the fabric can become stressed and when so stressed, it will contain broken and disordered fibers. Such fibers detrimentally impart a worn and dull appearance to the fabric. However, when treated in the method of this invention, the so stressed fabric is subject to fabric/color enhancement. This is believed to arise by removal of some of the broken and disordered fibers which has the effect of restoring the appearance of the fabric prior to becoming stressed.

[0072] Additionally, it is contemplated that by employing the cellulase composition described herein with pigment type dyed fabrics (e.g., denims), these cellulase compositions will cause less redeposition of dye on non-cotton containing cellulosic fabrics. It is also contemplated that these anti-redeposition properties can be enhanced for one or more specific EG type component(s) as compared to other components.

[0073] The fungal cellulase compositions described above are employed in an aqueous solution which contains cellulase and other optional ingredients including, for example, a buffer, a surfactant, a scouring agent, and the like. The concentration of the cellulase composition employed in this solution is generally a concentration sufficient for its intended purpose. That is to say that an amount of the cellulase composition is employed to provide the desired enhancement(s) to the non-cotton-containing fabric. The amount of the cellulase composition employed is also dependent on the equipment employed, the process parameters employed (the temperature of the cellulase solution, the exposure time to the cellulase solution, and the like), the cellulase activity (e.g., a cellulase solution will require a lower concentration of a more active cellulase composition as compared to a less active cellulase composition), and the like. The exact concentration of the cellulase composition can be readily determined by the skilled artisan based on the above factors as well as the desired effect. Preferably, the concentration of the cellulase composition in the cellulase solution employed herein is from about 0.01 gram/liter of cellulase solution to about 10.0 grams/liter of cellulase solution; and more preferably, from about 0.05 grams/liter of cellulase solution to about 2 gram/liter of cellulase solution. (The cellulase concentration recited above refers to the weight of total protein).

[0074] When a buffer is employed in the cellulase solution, the concentration of buffer in the aqueous cellulase solution is that which is sufficient to maintain the pH of the solution within the range wherein the employed cellulase exhibits activity which, in turn, depends on the nature of the cellulase employed. The exact concentration of buffer employed will depend on several factors which the skilled artisan can readily take into account. For example, in a preferred embodiment, the buffer as well as the buffer concentration are selected so as to maintain the pH of the cellulase solution within the pH range required for optimal cellulase activity. In general, buffer concentration in the cellulase solution is about 0.005 N and greater. Preferably, the concentration of the buffer in the cellulase solution is from about 0.01 to about 0.5 N, and more preferably, from about 0.05 to about 0.15 N. It is possible that increased buffer concentrations in the cellulase solution may enhance the rate of tensile strength loss of the treated fabric.

[0075] In addition to cellulase and a buffer, the cellulase solution can optionally contain a small amount of a surfactant, i.e., less than about 2 weight percent, and preferably from about 0.01 to about 2 weight percent. Suitable surfactants include any surfactant compatible with the cellulase and the fabric including, for example, anionic, non-ionic and ampholytic surfactants.

[0076] Suitable anionic surfactants for use herein include linear or branched alkylbenzenesulfonates; alkyl or alkenyl ether sulfates having linear or branched alkyl groups or alkenyl groups; alkyl or alkenyl sulfates; olefinsulfonates; alkanesulfonates and the like. Suitable counter ions for anionic surfactants include alkali metal ions such as sodium and potassium; alkaline earth metal ions such as calcium and magnesium; ammonium ion; and alkanolamines having 1 to 3 alkanol groups of carbon number 2 or 3.

[0077] Ampholytic surfactants include quaternary ammonium salt sulfonates, betaine-type ampholytic surfactants, and the like. Such ampholytic surfactants have both the positive and negative charged groups in the same molecule.

[0078] Nonionic surfactants generally comprise polyoxyalkylene ethers, as well as higher fatty acid alkanolamides or alkylene oxide adduct thereof, fatty acid glycerine monoesters, and the like. Mixtures of surfactants can also be employed.

[0079] The liquor ratios, i.e., the ratio of weight of cellulase solution to the weight of fabric, employed herein is generally an amount sufficient to achieve the desired enhancement in the non-cotton containing cellulosic fabric and is dependent upon the process used and the enhancement to be achieve. Preferably, the liquor ratios are generally from about 0.1:1 and greater, and more preferably greater than about 1:1 and even more preferably greater than about 10:1. Use of liquor ratios of greater than about 50:1 are usually not preferred from an economic viewpoint.

[0080] Reaction temperatures for cellulase treatment are governed by two competing factors. Firstly, higher temperatures generally correspond to enhanced reaction kinetics, i.e., faster reactions, which permit reduced reaction times as compared to reaction times required at lower temperatures. Accordingly, reaction temperatures are generally at least about 30°C and greater. Secondly, cellulase is a protein which loses activity beyond a given reaction temperature which temperature is dependent on the nature of the cellulase used. Thus, if the reaction temperature is permitted to go too high, then the cellulolytic activity is lost as a result of the denaturing of the cellulase. As a result, the maximum reaction temperatures employed herein are generally about 65°C. In view of the above, reaction temperatures are generally from about 30°C to about 65°C; preferably, from about 35°C to about 60°C; and more preferably, from about 35°C to about 50°C.

[0081] Reaction times are generally from about 0.1 hours to about 24 hours and, preferably, from about 0.25 hours to about 5 hours.

[0082] It is contemplated that non-cotton containing cellulosic fabrics treated with the above described methods using such cellulase compositions will also possess reduced strength loss as compared to the same non-cotton containing cellulosic fabric treated in the same manner with a complete fungal cellulase composition.

[0083] In a preferred embodiment, a concentrate can be prepared for use in the methods described herein. Such concentrates would contain concentrated amounts of the cellulase composition described above, buffer and surfactant, preferably in an aqueous solution. When so formulated, the concentrate can readily be diluted with water so as to quickly and accurately prepare cellulase solutions having the requisite concentration of these additives. Preferably, such concentrates will comprise from about 0.1 to about 20 weight percent of a cellulase composition described above (protein); from about 10 to about 50 weight percent buffer; from about 10 to about 50 weight percent surfactant; and from about 0 to 80 weight percent water. When aqueous concentrates are formulated, these concentrates can be diluted by factors of from about 2 to about 200 so as to arrive at the requisite concentration of the components in the cellulase solution. As is readily apparent, such concentrates will permit facile formulation of the cellulase solutions as well as permit feasible transportation of the concentration to the location where it will be used. The cellulase composition as described above can be added to the concentrate either in a liquid diluent, in granules, in emulsions, in gels, in pastes, and the like. Such forms are well known to the skilled artisan.

[0084] When a solid cellulase concentrate is employed, the cellulase composition is generally a granule, a powder, an agglomerate and the like. When granules are used, the granules are preferably formulated so as to contain a cellulase protecting agent. See, for instance, WO91/17235. Likewise, the granule can be formulated so as to contain materials to reduce the rate of dissolution of the granule into the wash medium. Such materials and granules are disclosed in WO92/13030

[0085] It is contemplated that the cellulase compositions described herein can additionally be used in a pre-wash and as a pre-soak either as a liquid or a spray. It is still further contemplated that the cellulase compositions described herein can also be used in home use as a stand alone composition suitable for enhancing color and appearance of fabrics. See, for example, U.S. Patent No. 4,738,682.

[0086] The following examples are offered to illustrate the present invention and should not be construed in any way as limiting its scope.

EXAMPLES

[0087] Examples 1-12 demonstrate the preparation of Trichoderma longibrachiatum genetically engineered so as to be incapable of producing one or more cellulase components or so as to overproduce specific cellulase components.

Example 1

Selection for pyr4 mutants of Trichoderma longibrachiatum

[0088] The pyr4 gene encodes orotidine-5'-monophosphate decarboxylase, an enzyme required for the biosynthesis of uridine. The toxic inhibitor 5-fluoroorotic acid (FOA) is incorporated into uridine by wild-type cells and thus poisons the cells. However, cells defective in the pyr4 gene are resistant to this inhibitor but require uridine for growth. It is, therefore, possible to select for pyr4 mutant strains using FOA. In practice, spores of Trichoderma longibrachiatum strain RL-P37 (Sheir-Neiss G. and Montenecourt, B. S., 1984, Appl. Microbiol. Biotechnol. 20:46-53) were spread on the surface of a solidified medium containing 2 mg/ml uridine and 1.2 mg/ml FOA. Spontaneous FOA-resistant colonies appeared within three to four days and it was possible to subsequently identify those FOA-resistant mutants which required uridine for growth. In order to identify those mutants which specifically had a defective pyr4 gene, protoplasts were generated and transformed with a plasmid containing a wild-type pyr4 gene (see Examples 3 and 4). Following transformation, protoplasts were plated on medium lacking uridine. Subsequent growth of transformed colonies demonstrated complementation of a defective pyr4 gene by the plasmid-borne pyr4 gene. In this way strain GC69 was identified as a pyr4 mutant of strain RL-P37.

Example 2

Preparation of CBHI Deletion Vector

[0089] A cbh1 gene encoding the CBHI protein was cloned from genomic DNA using strain RL-P37 by hybridization with an oligonucleotide probe designed on the basis of the published sequence for this gene using known probe synthesis methods (Shoemaker et al., "Molecular Cloning of Exo-cellobiohydrolase I Derived from Trichoderma longibrachiatum Strain L27", Bio/Technology 1, p. 691 (1983). The cbhI gene resides on a 6.5 kb PstI fragment and was inserted into PstI cut pUC4K (purchased from Pharmacia Inc., Piscataway, NJ) replacing the Kan gene of this vector. The resulting plasmid, pUC4K::cbhI was then cut with HindIII and the larger fragment of about 6 kb was isolated and religated to give pUC4K::cbhIΔH/H. This procedure removes the entire cbh1 coding sequence and approximately 1.2 kb upstream and 1.5 kb downstream of flanking DNA from either side of the original PstI fragment.

[0090] The Trichoderma longibrachiatum pyr4 gene was cloned as a 6.5 kb fragment of genomic DNA in pUC18 following the methods of Sanbrook et al., 1989, "Molecular Cloning, A Laboratory Manuel", 2nd Ed., Cold Springs Harbor Laboratory Press. The plasmid pUC4K::cbhIΔH/H was cut with HindIII and the ends were desphosphorylated with calf intestinal alkaline phosphatase. This end dephosphorylated DNA was ligated with the 6.5 kb HindIII fragment containing the Trichoderma longibrachiatum pyr4 gene to give pΔCBHIpyr4. See FIG. 1.

Example 3

Isolation of Protoplasts

[0091] Mycelium was obtained by inoculating 100 ml of YEG (0.5% yeast extract, 2% glucose) in a 500 ml flask with about 5 x 10^7 Trichoderma longibrachiatum GC69 spores (the pyr4 mutant strain). The flask was then incubated at 37°C with shaking for about 16 hours. The mycelium was harvested by centrifugation at 2,750 x g. The harvested mycelium was further washed in 1.2 M sorbitol solution and resuspended in 40 ml of Novozym^R 234 solution (which is the tradename for a multicomponent enzyme system containing 1,3-alpha-glucanase, 1,3-beta-glucanase, laminarinase, xylanase, chitinase and protease from Novo Biolabs, Danbury Ct.) containing 5 mg/ml Novozym^R 234; 5 mg/ml $MgSO_4.7H_2O$; 0.5 mg/ml bovine serum albumin; 1.2 M sorbitol. The protoplasts were removed from cellular debris by filtration through Miracloth (Calbiochem. Corp) and collected by centrifugation at 2,000 x g. The protoplasts were washed three times in 1.2 M sorbitol and once in 1.2 M sorbitol, 50 mM $CaCl_2$, centrifuged and resuspended. The protoplasts were finally resuspended at a density of 2 x 10^8 protoplasts per ml of 1.2 M sorbitol, 50 mM $CaCl_2$.

Example 4

Transformation of Fungal Protoplasts

[0092] 200 µl of the protoplast suspension prepared in Example 3 was added to 20 µl of EcoRI digested pΔCBHIpyr4 (prepared in Example 2) in TE buffer (10 mM Tris, pH 7.4; 1 mM EDTA) and 50 µl of a polyethylene glycol (PEG) solution containing 25% PEG 4000, 0.6 M KCl and 50 mM $CaCl_2$. This mixture was incubated on ice for 20 minutes. After this incubation period 2.0 ml of the above-identified PEG solution was added thereto, the solution was further mixed and incubated at room temperature for 5 minutes. After this second incubation, 4.0 ml of a solution containing 1.2 M sorbitol and 50 mM $CaCl_2$ was added thereto and this solution was further mixed. The protoplast solution was then immediately added to molten aliquots of Vogel's Medium N (3 grams sodium citrate, 5 grams KH_2PO_4, 2 grams NH_4NO_3, 0.2 grams $MgSO_4.7H_2O$, 0.1 gram $CaCl_2.2H_2O$, 5 µg α-biotin, 5 mg citric acid, 5 mg $ZnSO_4.7H_2O$, 1 mg $Fe(NH_4)_2.6H_2O$, 0.25 mg $CuSO_4.5H_2O$, 50 µg $MnSO_4.4H_2O$ per liter) containing an additional 1% glucose, 1.2 M sorbitol and 1% agarose. The protoplast/medium mixture was then poured onto a solid medium containing the same Vogel's medium as stated above. No uridine was present in the medium and therefore only transformed colonies were able to grow as a result of complementation of the pyr4 mutation of strain GC69 by the wild type pyr4 gene present in pΔCBHIpyr4. These colonies were subsequently transferred and stable transformants purified, on a solid Vogel's medium N containing as an additive, 1% glucose.

Example 5

Analysis of the Transformants

[0093] DNA was isolated from the transformants obtained in example 3 after they were grown in the liquid Vogel's medium N containing 1% glucose. These transformant DNA samples were further cut with a PstI restriction enzyme and subjected to agarose gel electrophoresis. The gel was then further blotted onto a Nytran membrane filter and hybridized with a ^{32}P labelled pΔCBHIpyr4 probe. The probe was selected to identify the native cbh1 gene as a 6.5 kb PstI fragment, the native pyr4 gene and any DNA sequences derived from the transforming DNA fragment. FIG. 2 outlines deletion of the Trichoderma longibrachiatum cbh1 gene by integration of the larger EcoR1 fragment from pΔCBHIpyr4 at the cbh1 locus on one of the Trichoderma longibrachiatum chromosomes.

[0094] The bands from the hybridization were visualized via autoradiography. The result of the autoradiograph is seen in Fig. 3. Five samples were run as described above, hence samples A, B, C, D, and E. Lane E is the untransformed strain GC69 and was used as a control in the present analysis. Lanes A-D represent transformants obtained from the methods described above. The numbers on the side of the autoradiograph represent the sizes of molecular weight markers. As can be seen from this autoradiograph, Lane D does not contain the 6.5 kb CBHI band, indicating that this gene has been totally deleted in the transformant. This cbh1 deleted strain is called P37PΔCBHI. The other transformants analyzed appear identical to the untransformed control strain. Presumably, this happened because the linear fragment from p△CBHIpyr4 integrated by a double cross-over at the native pyr4 locus to give a gene replacement event.

Example 6

[0095] The same procedure was used in this example as in Example 5, except that the probe used was changed to a ^{32}P labelled pIntCBHI probe. This probe is a pUC-type plasmid containing a 2 kb BglII fragment from the cbh1 locus within the region that was deleted in pUC4::cbhl△H/H. Two samples were run in this example including a control sample A, which is the untransformed strain GC69 and the transformant P37PΔCBHI, sample B. As can be seen in Fig. 4, sample A contained the cbh1 gene, as indicated by the band at 6.5 kb; however the transformant, sample B does not contain this 6.5 kb band and therefore does not contain the cbh1 gene.

Example 7

Protein Secretion by Strain P37PΔCBHI

[0096] Spores from the produced P37PΔCBHI strain were inoculated into 50 ml of a Trichoderma basal medium containing 1% glucose, 0.14% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.03% $MgSO_4$, 0.03% urea, 0.75% bactotryptone, 0.05% Tween 80, 0.000016% $CuSO_4.5H_2O$, 0.001% $FeSO_4.7H_2O$, 0.000128% $ZnSO_4.7H_2O$, 0.0000054% $Na_2MoO_4.2H_2O$, 0.0000007% MnCl.4H2O). The medium was incubated while shaking in a 250 ml flask at 37°C for about 48 hours. The resulting mycelium was collected by filtering through Miracloth (Calbiochem Corp.) and washed two or three times with 17 mM potassium phosphate. The mycelium was finally suspended in 17 mM potassium phosphate with 1 mM sopho-

rose and further incubated for 24 hours at 30°C while shaking. The supernatant was then collected from these cultures and the mycelium was discarded. Samples of the culture supernatant were analyzed by isoelectrofocusing using a Pharmacia Phastgel system and pH 3-9 precast gels according to the manufacturer's instructions. The gel was stained with silver stain to visualize the protein bands. The band corresponding to the cbh1 protein was absent from the sample derived from the strain P37PΔCBHI, as shown in Fig. 5. This isoelectric focusing gel shows various proteins in different supernatant cultures of Trichoderma longibrachiatum. Lane A is partially purified CBHI; Lane B is the supernatant from an untransformed Trichoderma longibrachiatum culture; Lane C is the supernatant from a strain deleted for the cbh1 gene produced according to the methods of the present invention. The position of various cellulase components are labelled. Since CBHI constitutes about 50% of the total extracellular protein, it is the major secreted protein and hence is the darkest band on the gel. This isoelectric focusing gel clearly shows depletion of the CBHI protein in the strain deleted for cbh1.

Example 8

Preparation of pPΔCBHII

[0097] The cbh2 gene of T longibrachiatum, encoding the CBHII protein, has been cloned as a 4.1 kb EcoRI fragment of genomic DNA which is shown diagrammatically in FIG. 6A (Chen et al., 1987, Biotechnology, 5:274-278). Using methods known in the art, a plasmid, pPΔCBHII (FIG. 6B), has been constructed in which a 3.2 kb central region of this clone between a HindIII site (at 74 bp 3' of the CBHII translation initiation site) and a ClaI site (at 265 bp 3' of the last codon of CBHII) has been removed and replaced by the Trichoderma longibrachiatum pyr4 gene.

[0098] Digestion of this plasmid with EcoRI will liberate a fragment having 0.7 kb of flanking DNA from the cbh2 locus at one end, 1.7 kb of flanking DNA from the cbh2 locus at the other end and the Trichoderma longibrachiatum pyr4 gene in the middle.

Example 9

Generation of a pyr4 mutant of P37PΔCBHI

[0099] Spores of the transformant (P37PΔCBHI) which was deleted for the cbhl gene were spread onto medium containing FOA. A pyr4 derivative of this transformant was subsequently obtained using the methods of Example 1. This pyr4 strain was designated P37P△CBHIPyr 26.

Example 10

Deletion of cbh2 gene in a strain previously deleted for cbhl

[0100] Protoplasts of strain P37PΔCBHIPyr 26 were generated and transformed with EcoRI digested pPΔCBHII according to the methods outlined in Examples 3 and 4.

[0101] Purified stable transformants were cultured in shake flasks as in Example 7 and the protein in the culture supernatants was examined by isoelectrofocusing. One transformant (designated P37PΔΔCBH67) was identified which did not produce any CBHII protein. Lane D of Figure 5 shows the supernatant from a strain deleted for both the cbh1 and cbh2 genes produced according to the methods of the present invention.

[0102] DNA was extracted from strain P37P△△CBH67, digested with EcoRI and Asp718, and subjected to agarose gel electrophoresis. The DNA from this gel was blotted to a membrane filter and hybridized with ^{32}P labelled pP△CBHII (Figure 7). Lane A of Figure 7 shows the hybridization pattern observed for DNA from an untransformed Trichoderma longibrachiatum strain. The 4.1 kb EcoRI fragment containing the wild-type cbh2 gene was observed. Lane B shows the hybridization pattern observed for strain P37PΔΔCBH67. The single 4.1 kb band has been eliminated and replaced by two bands of approximately 0.9 and 3.1 kb. This is the expected pattern if a single copy of the EcoRI fragment from pPΔCBHII had integrated precisely at the cbh2 locus.

[0103] The same DNA samples were also digested with EcoRI and Southern analysis was performed as above. In this example, the probe was ^{32}P labelled pIntCBHII. This plasmid contains a portion of the cbh2 gene coding sequence from within that segment of cbh2 DNA which was deleted in plasmid pPΔCBHII. No hybridization was seen with DNA from strain P37PΔΔCBH67 showing that the cbh2 gene was deleted and that no sequences derived from the pUC plasmid were present in this strain.

Example 11

Construction of pEGIpyr4

[0104] The Trichoderma longibrachiatum egl1 gene, which encodes EGI, has been cloned as a 4.2 kb HindIII fragment of genomic DNA from strain RL-P37 by hybridization with oligonucleotides synthesized according to the published sequence (Penttila et al., 1986, Gene 45:253-263). A 3.6 kb HindIII-BamHI fragment was taken from this clone and ligated with a 1.6 kb HindIII-BamHI fragment containing the Trichoderma longibrachiatum pyr4 gene and a pUC-based plasmid cut with HindIII to give the plasmid pEGIpyr4 (Figure 8). Digestion of pEGIpyr4 with HindIII would liberate a fragment of DNA containing only Trichoderma lonaibrachiatum genomic DNA (the egl1 and pyr4 genes) except for 24 bp of sequenced, synthetic DNA between the two genes and 6 bp of sequenced, synthetic DNA at one end (see Figure 8).

Example 12

Transformants of Trichoderma longibrachiatum containing pEGIpyr4

[0105] A pyr4 defective mutant of Trichoderma longibrachiatum strain RutC30 (Sheir-Neiss and Montenecourt, 1984, Appl. Microbiol. Biotechnol. 20:46-53) was obtained by the method outlined in Example 1. Protoplasts of this strain were transformed with undigested pEGIpyr4 and stable transformants were purified. Five of these transformants (designated EP2, EP4, EP5, EP6, EP11), as well as untransformed RutC30 were inoculated into 50 ml of YEG medium (yeast extract, 5 g/l; glucose, 20 g/l) in 250 ml shake flasks and cultured with shaking for 2 days at 28°C. The resulting mycelium was washed with sterile water and added to 50 ml of TSF medium (0.05M citrate-phosphate buffer, pH 5.0; Avicel microcrystalline cellulose, 10 g/1; KH_2PO_4, 2.0 g/l; $(NH_4)_2SO_4$, 1.4 g/l; proteose peptone, 1.0 g/l; Urea, 0.3 g/l; $MgSO_4.7H_2O$, 0.3 g/l; $CaCl_2$, 0.3 g/l; $FeSO_4.7H_2O$, 5.0 mg/l; $MnSO_4.H_2O$, 1.6 mg/l; $ZnSO_4$, 1.4 mg/l; $CoCl_2$, 2.0 mg/l; 0.1% Tween 80). These cultures were incubated with shaking for a further 4 days at 28°C. Samples of the supernatant were taken from these cultures and assays designed to measure the total amount of protein and of endoglucanase activity were performed as described below.

[0106] The endoglucanase assay relied on the release of soluble, dyed oligosaccharides from Remazol Brilliant Blue - carboxymethylcellulose (RBB-CMC, obtained from MegaZyme, North Rocks, NSW, Australia). The substrate was prepared by adding 2 g of dry RBB-CMC to 80 ml of just boiled deionized water with vigorous stirring. When cooled to room temperature, 5 ml of 2 M sodium acetate buffer (pH 4.8) was added and the pH adjusted to 4.5. The volume was finally adjusted to 100 ml with deionized water and sodium azide added to a final concentration of 0.02%. Aliquots of Trichoderma longibrachiatum culture supernatant or 0.1 M sodium acetate as a blank (10-20 µl) were placed in tubes, 250 µl of substrate was added and the tubes were incubated for 30 minutes at 37°C. The tubes were placed on ice for 10 minutes and 1 ml of cold precipitant (3.3 % sodium acetate, 0.4% zinc acetate, pH 5 with HCl, 76% ethanol) was then added. The tubes were vortexed and allowed to sit for 5 minutes before centrifuging for 3 minutes at approximately 13,000xg. The optical density was measured spectrophotometrically at a wavelength of 590-600 nm.

[0107] The protein assay used was the BCA (bicinchoninic acid) assay using reagents obtained from Pierce, Rockford, Illinois, USA. The standard was bovine serum albumin (BSA). BCA reagent was made by mixing 1 part of reagent B with 50 parts of reagent A. One ml of the BCA reagent was mixed with 50 µl of appropriately diluted BSA or Trichoderma longibrachiatum culture supernatant. Incubation was for 30 minutes at 37°C and the optical density was finally measured spectrophotometrically at a wavelength of 562 nm.

[0108] The results of the assays described above are shown in Table 1. It is clear that some of the transformants produced increased amounts of endoglucanase activity compared to untransformed strain RutC30. It is thought that the endoglucanases or exo-cellobiohydrolases produced by untransformed Trichoderma longibrachiatum constitute approximately 20% and 70% respectively of the total amount of protein secreted. Therefore a transformant such as EPS, which produces approximately four-fold more endoglucanase than strain RutC30, would be expected to secrete approximately equal amounts of endoglucanase type and exo-cellobiohydrolase type proteins.

[0109] The transformants described in this example were obtained using intact pEGIpyr4 and will contain DNA sequences integrated in the genome which were derived from the pUC plasmid. Prior to transformation it would be possible to digest pEGIpyr4 with HindIII and isolate the larger DNA fragment containing only Trichoderma longibrachiatum DNA. Transformation of Trichoderma longibrachiatum with this isolated fragment of DNA would allow isolation of transformants which overproduced EGI and contained no heterologous DNA sequences except for the two short pieces of synthetic DNA shown in Figure 8. It would also be possible to use pEGIpyr4 to transform a strain which was deleted for either the cbh1 gene, or the cbh2 gene, or for both genes. In this way a strain could be constructed which would overproduce EGI and produce either a limited range of, or no, exo-cellobiohydrolases.

[0110] The methods of example 12 could be used to produce Trichoderma longibrachiatum strains which would over-

produce any of the other endoglucanases normally produced by Trichoderma longibrachiatum (T. longibrachiatum).

Table 1

Secreted endoglucanase activity of T. longibrachiatum transformants			
STRAIN	ENDOGLUCANASE ACTIVITY (O.D. AT 590 nm)	PROTEIN (μg/ml)	ENDOGLUCANASE/ μG PROTEIN
RutC30	0.32	4.1	0.078
EP2	0.70	3.7	0.189
EP4	0.76	3.65	0.208
EP5	1.24	4.1	0.302
EP6	0.52	2.93	0.177
EP11	0.99	4.11	0.241

[0111] The above results are presented for the purpose of demonstrating the overproduction of the EGI component and not for the purpose of the extent of overproduction. In this regard, the extent of overproduction is expected to vary with each experiment.

[0112] Example 13 demonstrates the isolation of the components of Cytolase 123 Cellulase (a complete fungal cellulase composition obtained from richoderma longibrachiatum and available from Genencor International, Inc., South San Francisco, CA) via purification procedures.

Example 13

Purification of Cvtolase 123 Cellulase into Cellulase Components

[0113] CYTOLASE 123 cellulase was fractionated in the following manner. The normal distribution of cellulase components in this cellulase system is as follows:

CBH I	45-55 weight percent
CBH II	13-15 weight percent
EG I	11-13 weight percent
EG II	8-10 weight percent
EG III	1-4 weight percent
BG	0.5-1 weight percent.

[0114] The fractionation was done using columns containing the following resins: Sephadex G-25 gel filtration resin from Sigma Chemical Company (St. Louis, Mo), QA Trisacryl M anion exchange resin and SP Trisacryl M cation exchange resin from IBF Biotechnics (Savage, Md). CYTOLASE 123 cellulase, 0.5g, was desalted using a column of 3 liters of Sephadex G-25 gel filtration resin with 10 mM sodium phosphate buffer at pH 6.8. The desalted solution, was then loaded onto a column of 20 ml of QA Trisacryl M anion exchange resin. The fraction bound on this column contained CBH I and EG I. These components were separated by gradient elution using an aqueous gradient containing from 0 to about 500 mM sodium chloride. The fraction not bound on this column contained CBH II and EG II. These fractions were desalted using a column of Sephadex G-25 gel filtration resin equilibrated with 10 mM sodium citrate, pH 3.3. This solution, 200 ml, was then loaded onto a column of 20 ml of SP Trisacryl M cation exchange resin. CBH II and EG II were eluted separately using an aqueous gradient containing from 0 to about 200 mM sodium chloride.

[0115] Following procedures similar to that of Example 13 above, other cellulase systems which can be separated into their components include CELLUCAST (available from Novo Industry, Copenhagen, Denmark), RAPIDASE (available from Gist Brocades, N.V., Delft, Holland), and cellulase systems derived from Trichoderma koningii, Penicillum sp. and the like.

Example 14

Purification of EG III from Cytolase 123 Cellulase

[0116] Example 13 above demonstrated the isolation of several components from Cytolase 123 Cellulase. However, because EG III is present in very small quantities in Cytolase 123 Cellulase, the following procedures were employed

to isolate this component.

A. Large Scale Extraction of EG III Cellulase Enzyme

[0117] One hundred liters of cell free cellulase filtrate were heated to about 30°C. The heated material was made about 4% wt/vol PEG 8000 (polyethylene glycol, MW of about 8000) and about 10% wt/vol anhydrous sodium sulfate. The mixture formed a two phase liquid mixture. The phases were separated using an SA-1 disk stack centrifuge. The phases were analyzed using silver staining isoelectric focusing gels. Separation was obtained for EG III and xylanase. The recovered composition contained about 20 to 50 weight percent of EG III.

[0118] Regarding the above procedure, use of a polyethylene glycol having a molecular weight of less than about 8000 gave inadequate separation; whereas, use of polyethylene glycol having a molecular weight of greater than about 8000 resulted in the exclusion of desired enzymes in the recovered composition. With regard to the amount of sodium sulfate, sodium sulfate levels greater than about 10% wt/vol caused precipitation problems; whereas, sodium sulfate levels less than about 10% wt/vol gave poor separation or the solution remained in a single phase.

B. Purification of EG III Via Fractionation

[0119] The purification of EG III is conducted by fractionation from a complete fungal cellulase composition (CYTOLASE 123 cellulase, commercially available from Genencor International, South San Francisco, CA) which is produced by wild type Trichoderma longibrachiatum. Specifically, the fractionation is done using columns containing the following resins: Sephadex G-25 gel filtration resin from Sigma Chemical Company (St. Louis, Mo), QA Trisacryl M anion exchange resin and SP Trisacryl M cation exchange resin from IBF Biotechnics (Savage, Md). CYTOLASE 123 cellulase, 0.5g, is desalted using a column of 3 liters of Sephadex G-25 gel filtration resin with 10 mM sodium phosphate buffer at pH 6.8. The desalted solution, is then loaded onto a column of 20 ml of QA Trisacryl M anion exchange resin. The fraction bound on this column contained CBH I and EG I. The fraction not bound on this column contains CBH II, EG II and EG III. These fractions are desalted using a column of Sephadex G-25 gel filtration resin equilibrated with 10 mM sodium citrate, pH 4.5. This solution, 200 ml, is then loaded onto a column of 20 ml of SP Trisacryl M cation exchange resin. The EG III was eluted with 100 mL of an aqueous solution of 200 mM sodium chloride.

[0120] In order to enhance the efficiency of the isolation of EG III, it may be desirable to employ Trichoderma longibrachiatum genetically modified so as to be incapable of producing one or more of EG I, EG II, CBH I and/or CBH II. The absence of one or more of such components will necessarily lead to more efficient isolation of EG III.

[0121] Likewise, it may be desirable for the EG III compositions described above to be further purified to provide for substantially pure EG III compositions, i.e., compositions containing EG III at greater than about 80 weight percent of protein. For example, such a substantially pure EG III protein can be obtained by utilizing material obtained from procedure A in procedure B or vica versa. One particular method for further purifying EG III is by further fractionation of an EG III sample obtained in part b) of this Example 14. The further fraction was done on a FPLC system using a Mono-S-HR 5/5 column (available from Pharmacia LKB Biotechnology, Piscataway, NJ). The FPLC system consists of a liquid chromatography controller, 2 pumps, a dual path monitor, a fraction collector and a chart recorder (all of which are available from Pharmacia LKB Biotechnology, Piscataway, NJ). The fractionation was conducted by desalting 5 ml of the EG III sample prepared in part b) of this Example 14 with a 20 ml Sephadex G-25 column which had been previously equilibrated with 10 mM sodium citrate pH 4. The column was then eluted with 0-200 mM aqueous gradient of NaCl at a rate of 0.5 ml/minute with samples collected in 1 ml fractions. EG III was recovered in fractions 10 and 11 and was determined to be greater than 90% pure by SDS gel electrophoresis. EG III of this purity is suitable for determining the N-terminal amino acid sequence by known techniques.

[0122] Substantially pure EG III as well as EG I and EG II components purified in Example 13 above can be used singularly or in mixtures in the methods of this invention. These EG components have the following characteristics:

	MW	pI	pH optimum[1]
EG I	~47-49 kD	4.7	~5
EG II	-35 kD	5.5	~5
EG III	~25-28 kD	7.4	~5.5-6.0

1. pH optimum determined by RBB-CMC activity as per Example 15 below.

[0123] The use of a mixture of these components in the practice of this invention may give a synergistic response in improving softening, feel, appearance, etc., as compared to a single component. On the other hand, the use of a single component in the practice of this invention may be more stable or have a broader spectrum of activity over a

range of pHs. For instance, Example 15 below shows that EG III has considerable activity against RBB-CMC under alkaline conditions.

Example 15

Activity of Cellulase Compositions Over a pH Range

[0124] The following procedure was employed to determine the pH profiles of two different cellulase compositions. The first cellulase composition was a CBH I and II deleted cellulase composition prepared from Trichoderma longibrachiatum genetically modified in a manner similar to that described above so as to be unable to produce CBH I and CBH II components. Insofar as this cellulase composition does not contain CBH I and CBH II which generally comprise from about 58 to 70 percent of a cellulase composition derived from Trichoderma longibrachiatum, this cellulase composition is necessarily substantially free of CBH I type and CBH II type cellulase components and accordingly, is enriched in EG components, i.e., EG I, EG II, EG III and the like.

[0125] The second cellulase composition was an approximately 20 to 40% pure fraction of EG III isolated from a cellulase composition derived from Trichoderma longibrachiatum via purification methods similar to part b) of Example 14.

[0126] The activity of these cellulase compositions was determined at 40°C and the determinations were made using the following procedures.

[0127] Add 5 to 20 μl of an appropriate enzyme solution at a concentration sufficient to provide the requisite amount of enzyme in the final solution. Add 250 μl of 2 weight percent RBB-CMC (Remazol Brilliant Blue R-Carboxymethylcellulose -- commercially available from MegaZyme, 6 Altona Place, North Rocks, N.S.W. 2151, Australia) in 0.05M citrate/phosphate buffer at pH 4, 5, 5.5, 6, 6.5, 7, 7.5 and 8.

[0128] Vortex and incubate at 40°C for 30 minutes. Chill in an ice bath for 5 to 10 minutes. Add 1000 μl of methyl cellosolve containing 0.3M sodium acetate and 0.02M zinc acetate. Vortex and let sit for 5-10 minutes. Centrifuge and pour supernatant into cuvets. Measure the optical density (OD) of the solution in each cuvet at 590 nm. Higher levels of optical density correspond to higher levels of enzyme activity.

[0129] The results of this analysis are set forth in FIG. 9 which illustrates the relative activity of the CBH I and II deleted cellulase composition compared to the EG III cellulase composition. From this figure, it is seen that the cellulase composition deleted in CBH I and CBH II possesses optimum cellulolytic activity against RBB-CMC at near pH 5.5 and has some activity at alkaline pHs, i.e., at pHs from above 7 to 8. On the other hand, the cellulase composition enriched in EG III possesses optimum cellulolytic activity at pH 5.5 - 6 and possesses significant activity at alkaline pHs.

[0130] From the above example, one skilled in the art would merely need to adjust and maintain the pH of the aqueous textile composition so that the cellulase composition is active and preferably, possesses optimum activity. As noted above, such adjustments and maintenance may involve the use of a suitable buffer.

Example 16

Enhanced properties of non-cotton containing cellulosic fabrics

[0131] This example demonstrates the ability of EG cellulase composition to enhance appearance, softness and surface polishing of non-cotton containing cellulosic fabrics. A 200 kg Jet Dyer machine was used to evaluate the enhanced properties of the non-cotton containing cellulosic fabric TENCEL™. Approximately 10 kg of 100% TENCEL™ mid-weight woven fabric was loaded into the machine in rope form and sewn end-to-end. This process may be performed on greige or dyed fabric. The jet machine was filled with 150 - 200 liters of water (which represents approximately 15-20:1 liquor to fabric ratio) and heated to 120 - 1400F (500 - 600C). The pH was adjusted to 4.5 - 5.0 by the addition of 3.6 g/l (56%) acetic acid and 1.9 g/l (50%) sodium hydroxide. The sodium hydroxide was added slowly to a dilute acetic acid solution before putting into the machine. Next, 0.25 - 0.5 ml/l of a nonionic wetting agent (Triton X-100) was added to the liquor. The pH and temperature was checked to ensure that the pH was between 4.5 and 5.0, and the temperature was between 500 - 60°C. Next, 3 - 4 g/l of an enriched EG cellulase composition was added. The enriched EG cellulase composition comprised a cellulase composition free of all CBH type components, which composition is derived from Trichoderma longibrachiatum genetically engineered in the manner described above so as to be incapable of producing CBH I and II components and overproduces EG I.

[0132] After adding the enriched EG cellulase composition, the jet was run for 30 - 60 minutes. At the end of the cycle, 0.25 g/l soda ash was added to the liquor and run for 10 minutes. The liquor was dropped from the jet, then the jet was filled again with water and the fabric rinsed one more time. The fabric was removed from the jet and dried. Finally, a silicone-based finish was exhausted onto the fabric.

[0133] Swatches were analyzed for softness and surface appearance by evaluation in a preference test. Specifically,

four panelists were given their own set of swatches and asked to rate them with respect to softness and surface appearance. Softness was based on the softness criteria such as pliability of the whole fabric. Surface appearance was based on the amount of loose fibers or fuzz present on the fabric. Swatches were compared to a non-enzyme treated fabric control and in the measurement of softness, an additional control was included i.e. a fabric treated with a complete fungal cellulase composition. Scores were assigned to each swatch and the average score was tabulated from the four panelists. The highest score for softness and surface appearance was assigned the value 5.0. The lowest score for least soft and most fuzz was assigned the value 0. The results of this averaging are set forth in FIG. 10 and 11. Specifically, these results demonstrate that softness and surface appearance were both improved following EG cellulase treatment. Additionally, the surface appearance of the TENCEL™ fabric was maintained following 10 home launderings whereas the control fabrics' surface appearance declined substantially.

[0134] An additional comparison of the EG enriched cellulase composition treated TENCEL™ fabric was compared to whole cellulase treated TENCEL™ fabric (FIG. 10). In this example, swatches were analyzed for softness by evaluation in a preference test. Four panelists were given their own set of swatches and asked to rate them with respect to softness. Softness was based on the above-mentioned criteria and panel score scale. Swatches were compared to a whole cellulase treated fabric control. Scores were assigned to each swatch and an average score was tabulated from the four panelists. The results of this averaging are set forth in FIG. 10. Specifically, these results demonstrate that EG enriched cellulase treated TENCEL™ fabric was on average softer than the whole cellulase treated fabric control.

Claims

1. A method for enhancing the feel and/or appearance and/or for providing color enhancement and/or a stone washed appearance to non-cotton containing cellulosic fabrics during manufacture of the fabric by treatment of the fabric with a composition comprising a complete fungal cellulase composition which comprises exo-cellobiohydrolase I type component(s) and endoglucanase type component(s), wherein the method comprises employing a composition comprising a fungal cellulase composition comprising one or more EG type components and one or more CBH I type components wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH I type components of greater than 5:1, and wherein said non-cotton containing cellulosic fabric comprises jute, flax, ramie, acetate derivatized cellulose or solvent-spun cellulosic fibres.

2. The method according to claim 1 wherein said fungal cellulase composition comprises one or more EG type components and one or more CBH I type components, wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH type components of greater than 5:1.

3. The method according to claim 2 wherein said fungal cellulase composition has a protein weight ratio of all EG type components to all CBH type components of greater than 10:1.

4. The method according to any one of claims 1 to 3 wherein said fungal cellulase composition comprises at least about 20 weight percent EG type components based on the total weight of protein in the cellulase composition.

5. The method of any one of the preceding claims wherein said method is conducted with agitation of the cellulase composition under conditions so as to produce a cascading effect of the cellulase composition over the fabric.

6. A non-cotton containing cellulosic fabric prepared by the method of any one of the preceding claims.

7. Use of a composition comprising a fungal cellulase composition comprising one or more EG type components and one or more CBH I type components, wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH I type components of greater than 5:1, to enhance the feel and/or appearance and/or for providing color enhancement and/or a stone washed appearance to a non-cotton containing cellulosic fabric during manufacture of the fabric, wherein said non-cotton containing cellulosic fabric comprises jute, flax, ramie, acetate derivatized cellulose or solvent-spun cellulosic fibres.

8. The use of claim 7 wherein said fungal cellulase composition comprises one or more EG type components and one or more CBH I type components wherein said cellulase composition has a protein weight ratio of all EG type components to all CBH type components of greater than 5:1.

9. The use of claim 8 wherein said fungal cellulase composition has a protein weight ratio of all EG type components

to all CBH type components of greater than 10:1.

10. The use of any one of claims 7 to 9 wherein said fungal cellulase composition comprises at least about 20 weight percent EG type components based on the total weight of protein in the cellulase composition.

11. The use of any one of claims 7 to 10 wherein the fabric is contacted with the cellulase composition under conditions so as to produce a cascading effect of the cellulase composition over the fabric.

Patentansprüche

1. Verfahren zur Verbesserung des Griffs und/oder des Erscheinungsbildes und/oder zur Farbverstärkung und/oder zum Verleihen eines "Stonewashed"-Erscheinungsbildes von nicht-baumwollhältigen Cellulosegeweben während der Herstellung des Gewebes durch Behandlung des Gewebes mit einer Zusammensetzung, die eine komplette Pilz-Cellulase-Zusammensetzung umfasst, die eine oder mehrere Komponenten vom Exo-Cellobiohydrolase I-Typ und eine oder mehrere Komponenten vom Endoglucanase-Typ umfasst, worin das Verfahren den Einsatz einer Zusammensetzung umfasst, die eine Pilz-Cellulase-Zusammensetzung umfasst, die eine oder mehrere Komponenten vom EG-Typ und eine oder mehrere Komponenten von CBH I-Typ umfasst, worin die Cellulase-Zusammensetzung ein Protein-Gewichtsverhältnis zwischen allen Komponenten vom EG-Typ und allen Komponenten vom CBH I-Typ von über 5:1 aufweist, und worin das nicht-baumwollhältige Cellulosegewebe Jute-, Flachs-, Ramie-, Acetat-derivatisierte Cellulose- oder Solvent-gesponnene Cellulosefasern umfasst.

2. Verfahren nach Anspruch 1, worin die Pilz-Cellulase-Zusammensetzung eine oder mehrere Komponenten vom EG-Typ und eine oder mehrere Komponenten vom CBH I-Typ umfasst, worin die Cellulase-Zusammensetzung ein Protein-Gewichtsverhältnis zwischen allen Komponenten vom EG-Typ und allen Komponenten vom CBH-Typ von über 5:1 aufweist.

3. Verfahren nach Anspruch 2, worin die Pilz-Cellulase-Zusammensetzung ein Protein-Gewichtsverhältnis zwischen allen Komponenten vom EG-Typ und allen Komponenten vom CBH-Typ von über 10:1 aufweist.

4. Verfahren nach einem der Ansprüche 1 bis 3, worin die Pilz-Cellulase-Zusammensetzung zumindest etwa 20 Gew.-% Komponenten vom EG-Typ, bezogen auf das Gesamtgewicht an Protein in der Cellulasezusammensetzung, umfasst.

5. Verfahren nach einem der vorangegangenen Ansprüche, worin das Verfahren unter Bewegung der Cellulase-Zusammensetzung unter Bedingungen durchgeführt wird, um eine kaskadierende Wirkung der Cellulase-Zusammensetzung auf dem Gewebe hervorzurufen.

6. Nicht-baumwollhältiges Cellulosegewebe, hergestellt nach dem Verfahren nach einem der vorangegangenen Ansprüche.

7. Verwendung einer eine Pilz-Cellulase-Zusammensetzung umfassenden Zusammensetzung, die eine oder mehrere Komponenten vom EG-Typ und eine oder mehrere Komponenten vom CBH I-Typ umfasst, worin die Cellulase-Zusammensetzung ein Protein-Gewichtsverhältnis zwischen allen Komponenten vom EG-Typ und allen Komponenten vom CBH I-Typ von über 5:1 aufweist, um den Griff und/oder das Erscheinungsbild eines nicht-baumwollhältigen Cellulosegewebes während der Herstellung des Gewebes zu verbessern und/oder dessen Farbe zu verstärken und/oder ihm ein "Stone-washed"-Erscheinungsbild zu verleihen, worin das nicht-baumwollhältige Cellulosegewebe Jute-, Flachs-, Ramie-, Acetat-derivatisierte Cellulose- oder Solvent-gesponnene Cellulosefasern umfasst.

8. Verwendung nach Anspruch 7, worin die Pilz-Cellulase-Zusammensetzung eine oder mehrere Komponenten vom EG-Typ und eine oder mehrere Komponenten vom CBH I-Typ umfasst, worin die Cellulase-Zusammensetzung ein Protein-Gewichtsverhältnis zwischen allen Komponenten vom EG-Typ und allen Komponenten vom CBH-Typ von über 5:1 aufweist.

9. Verwendung nach Anspruch 8, worin die Pilz-Cellulase-Zusammensetzung ein Protein-Gewichtsverhältnis zwischen allen Komponenten vom EG-Typ und allen Komponenten von CBH-Typ von über 10:1 aufweist.

10. Verwendung nach einem der Ansprüche 7 bis 9, worin die Pilz-Cellulase-Zusammensetzung zumindest etwa 20 Gew.-% an Komponenten vom EG-Typ, bezogen auf das Gesamtgewicht von Protein in der Cellulasezusammensetzung, umfasst.

11. Verwendung nach einem der Ansprüche 7 bis 10, worin das Gewebe mit der Cellulase-Zusammensetzung unter solchen Bedingungen in Kontakt gebracht wird, dass eine kaskadierende Wirkung der Cellulase-Zusammensetzung auf dem Gewebe hervorgerufen wird.

Revendications

1. Méthode pour améliorer le toucher et/ou l'aspect et/ou pour produire un enrichissement des couleurs et/ou un aspect lavé à la pierre d'étoffes cellulosiques ne contenant pas de coton pendant la fabrication de l'étoffe, par traitement de l'étoffe avec une composition comprenant une composition de cellulase fongique complète qui comprend un ou des composants du type exocellobiohydrolase I et un ou des composants du type endoglucanase, où la méthode comprend l'emploi d'une composition comprenant une composition de cellulase fongique comprenant un ou plusieurs composants du type EG et un ou plusieurs composants du type CBH I où ladite composition de cellulase a un rapport pondéral des protéines de tous les composants du type EG à tous les composants du type CBH I de plus de 5:1, et où ladite étoffe cellulosique ne contenant pas de coton comprend des fibres de jute, de lin, de ramie, de cellulose dérivée à l'acétate ou cellulosiques filées au solvant.

2. Méthode selon la revendication 1 où ladite composition de cellulase fongique comprend un ou plusieurs composants du type EG et un ou plusieurs composants du type CBH I, où ladite composition de cellulase a un rapport pondéral des protéines de tous les composants du type EG à tous les composants du type CBH de plus de 5:1.

3. Méthode selon la revendication 2 où ladite composition de cellulase fongique a un rapport pondéral des protéines de tous les composants du type EG à tous les composants du type CBH de plus de 10:1.

4. Méthode selon l'une quelconque des revendications 1 à 3 où ladite composition de cellulase fongique comprend au moins environ 20 pour cent en poids de composants du type EG en se basant sur le poids total de la protéine dans la composition de cellulase.

5. Méthode selon l'une quelconque des revendications précédentes, où ladite méthode est entreprise avec agitation de la composition de cellulase dans des conditions afin de produire un effet de cascade de la composition de cellulase sur l'étoffe.

6. Fibre cellulosique ne contenant pas de coton, préparée par la méthode selon l'une quelconque des revendications précédentes.

7. Utilisation d'une composition comprenant une composition de cellulase fongique comprenant un ou plusieurs composants du type EG et un ou plusieurs composants du type CBHI, où ladite composition de cellulase a un rapport pondéral des protéines de tous les composants du type EG à tous les composants du type CBHI de plus de 5:1, pour améliorer le toucher et/ou l'aspect et/ou pour produire un enrichissement des couleurs et/ou un aspect lavé à la pierre d'une étoffe cellulosique ne contenant. pas de coton, pendant la fabrication de l'étoffe où ladite étoffe cellulosique ne contenant pas de coton comprend des fibres de jute, de lin, de ramie, de cellulose dérivée à l'acétate ou cellulosiques filées au solvant.

8. Utilisation de la revendication 7 où ladite composition de cellulase fongique comprend un ou plusieurs composants du type EG et un ou plusieurs composants du type CBH I, où ladite composition de cellulase a un rapport pondéral des protéines de tous les composants du type EG à tous les composants du type CBH de plus de 5:1.

9. Utilisation de la revendication 8 où ladite composition de cellulase fongique a un rapport pondéral des protéines de tous les composants du type EG à tous les composants du type CBH de plus de 10:1.

10. Utilisation selon l'une quelconque des revendications 7 à 9 où ladite composition de cellulase fongique comprend au moins environ 20 pour cent en poids de composants du type EG en se basant sur le poids total de la protéine dans la composition de cellulase.

11. Utilisation selon l'une quelconque des revendications 7 à 10 où l'étoffe est mise en contact avec la composition de cellulase dans des conditions afin de produire un effet de cascade de la composition de cellulase sur l'étoffe.

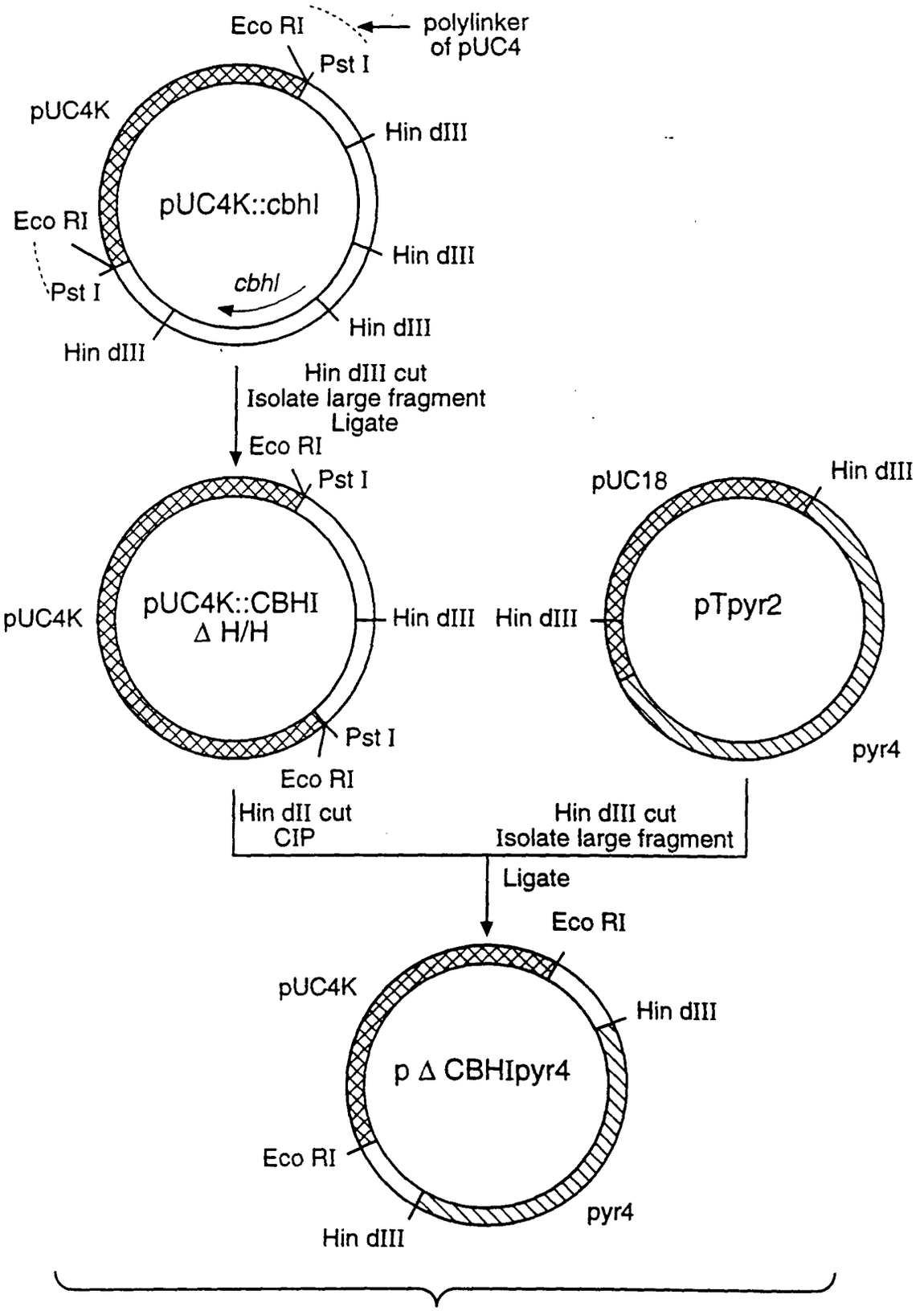
Eco RI
polylinker of pUC4
Pst I
pUC4K
Hin dIII
pUC4K::cbhl
Eco RI
Hin dIII
Pst I
cbhl
Hin dIII
Hin dIII
Hin dIII cut
Isolate large fragment
Ligate
Eco RI
Pst I
pUC4K
pUC4K::CBHI
Δ H/H
Hin dIII
Pst I
Eco RI
pUC18
Hin dIII
Hin dIII
pTpyr2
pyr4
Hin dII cut
CIP
Hin dIII cut
Isolate large fragment
Ligate
Eco RI
pUC4K
Hin dIII
p Δ CBHIpyr4
Eco RI
pyr4
Hin dIII

FIG._1

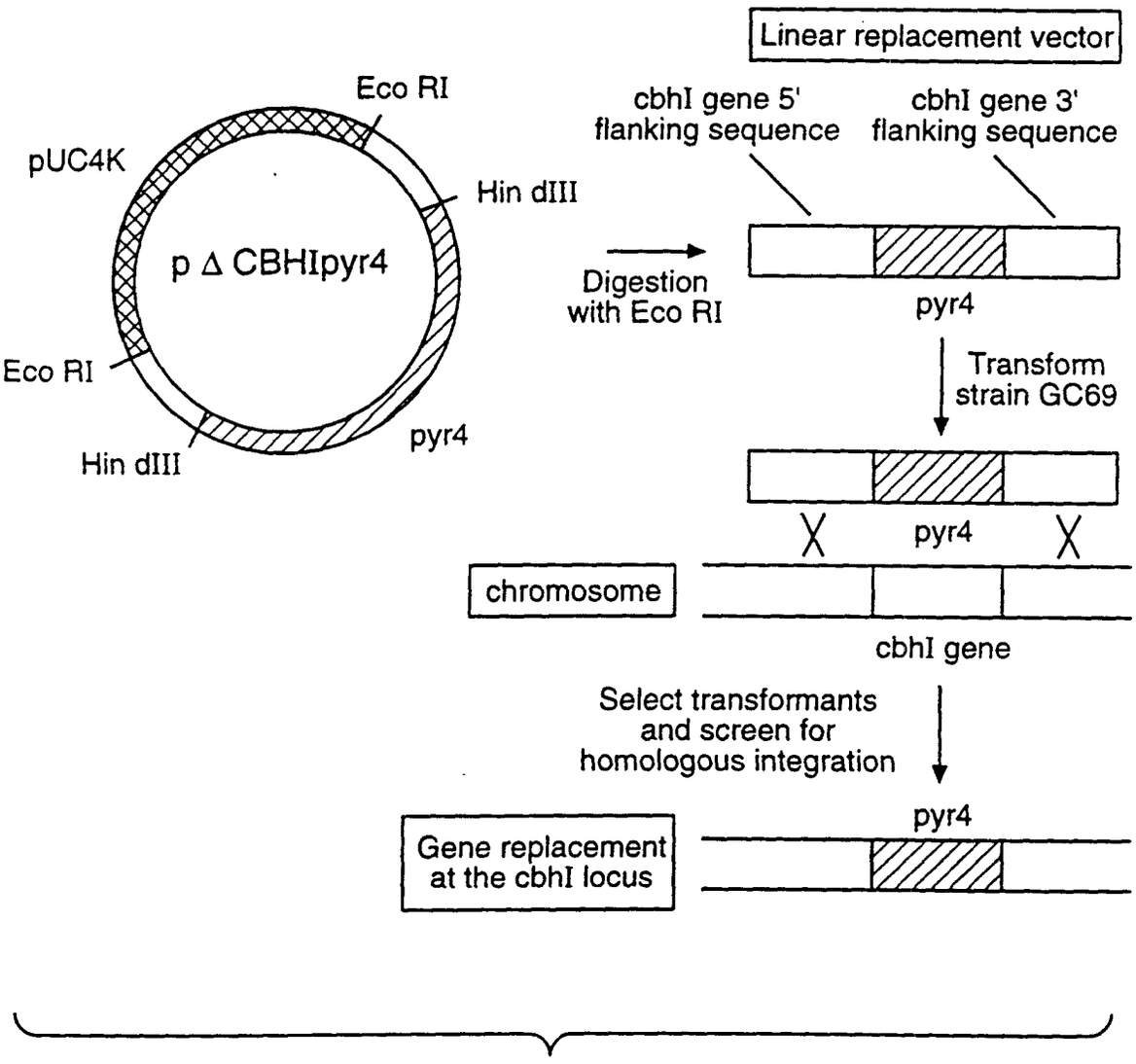
Linear replacement vector
Eco RI
pUC4K
Hin dIII
p Δ CBHIpyr4
Eco RI
Hin dIII
pyr4
cbhI gene 5' flanking sequence
cbhI gene 3' flanking sequence
Digestion with Eco RI
pyr4
Transform strain GC69
pyr4
chromosome
cbhI gene
Select transformants and screen for homologous integration
pyr4
Gene replacement at the cbhI locus

FIG._2

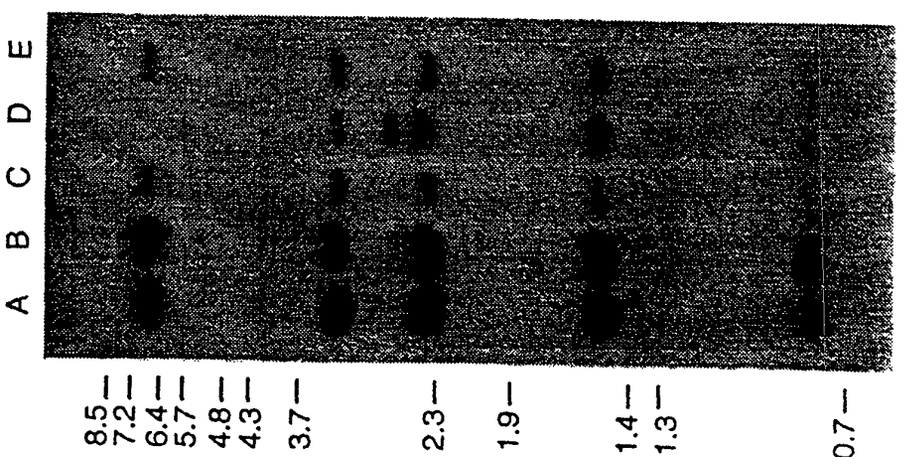

FIG._3

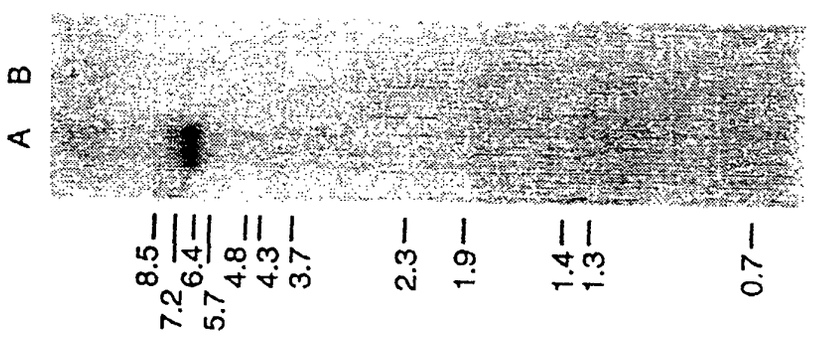

FIG._4

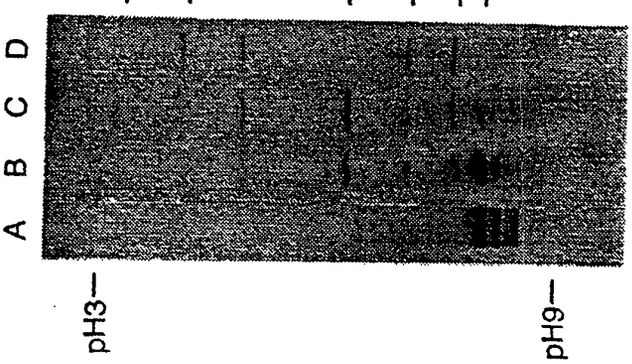

FIG._5

FIG._7

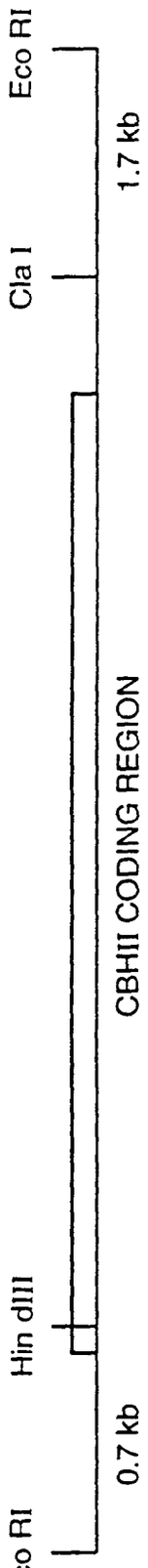
Eco RI
Hin dIII
Cla I
Eco RI
0.7 kb
CBHII CODING REGION
1.7 kb

FIG._6A

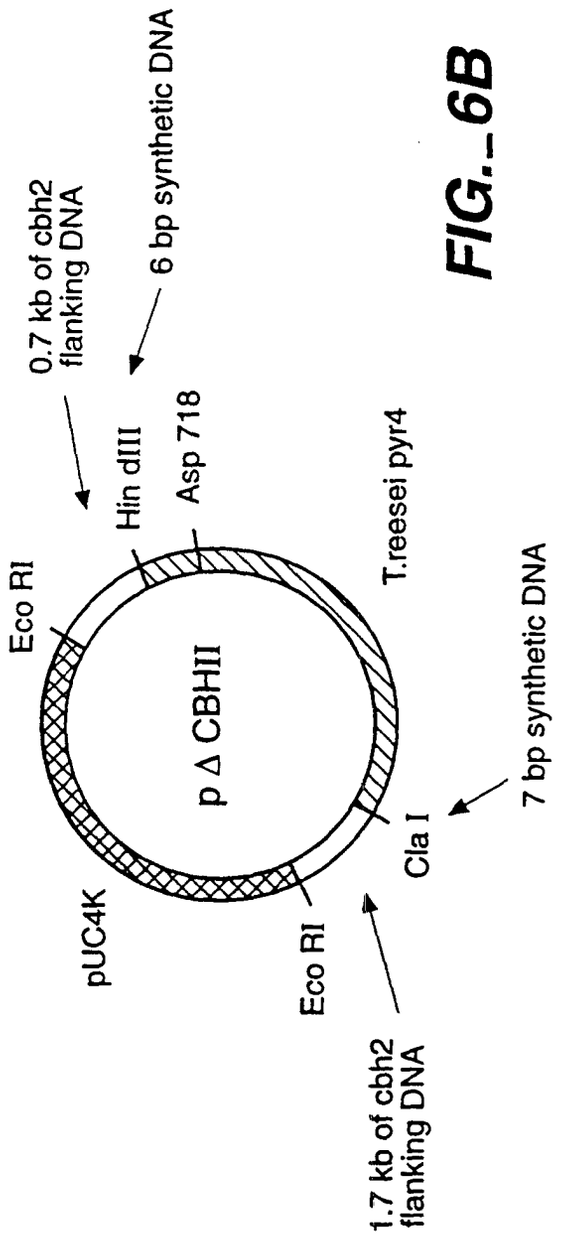
0.7 kb of cbh2 flanking DNA
6 bp synthetic DNA
Hin dIII
Asp 718
Eco RI
p Δ CBHII
T.reesei pyr4
pUC4K
Eco RI
Cla I
7 bp synthetic DNA
1.7 kb of cbh2 flanking DNA

FIG._6B

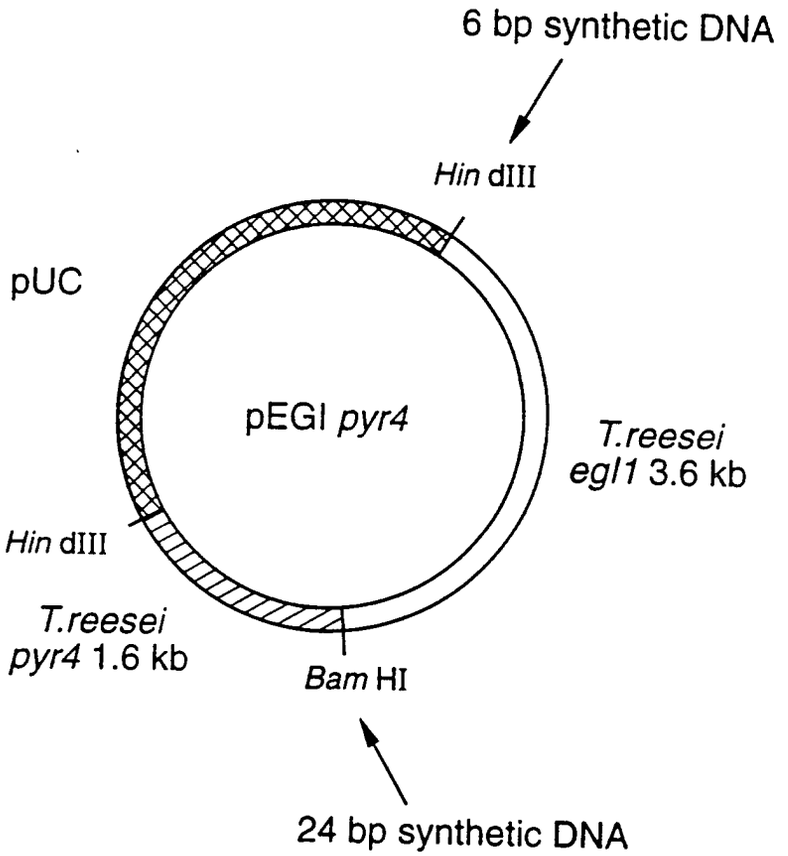
6 bp synthetic DNA
Hin dIII
pUC
pEGI pyr4
T.reesei egl1 3.6 kb
Hin dIII
T.reesei pyr4 1.6 kb
Bam HI
24 bp synthetic DNA

FIG._8

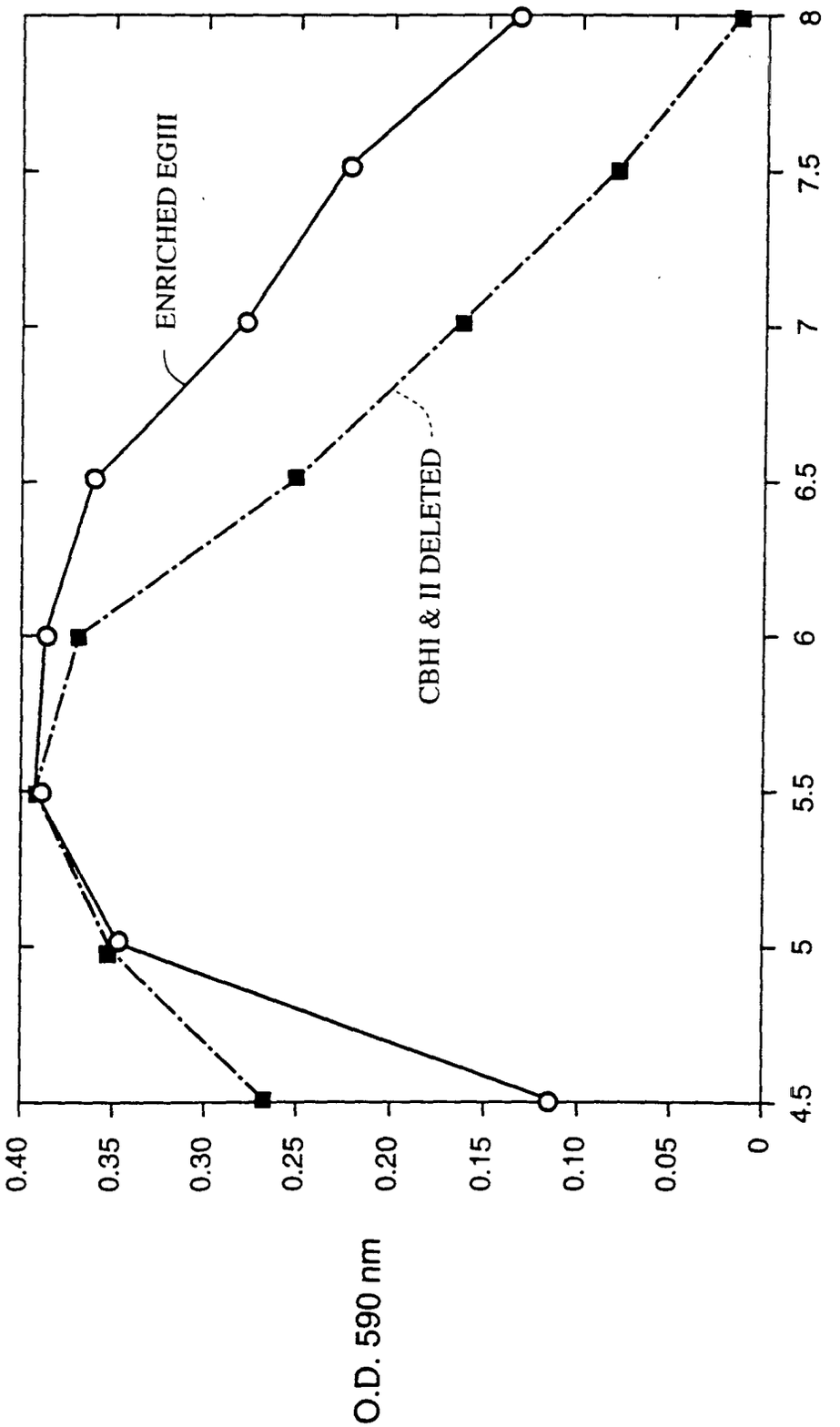
ENRICHED EGIII
CBHI & II DELETED
O.D. 590 nm
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
4.5
5
5.5
6
6.5
7
7.5
8
pH

FIG._9

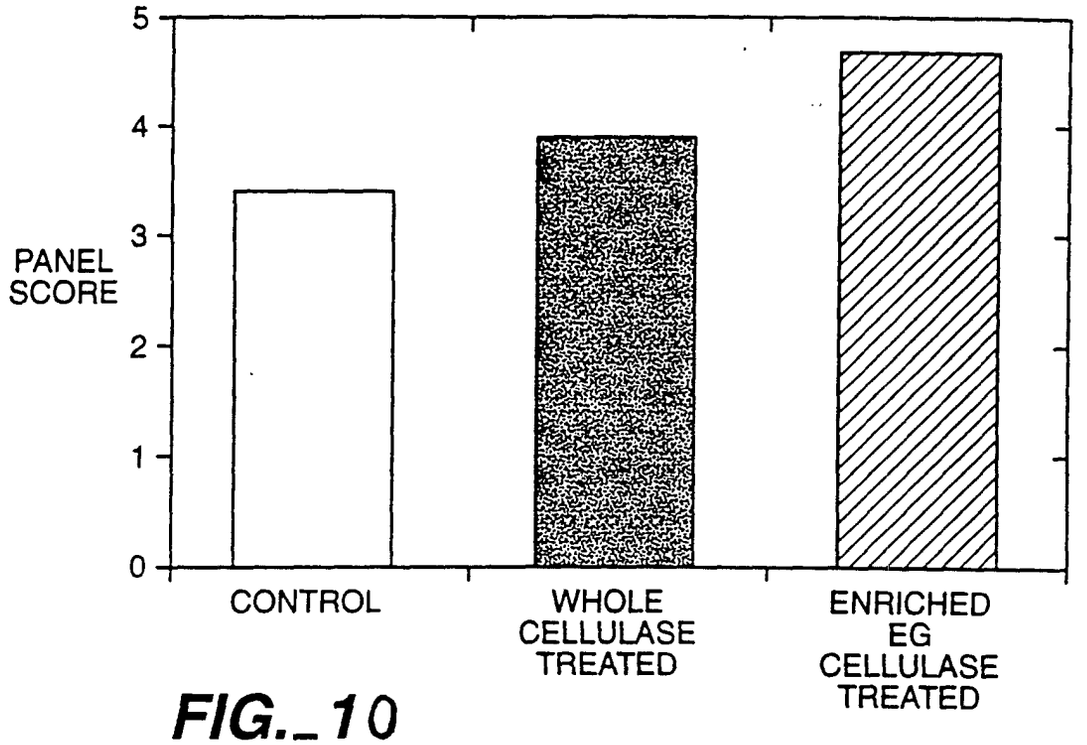
5
4
3
2
1
0
PANEL SCORE
CONTROL
WHOLE CELLULASE TREATED
ENRICHED EG CELLULASE TREATED

FIG._10

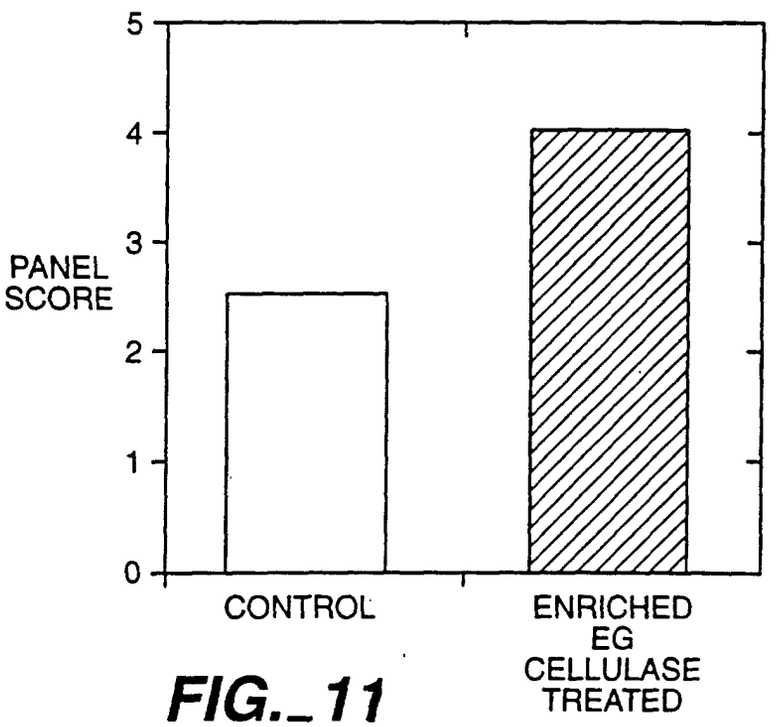
5
4
3
2
1
0
PANEL SCORE
CONTROL
ENRICHED EG CELLULASE TREATED

FIG._11