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(54) **DETERGENT COMPOSITION COMPRISING POLYPEPTIDE COMPRISING CARBOHYDRATE-BINDING DOMAIN**

(57) The present invention relates to detergent compositions comprising a polypeptide comprising a carbohydrate-binding module (CBM). The detergent compositions, such as laundry and dish wash compositions, including hand wash and automatic dish wash compositions, are suitable for use in cleaning processes.

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Description

REFERENCETO A SEQUENCE LISTING

[0001] This application contains a Sequence Listing in computer readable form, which is incorporated herein by reference.

BACKGROUNDOF THE INVENTION

Field of the Invention

[0002] The present invention relates to detergent compositions comprising a polypeptide comprising a carbohydrate-binding module (CBM). The detergent compositions, such as laundry and dish wash compositions, including hand wash and automatic dish wash compositions, are suitable for use in cleaning processes.

Description of the Related Art

[0003] Xanthan gum is a polysaccharide derived from the bacterial coat of *Xanthomonas campestris.* It is produced by the fermentation of glucose, sucrose, or lactose by the *Xanthomonas campestris* bacterium. After a fermentation period, the polysaccharide is precipitated from a growth medium with isopropyl alcohol, dried, and ground into a fine powder. Later, it is added to a liquid medium to form the gum. Xanthan gum is a natural polysaccharide consisting of different sugars which are connected by several different bonds, such as β-D-mannosyl-β-D-1,4-glucuronosyl bonds and β-D-glucosyl-β-D-1,4-glucosyl bonds. Xanthan gum is at least partly soluble in water and forms highly viscous solutions or gels. Complete enzymatic degradation of xanthan gum requires several enzymatic activities including xanthan lyase activity and endo-β-1,4-glucanase activity. Xanthan lyases are enzymes that cleaves the linkage between mannose and glucuronic acid in β-D-mannosyl-β-D-1,4-glucuronosyl residues of xanthan and have been described in the literature. Xanthan degrading enzymes are known in the art, e.g. two xanthan lyases have been isolated from *Paenibacillus alginolyticus* XL-1. Glycoside hydrolases are enzymes that catalyse the hydrolysis of the glycosyl bond to release smaller sugars. There are over 100 classes of glycoside hydrolases, which have been classified, see the Uniprot website at www.cazy.org. The glycoside hydrolase family 9 (GH9) consists of over 70 different enzymes that are mostly endo-glucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91), β-glucosidases (EC 3.2.1.21) and exo-β-glucosaminidase (EC 3.2.1.165). In recent years xanthan gum has been used as an ingredient in many consumer products including foods (e.g. as thickening agent in salad dressings and dairy products) and cosmetics (e.g. as stabilizer and thickener in toothpaste and make-up, creams and lotions to prevent ingredients from separating and to provide the right texture of the product). Further, xanthan gum has found use in the oil industry as an additive to regulate the viscosity of drilling fluids *etc.* The widespread use of xanthan gum has led to a desire to degrade solutions, gels or mixtures containing xanthan gum thereby allowing easier removal of such products.

[0004] Accordingly, there is a continued need to optimize the degradation of xanthan gum either by directing enzymes to the xanthan gum or speed up the process of traditional polypeptides capable of degrading xanthan gum. The present invention provides a detergent composition comprising a CBM domain, which improves the performance or properties of such traditional polypeptides by attaching the CBM domain to such enzymes, and thereby allowing the direction of the enzyme to xanthan. Thus, this allows the enzyme specificity to be increased, controlled, or allowing improvement of specific properties of xanthan.

SUMMARY OF THE INVENTION

[0005] The present invention relates to a detergent composition comprising a polypeptide comprising a carbohydrate-binding module (CBM), wherein the carbohydrate-binding module is selected from the group consisting of:

(a) a polypeptide comprising (or consisting essentially of) one or more of the following motifs:

i) GIYQZ1 (SEQ ID NO: 1), wherein Z1 is independently selected from the group of amino acids L and F; preferably said GIYQZ1 motif is either GIYQL (SEQ ID NO: 2) or GIYQF (SEQ ID NO: 3);
ii) TGKB1 (SEQ ID NO: 4), wherein B1 is independently selected from the group of amino acids N, H and S; preferably said TGKB1 motif is either TGKN (SEQ ID NO: 5) or TGKH (SEQ ID NO: 6) or TGKS (SEQ ID NO: 7);
iii) GX1X2QLX3 (SEQ ID NO: 8), wherein X1 is independently selected from the group consisting of amino acids I, T and K; wherein X2 is independently selected from the group consisting of amino acids F and Y; wherein X3 is independently selected from the group consisting of amino acids S and Q; preferably said GX1X2QLX3

motif is either GIYQLQ (SEQ ID NO: 9) or GIYQLS (SEQ ID NO: 10);

(b) a polypeptide having at least 34% (e.g., at least 35%, at least 40%, at least 45%, at least 46%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100%) sequence identity to the carbohydrate-binding module having either

i') SEQ ID NO: 11, preferably said polypeptide has at least 34% sequence identity to the SEQ ID NO: 11; or
ii') SEQ ID NO: 12, preferably said polypeptide has at least 46% sequence identity to the SEQ ID NO: 12;

(c) the polypeptide according to (b), wherein said polypeptide comprises one or more of motifs according to (a);
(d) preferably the carbohydrate-binding module does not have an enzymatic activity (e.g., does not have xanthan lyase or endoglucanase activity).

[0006] The carbohydrate-binding module comprised in the detergent composition comprises two or three of said motifs; preferably said carbohydrate-binding module comprises two motifs, such as said motif of ii) and iii); further preferably said carbohydrate-binding module comprises three motifs, such as said motifs of i), ii), and iii).

[0007] In one embodiment of present invention the carbohydrate-binding module comprised in the detergent composition can be operably linked to a catalytic domain, wherein said catalytic domain is obtained from a xanthan lyase or an endoglucanase.

[0008] In a preferred embodiment the polypeptide comprised in the detergent composition is selected from the group consisting of: SEQ ID NO: 13 and SEQ ID NO: 14.

[0009] In one embodiment of the present invention the detergent composition is in form of a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

[0010] In one embodiment, the composition further comprises one or more additional enzymes selected among protease, lipase, cutinase, amylase, carbohydrase, cellulase, pectinase, pectatlyase, mannanase, arabinase, galactanase, xylanase, oxidase, xanthanase, laccase, and/or peroxidase.

[0011] In one embodiment, the detergent composition of present invention is a laundry detergent composition or a dishwashing composition, preferably a machine dishwashing composition.

[0012] The invention furthermore relates to the use of the detergent composition for degrading xanthan gum and the use of the detergent composition in a cleaning process, preferably in laundry or hard surface cleaning such as dish wash.

[0013] Furthermore, methods for removing a stain from a surface, which comprises contacting the surface with the detergent composition of present invention, as well as for degrading xanthan gum comprising applying the detergent composition to xanthan gum, preferably wherein the xanthan gum is on the surface of a textile or of a hard surface, such as in dish wash, are envisaged.

[0014] The invention further relates to the use of the carbohydrate-binding module comprised in the detergent composition according to present invention for one or more of the following:

i) binding or facilitating binding to xanthan gum in a cleaning process;
ii) facilitating or improving degradation of xanthan gum in a cleaning process;
iii) facilitating or improving binding affinity and/or specificity and/or adhesion of a polypeptide to xanthan gum in a cleaning process;
iv) facilitating or improving adhesion of a detergent composition to a textile in a cleaning process;
v) washing or cleaning a textile and/or a hard surface such as dish wash including Automatic Dish Wash (ADW);
vi) in a cleaning process such as laundry or hard surface cleaning including dish wash including Automatic Dish Wash (ADW) and industrial cleaning;
vii) laundering and/or hard surface cleaning including dish wash including Automatic Dish Wash (ADW);
viii) facilitating or improving removal of xanthan gum containing soil in a cleaning process;
ix) facilitating or improving removal of xanthan gum containing soil in the presence of xanthan lyase and/or endoglucanase activity in a cleaning process.

OVERVIEW OF SEQUENCE LISTING

[0015]

SEQ ID NO: 1 is the first motif of the present invention.
SEQ ID NO: 2 is a preferred embodiment of the first motif of SEQ ID NO: 1.

SEQ ID NO: 3 is another preferred embodiment of the first motif of SEQ ID NO: 1.
SEQ ID NO: 4 is the second motif of the present invention.
SEQ ID NO: 5 is a preferred embodiment of the second motif of SEQ ID NO: 4.
SEQ ID NO: 6 is another preferred embodiment of the second motif of SEQ ID NO: 4.
SEQ ID NO: 7 is another preferred embodiment of the second motif of SEQ ID NO: 4.
SEQ ID NO: 8 is the third motif of the present invention.
SEQ ID NO: 9 is a preferred embodiment of the third motif of SEQ ID NO: 8.
SEQ ID NO: 10 is another preferred embodiment of the third motif of SEQ ID NO: 8.
SEQ ID NO: 11 is a carbohydrate-binding module (CBM#1).
SEQ ID NO: 12 is a carbohydrate-binding module (CBM#2).
SEQ ID NO: 13 is an amino acid sequence of an Endoglucanase.
SEQ ID NO: 14 is an amino acid sequence of a Xanthan lyase.
SEQ ID NO: 15 is a DNA encoding sequence for the CBM#1 of SEQ ID NO: 11.
SEQ ID NO: 16 is a DNA encoding sequence for the CBM#2 of SEQ ID NO: 12.
SEQ ID NO: 17 is the DNA encoding sequence for the endoglucanase of SEQ ID NO: 13.
SEQ ID NO: 18 is the DNA encoding sequence for the xanthan lyase of SEQ ID NO: 14.
SEQ ID NO: 19 is the D2003 forward primer.
SEQ ID NO: 20 is the D2004 reverse primer.
SEQ ID NO: 21 is an amino acid sequence of a His tag.
SEQ ID NO: 22 is an amino acid sequence of a secretion signal.

DEFINITIONS

[0016] cDNA: The term "cDNA" means a DNA molecule that can be prepared by reverse transcription from a mature, spliced, mRNA molecule obtained from a eukaryotic or prokaryotic cell. cDNA lacks intron sequences that may be present in the corresponding genomic DNA. The initial, primary RNA transcript is a precursor to mRNA that is processed through a series of steps, including splicing, before appearing as mature spliced mRNA.

[0017] Cleaning or Detergent Application: the term "cleaning or detergent application" means applying the xanthan lyase of the application in any composition for the purpose of cleaning or washing, by hand, machine or automated, a hard surface or a textile.

[0018] Cleaning Composition: the term "cleaning composition" refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles, dishes, and hard surfaces. The terms encompass any materials/compounds selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, gel, powder, granulate, paste, or spray compositions) and includes, but is not limited to, detergent compositions (e.g., liquid and/or solid laundry detergents and fine fabric detergents; hard surface cleaning formulations, such as for glass, wood, ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; fabric fresheners; fabric softeners; and textile and laundry pre-spotters, as well as dish wash detergents). In addition to the xanthan lyase, the detergent formulation may contain one or more additional enzymes (such as xanthan lyases, proteases, amylases, lipases, cutinases, cellulases, xanthan lyases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases and mannanases, or any mixture thereof), and/or components such as surfactants, builders, chelators or chelating agents, bleach system or bleach components, polymers, fabric conditioners, foam boosters, suds suppressors, dyes, perfume, tannish inhibitors, optical brighteners, bactericides, fungicides, soil suspending agents, anticorrosion agents, enzyme inhibitors or stabilizers, enzyme activators, transferase(s), hydrolytic enzymes, oxido reductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers.

[0019] Coding sequence: The term "coding sequence" means a polynucleotide, which directly specifies the amino acid sequence of a polypeptide. The boundaries of the coding sequence are generally determined by an open reading frame, which begins with a start codon such as ATG, GTG, or TTG and ends with a stop codon such as TAA, TAG, or TGA. The coding sequence may be a genomic DNA, cDNA, synthetic DNA, or a combination thereof.

[0020] Color clarification: During washing and wearing loose or broken fibers can accumulate on the surface of the fabrics. One consequence can be that the colors of the fabric appear less bright or less intense because of the surface contaminations. Removal of the loose or broken fibers from the textile will partly restore the original colors and looks of the textile. By the term "color clarification", as used herein, is meant the partial restoration of the initial colors of textile.

[0021] Control sequences: The term "control sequences" means nucleic acid sequences necessary for expression of a polynucleotide encoding a mature polypeptide of the present invention. Each control sequence may be native (i.e., from the same gene) or foreign (i.e., from a different gene) to the polynucleotide encoding the polypeptide or native or foreign to each other. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with

linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the polynucleotide encoding a polypeptide.

[0022] Degrading xanthan gum and xanthan gum degrading activity: The terms "degrading xanthan gum" and "xanthan gum degrading activity" are used interchangeably and are defined as the depolymerisation, degradation or breaking down of xanthan gum into smaller components. The degradation of xanthan gum can either be the removal of one or more side chain saccharides, the cutting of the backbone of xanthan gum into smaller components or the removal of one or more side chain saccharides and the cutting of the backbone of xanthan gum into smaller components. Non-limiting examples of the xanthan gum degrading activity include xanthan lyase EC 4.2.2.12 activity.

[0023] Delta remission value (ΔRem): The terms "Delta remission" or "Delta remission value" are defined herein as the result of a reflectance or remission measurement at 460 nm. The swatch is measured with one swatch of similar color as background, preferably a swatch from a repetition wash. A swatch representing each swatch type is measured before wash. The Delta remission is the remission value of the washed swatch minus the remission value of the unwashed swatch.

[0024] Delta enzyme performance value (ΔRem enzyme value): The term "Delta enzyme remission value" is defined herein as the result of a reflectance or remission measurement at 460 nm. The swatch is measured with one swatch of similar color as background, preferably a swatch from a repetition wash. A swatch representing each swatch type is measured before wash. The Delta remission is the remission value of the swatch washed in detergent with an enzyme present minus the remission value of a similar swatch washed in a detergent without enzyme present.

[0025] Delta enzyme intensity value (**ΔInt enzyme value**)**:** The terms "Delta enzyme intensity" or "Delta enzyme intensity value" are defined herein as the result of an enzyme intensity value as defined in AMSA assay. The Delta intensity is the intensity value of the swatch area washed in detergent with an enzyme present minus the intensity value of the swatch area washed in detergent without enzyme present.

[0026] Detergent component: the term "detergent component" is defined herein to mean the types of chemicals which can be used in detergent compositions. Examples of detergent components are surfactants, hydrotropes, builders, co-builders, chelators or chelating agents, bleaching system or bleach components, polymers, fabric hueing agents, fabric conditioners, foam boosters, suds suppressors, dispersants, dye transfer inhibitors, fluorescent whitening agents, perfume, optical brighteners, bactericides, fungicides, soil suspending agents, soil release polymers, anti-redeposition agents, enzyme inhibitors or stabilizers, enzyme activators, antioxidants, and solubilizers. The detergent composition may comprise of one or more of any type of detergent component.

[0027] Detergent composition: the term "detergent composition" refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles, dishes, and hard surfaces. The detergent composition may be used to e.g. clean textiles, dishes and hard surfaces for both household cleaning and industrial cleaning. The terms encompass any materials/compounds selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, gel, powder, granulate, paste, or spray compositions) and includes, but is not limited to, detergent compositions (e.g., liquid and/or solid laundry detergents and fine fabric detergents; hard surface cleaning formulations, such as for glass, wood, ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; fabric fresheners; fabric softeners; and textile and laundry pre-spotters, as well as dish wash detergents). In addition to containing a xanthan lyase of the invention and/or a GH9 endoglucanase, the detergent formulation may contain one or more additional enzymes (such as endoglucanases, xanthan lyases, proteases, amylases, lichenases, lipases, cutinases, cellulases, xanthan lyases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases and mannanases, or any mixture thereof), and/or components such as surfactants, builders, chelators or chelating agents, bleach system or bleach components, polymers, fabric conditioners, foam boosters, suds suppressors, dyes, perfume, tannish inhibitors, optical brighteners, bactericides, fungicides, soil suspending agents, anti-corrosion agents, enzyme inhibitors or stabilizers, enzyme activators, transferase(s), hydrolytic enzymes, oxido reductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers.

[0028] Dish wash: The term "dish wash" refers to all forms of washing dishes, e.g. by hand or automatic dish wash. Washing dishes includes, but is not limited to, the cleaning of all forms of crockery such as plates, cups, glasses, bowls, all forms of cutlery such as spoons, knives, forks and serving utensils as well as ceramics, plastics, metals, china, glass and acrylics.

[0029] Dish washing composition: The term "dish washing composition" refers to all forms of compositions for cleaning hard surfaces. The present invention is not restricted to any particular type of dish wash composition or any particular detergent.

[0030] Endoglucanase: The term "endoglucanase" or "EG" means an endo-1,4- or endo-1,3;1,4-beta-D-glucan 4-glucanohydrolase (*e.g.*, EC 3.2.1.4) that catalyses endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenin, beta-1,4 bonds in mixed beta-1,3/beta-1,4 glucans such as cereal beta-D-glucans, xyloglucans, xanthans and other plant material containing cellulosic components. Endoglucanase activity can be determined by measuring reduction in substrate viscosity or increase in reducing ends determined by a reducing sugar assay.

[0031] Enzyme detergency benefit: The term "enzyme detergency benefit" is defined herein as the advantageous effect an enzyme may add to a detergent compared to the same detergent without the enzyme. Important detergency benefits which can be provided by enzymes are stain removal with no or very little visible soils after washing and or cleaning, prevention or reduction of redeposition of soils released in the washing process an effect that also is termed anti-redeposition, restoring fully or partly the whiteness of textiles, which originally were white but after repeated use and wash have obtained a greyish or yellowish appearance an effect that also is termed whitening. Textile care benefits, which are not directly related to catalytic stain removal or prevention of redeposition of soils are also important for enzyme detergency benefits. Examples of such textile care benefits are prevention or reduction of dye transfer from one fabric to another fabric or another part of the same fabric an effect that is also termed dye transfer inhibition or anti-backstaining, removal of protruding or broken fibers from a fabric surface to decrease pilling tendencies or remove already existing pills or fuzz an effect that also is termed anti-pilling, improvement of the fabric-softness, color clarification of the fabric and removal of particulate soils which are trapped in the fibers of the fabric or garment. Enzymatic bleaching is a further enzyme detergency benefit where the catalytic activity generally is used to catalyze the formation of bleaching component such as hydrogen peroxide or other peroxides.

[0032] Expression: The term "expression" includes any step involved in the production of a polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

[0033] Expression vector: The term "expression vector" means a linear or circular DNA molecule that comprises a polynucleotide encoding a polypeptide and is operably linked to control sequences that provide for its expression.

[0034] Fragment: The term "fragment" means a polypeptide having one or more (e.g., several) amino acids absent from the amino and/or carboxyl terminus of a mature polypeptide; wherein the fragment has xanthan lyase activity. In one aspect, a fragment contains at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% or 95% of the number of amino acids of the mature polypeptide.

[0035] Half-life improvement factor: the term "Half-life improvement factor" or "HIF" can be defined according to the following formula: HIF = T½ (Variant) / T½ (Wild-type) = Ln(RA-Wild-type/100) / Ln(RA-Variant/100), wherein T½ (Variants) = (Ln (0.5)/Ln (RA-Variant/100))*Time, wherein T½ (Wild-type) = (Ln (0.5)/Ln (RA-Wild-type/100))*Time, wherein "RA" is residual activity. A preferred way of calculating HIF is also described in example 4 herein.

[0036] Hard surface cleaning: The term "Hard surface cleaning" is defined herein as cleaning of hard surfaces wherein hard surfaces may include floors, tables, walls, roofs etc. as well as surfaces of hard objects such as cars (car wash) and dishes (dish wash). Dish washing includes but are not limited to cleaning of plates, cups, glasses, bowls, and cutlery such as spoons, knives, forks, serving utensils, ceramics, plastics, metals, china, glass and acrylics.

[0037] Host cell: The term "host cell" means any cell type that is susceptible to transformation, transfection, transduction, or the like with a nucleic acid construct or expression vector comprising a polynucleotide of the present invention. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication.

[0038] Improved property: The term "improved property" means a characteristic associated with a variant that is improved compared to the parent. Such improved properties include, but are not limited to, catalytic efficiency, catalytic rate, chemical stability, oxidation stability, pH activity, pH stability, specific activity, stability under storage conditions, chelator stability, substrate binding, substrate cleavage, substrate specificity, substrate stability, surface properties, thermal activity, and thermostability.

[0039] Improved wash performance: The term "improved wash performance" is defined herein as a (variant) enzyme (also a blend of enzymes, not necessarily only variants but also backbones, and in combination with certain cleaning composition etc.) displaying an alteration of the wash performance of a protease variant relative to the wash performance of the parent protease variant e.g. by increased stain removal. The term "wash performance" includes wash performance in laundry but also e.g. in dish wash.

[0040] Isolated: The term "isolated" means a substance in a form or environment that does not occur in nature. Non-limiting examples of isolated substances include (1) any non-naturally occurring substance, (2) any substance including, but not limited to, any enzyme, variant, nucleic acid, protein, peptide or cofactor, that is at least partially removed from one or more or all of the naturally occurring constituents with which it is associated in nature; (3) any substance modified by the hand of man relative to that substance found in nature; or (4) any substance modified by increasing the amount of the substance relative to other components with which it is naturally associated (e.g., multiple copies of a gene encoding the substance; use of a stronger promoter than the promoter naturally associated with the gene encoding the substance). An isolated substance may be present in a fermentation broth sample.

[0041] Laundering: The term "laundering" relates to both household laundering and industrial laundering and means the process of treating textiles with a solution containing a cleaning or detergent composition of the present invention. The laundering process can for example be carried out using e.g. a household or an industrial washing machine or can be carried out by hand.

[0042] Mature polypeptide: The term "mature polypeptide" means a polypeptide in its final form following translation

and any post-translational modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc. In one aspect, the mature polypeptide is the amino acid sequence put forth in SEQ ID NO: 13 or 14.

[0043] It is known in the art that a host cell may produce a mixture of two of more different mature polypeptides (i.e., with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide. It is also known in the art that different host cells process polypeptides differently, and thus, one host cell expressing a polynucleotide may produce a different mature polypeptide (e.g., having a different C-terminal and/or N-terminal amino acid) as compared to another host cell expressing the same polynucleotide.

[0044] **Mature polypeptide coding sequence**: The term "mature polypeptide coding sequence" means a polynucleotide that encodes a mature polypeptide having enzymatic activity such as activity on xanthan gum pre-treated with xanthan lyase or xanthan lyase activity.

[0045] **Mutant**: The term "mutant" means a polynucleotide encoding a variant.

[0046] **Nucleic acid construct**: The term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic, which comprises one or more control sequences.

[0047] **Operably linked:** The term "operably linked" means a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs expression of the coding sequence.

[0048] **Parent**: The term "parent" or "parent polypeptide " means any polypeptide herein disclosed having either xanthan lyase activity or xanthan endoglucanase activity, to which an alteration is made to produce the enzyme variants of the present invention. In one aspect, the parent is a xanthan lyase having the identical amino acid sequence of the variant, but not having the alterations at one or more of the specified positions. In one aspect, the parent is a xanthan endoglucanase having the identical amino acid sequence of the variant, but not having the alterations at one or more of the specified positions. It will be understood, that the expression "having identical amino acid sequence" relates to 100% sequence identity. Non-limiting examples of parent xanthan lyases include the mature parent xanthan lyase having SEQ ID NO: 14. Non-limiting examples of parent xanthan endoglucanase include the mature parent xanthan endoglucanase having SEQ ID NO: 13.

[0049] **Sequence identity**: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity". For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm as implemented in the Needle program of the EMBOSS package, preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

$$\text{(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)}$$

[0050] For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.,* 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labelled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

$$\text{(Identical Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment)}$$

[0051] **Stringency conditions**: The different stringency conditions are defined as follows.

[0052] The term "very low stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 45°C.

[0053] The term "low stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 50°C.

[0054] The term "medium stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and

35% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 55°C.

[0055] The term "medium-high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 35% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 60°C.

[0056] The term "high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 50% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 65°C.

[0057] The term "very high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 50% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 70°C.

[0058] **Subsequence**: The term "subsequence" means a polynucleotide having one or more (e.g., several) nucleotides absent from the 5' and/or 3' end of a mature polypeptide coding sequence; wherein the subsequence encodes a fragment having enzymatic activity, such as activity on xanthan gum pre-treated with xanthan lyase or xanthan lyase activity.

[0059] **Textile**: The term "textile" means any textile material including yarns, yarn intermediates, fibers, nonwoven materials, natural materials, synthetic materials, and any other textile material, fabrics made of these materials and products made from fabrics (e.g., garments and other articles). The textile or fabric may be in the form of knits, wovens, denims, non-wovens, felts, yarns, and towelling. The textile may be cellulose based such as natural cellulosics, including cotton, flax/linen, jute, ramie, sisal or coir or manmade cellulosics (e.g. originating from wood pulp) including viscose/rayon, ramie, cellulose acetate fibers (tricell), lyocell or blends thereof. The textile or fabric may also be non-cellulose based such as natural polyamides including wool, camel, cashmere, mohair, rabit and silk or synthetic polymer such as nylon, aramid, polyester, acrylic, polypropylen and spandex/elastane, or blends thereof as well as blend of cellulose based and non-cellulose based fibers. Examples of blends are blends of cotton and/or rayon/viscose with one or more companion material such as wool, synthetic fibers (e.g. polyamide fibers, acrylic fibers, polyester fibers, polyvinyl alcohol fibers, polyvinyl chloride fibers, polyurethane fibers, polyurea fibers, aramid fibers), and cellulose-containing fibers (e.g. rayon/viscose, ramie, flax/linen, jute, cellulose acetate fibers, lyocell). Fabric may be conventional washable laundry, for example stained household laundry. When the term fabric or garment is used it is intended to include the broader term textiles as well.

[0060] **Textile care benefit**: "Textile care benefits", which are not directly related to catalytic stain removal or prevention of redeposition of soils, are also important for enzyme detergency benefits. Examples of such textile care benefits are prevention or reduction of dye transfer from one textile to another textile or another part of the same textile an effect that is also termed dye transfer inhibition or anti-backstaining, removal of protruding or broken fibers from a textile surface to decrease pilling tendencies or remove already existing pills or fuzz an effect that also is termed anti-pilling, improvement of the textile-softness, colour clarification of the textile and removal of particulate soils which are trapped in the fibers of the textile. Enzymatic bleaching is a further enzyme detergency benefit where the catalytic activity generally is used to catalyse the formation of bleaching component such as hydrogen peroxide or other peroxides or other bleaching species.

[0061] **Variant**: The term "variant" means a polypeptide (*e.g.*, a xanthan lyase polypeptide) comprising an alteration, *i.e.*, a substitution, insertion, and/or deletion, at one or more (e.g., several) positions. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding one or more (*e.g.* several) amino acids, *e.g.*, 1-5 amino acids adjacent to and immediately following the amino acid occupying a position. Non-limiting examples of variants of the present invention further include variants having at least 20%, *e.g.,* at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% xanthan lyase activity of the mature parent xanthan lyase of SEQ ID NO: 14, or xanthan endoglucanase activity of the mature parent xanthan endoglucanase of SEQ ID NO: 13.

[0062] **Stability**: The term "stability" means resistance or the degree of resistance to change, unfolding, disintegration, denaturation or activity loss. Non-limiting examples of stability include conformational stability, storage stability and stability during use, *e.g.* during a wash process and reflects the stability of a polypeptide (as a function of time, *e.g.* how much activity is retained when said polypeptide (*e.g.* said xanthan lyase variant) is kept in solution, in particular in a detergent solution. The stability is influenced by many factors, *e.g.* presence of chelator(s), pH, temperature, detergent composition, *e.g.* amount of builder(s), surfactant(s), chelator(s) etc. The xanthan lyase stability may be measured using a half-life improvement factor (HIF) as described in example 4 herein.

[0063] **Improved stability:** The term "improved stability" or "increased stability" is defined herein as increased stability in a detergent composition (*e.g.*, in solutions, *e.g.* in the presence of a chelator, e.g. EDTA or citrate), relative to the stability of the parent polypeptide or a variant thereof, but not having the alterations at one or more of the specified positions. The terms "improved stability" and "increased stability" includes "improved chemical stability", "detergent

stability" and "improved detergent stability.

[0064] Improved chemical stability: The term "improved chemical stability" is defined herein as a variant enzyme displaying retention of enzymatic activity after a period of incubation in the presence of a chemical or chemicals, either naturally occurring or synthetic, which reduces the enzymatic activity of the parent enzyme. Improved chemical stability may also result in variants being more able (*e.g.*, better that the parent) to catalyze a reaction in the presence of such chemicals. In a particular aspect of the invention the improved chemical stability is an improved stability in a detergent, in particular in a liquid detergent. The term "detergent stability" or "improved detergent stability is in particular an improved stability of the variant polypeptide compared to the parent polypeptide, when a variant polypeptide is mixed into a liquid detergent formulation, especially into a liquid detergent formulation comprising a chelator (e.g. EDTA or citrate).

[0065] Conformational stability: The term "conformational stability" means a resistance or a degree of resistance to conformational change, unfolding or disintegration. Accordingly, the term "less conformationally stable" means less resistant or having lesser degree of resistance to conformational change, unfolding or disintegration.

[0066] Instability: The term "instability" means lack of stability. Non-limiting examples of instability include conformational instability, unfolding, denaturation, disintegration, activity loss.

[0067] Conformational dynamics: The term "conformational dynamics" encompasses vibrations, structural rearrangements and transitions of a polypeptide (e.g. in solution). Accordingly, in the context of the present invention the term "more conformationally dynamic" means that conformational dynamics of a particular region (*e.g.* a chelator-induced instability region) is greater than conformational dynamics of a different region (e.g. an adjacent region).

[0068] Receptiveness to deuterium incorporation: The term "receptiveness to deuterium incorporation" means amount of hydrogen atoms replaced by a deuterium atoms during hydrogen-deuterium exchange. Said amount can be measured in relative (e.g. compared to another amount) or absolute (e.g. expressed numerically) terms. Accordingly, in the context of the present invention the term "more receptive to deuterium incorporation" means that receptiveness to deuterium incorporation of a particular region (e.g. a chelator-induced instability region) is greater than receptiveness to deuterium incorporation of a different region (e.g. an adjacent region).

[0069] Wash performance: The term "wash performance" is used as an enzyme's ability to remove stains present on the object to be cleaned during e.g. wash or hard surface cleaning. The improvement in the wash performance may be quantified by calculating the so-called intensity value (Int) in 'Automatic Mechanical Stress Assay (AMSA) for laundry' or the remission value (Rem) as defined herein.

[0070] Whiteness: The term "Whiteness" is defined herein as a broad term with different meanings in different regions and for different customers. Loss of whiteness can e.g. be due to greying, yellowing, or removal of optical brighteners/hueing agents. Greying and yellowing can be due to soil redeposition, body soils, colouring from, e.g. iron and copper ions or dye transfer. Whiteness might include one or several issues from the list below: Colorant or dye effects; Incomplete stain removal (e.g. body soils, sebum ect.); Re-deposition (greying, yellowing or other discolorations of the object) (removed soils re-associates with other part of textile, soiled or unsoiled); Chemical changes in textile during application; and Clarification or brightening of colours.

[0071] Xanthan lyase: The term "xanthan lyase" is defined herein as an enzyme that has activity on xanthan gum (e.g., enzymatic, activity, a xanthan gum degrading activity). Non-limiting examples of xanthan lyases include an enzyme that cleaves the linkage between mannose and glucuronic acid in β-D-mannosyl-β-D-1,4-glucuronosyl residues in xanthan gum (EC 4.2.2.12).

Conventions for Designation of Variants

[0072] For purposes of the present invention, the mature polypeptide disclosed in SEQ ID NOs: 13 and 14 is used to determine the corresponding amino acid residue in another xanthan lyase. The amino acid sequence of another polypeptide is aligned with the polypeptide disclosed in SEQ ID NOs: 13 or 14, and based on the alignment, the amino acid position number corresponding to any amino acid residue in the mature polypeptide disclosed in SEQ ID NO: 13 or 14 is determined using the Needleman-Wunsch algorithm as implemented in the Needle program of the EMBOSS package, preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix.

[0073] Identification of the corresponding amino acid residue in another xanthan lyase can be determined by an alignment of multiple polypeptide sequences using several computer programs. including, but not limited to, MUSCLE, MAFFT, and EMBOSS EMMA employing ClustalW, using their respective default parameters.

[0074] When the other enzyme has diverged from the polypeptide of SEQ ID NOs: 13 or 14 such that traditional sequence-based comparison fails to detect their relationship, other pairwise sequence comparison algorithms can be used. Greater sensitivity in sequence-based searching can be attained using search programs that utilize probabilistic representations of polypeptide families (profiles) to search databases. For example, the PSI-BLAST program generates profiles through an iterative database search process and is capable of detecting remote homologs. Even greater sensitivity can be achieved if the family or superfamily for the polypeptide has one or more representatives in the protein

structure databases. Programs such as GenTHREADER utilize information from a variety of sources (PSI-BLAST, secondary structure prediction, structural alignment profiles, and solvation potentials) as input to a neural network that predicts the structural fold for a query sequence. These alignments can in turn be used to generate homology models for the polypeptide, and such models can be assessed for accuracy using a variety of tools developed for that purpose.

[0075] For proteins of known structure, several tools and resources are available for retrieving and generating structural alignments. For example, the SCOP superfamilies of proteins have been structurally aligned, and those alignments are accessible and downloadable. Two or more protein structures can be aligned using a variety of algorithms such as the distance alignment matrix or combinatorial extension, and implementation of these algorithms can additionally be utilized to query structure databases with a structure of interest in order to discover possible structural homologs.

[0076] In describing the variants of the present invention, the nomenclature described below is adapted for ease of reference. The accepted IUPAC single letter or three letter amino acid abbreviation is employed.

[0077] Substitutions. For an amino acid substitution, the following nomenclature is used: Original amino acid, position, substituted amino acid. Accordingly, the substitution of threonine at position 226 with alanine is designated as "Thr226Ala" or "T226A". Multiple mutations are separated by addition marks ("+"), *e.g.,* "Gly205Arg + Ser411 Phe" or "G205R + S411 F", representing substitutions at positions 205 and 411 of glycine (G) with arginine (R) and serine (S) with phenylalanine (F), respectively.

[0078] Deletions. For an amino acid deletion, the following nomenclature is used: Original amino acid, position, *. Accordingly, the deletion of glycine at position 195 is designated as "Gly195*" or "G195*". Multiple deletions are separated by addition marks ("+"), e.g., "Gly195* + Ser411*" or "G195* + S411*".

[0079] Insertions. For an amino acid insertion, the following nomenclature is used: Original amino acid, position, original amino acid, inserted amino acid. Accordingly the insertion of lysine after glycine at position 195 is designated "Gly195GlyLys" or "G195GK". An insertion of multiple amino acids is designated [Original amino acid, position, original amino acid, inserted amino acid #1, inserted amino acid #2; etc.]. For example, the insertion of lysine and alanine after glycine at position 195 is indicated as "Gly195GlyLysAla" or "G195GKA".

[0080] In such cases the inserted amino acid residue(s) are numbered by the addition of lower case letters to the position number of the amino acid residue preceding the inserted amino acid residue(s). In the above example, the sequence would thus be:

Parent:	Variant:
195	195 195a 195b
G	G - K - A

[0081] Multiple alterations. Variants comprising multiple alterations are separated by addition marks ("+"), *e.g.,* "Arg170Tyr+Gly195Glu" or "R170Y+G195E" representing a substitution of arginine and glycine at positions 170 and 195 with tyrosine and glutamic acid, respectively.

[0082] Different alterations. Where different alterations can be introduced at a position, the different alterations are separated by a comma, e.g., "Arg170Tyr,Glu" represents a substitution of arginine at position 170 with tyrosine or glutamic acid. Thus, "Tyr167Gly,Ala + Arg170Gly,Ala" designates the following variants:
"Tyr167Gly+Arg170Gly", "Tyr167Gly+Arg170Ala", "Tyr167Ala+Arg170Gly", and "Tyr167 Ala+Arg170Ala".

DETAILED DESCRIPTION OF THE INVENTION

[0083] The present invention relates to a detergent composition comprising a polypeptide comprising a carbohydrate-binding module (CBM) which has been found by the inventors to bind particular well to xanthan gum. In particular, the present invention relates to a detergent composition comprising a polypeptide comprising a carbohydrate-binding module (CBM), wherein the carbohydrate-binding module is selected from the group consisting of:

a) a polypeptide comprising (or consisting essentially of) one or more of the following motifs:

i) GIYQZ1 (SEQ ID NO: 1), wherein Z1 is independently selected from the group of amino acids L and F; preferably said GIYQZ1 motif is either GIYQL (SEQ ID NO: 2) or GIYQF (SEQ ID NO: 3);
ii) TGKB1 (SEQ ID NO: 4), wherein B1 is independently selected from the group of amino acids N, H and S; preferably said TGKB1 motif is either TGKN (SEQ ID NO: 5) or TGKH (SEQ ID NO: 6) or TGKS (SEQ ID NO: 7);
iii) GX1X2QLX3 (SEQ ID NO: 8), wherein X1 is independently selected from the group consisting of amino acids I, T and K; wherein X2 is independently selected from the group consisting of amino acids F and Y; wherein X3 is independently selected from the group consisting of amino acids S and Q; preferably said GX1X2QLX3

motif is either GIYQLQ (SEQ ID NO: 9) or GIYQLS (SEQ ID NO: 10);

b) a polypeptide having at least 34% (e.g., at least 35%, at least 40%, at least 45%, at least 46%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100%) sequence identity to the carbohydrate-binding module having either

i') SEQ ID NO: 11, preferably said polypeptide has at least 34% sequence identity to the SEQ ID NO: 11; or
ii') SEQ ID NO: 12, preferably said polypeptide has at least 46% sequence identity to the SEQ ID NO: 12;

c) the polypeptide according to (b), wherein said polypeptide comprises one or more of motifs according to (a);
d) preferably the carbohydrate-binding module does not have an enzymatic activity (e.g., does not have xanthan lyase or endoglucanase activity).

[0084] In one embodiment, the carbohydrate-binding module comprised in the detergent composition of present invention comprises (or consisting essentially of) two or three of said motifs; preferably said carbohydrate-binding module comprises (or consisting essentially of) two motifs, such as said motif of ii) and iii); further preferably said carbohydrate-binding module comprises (or consisting essentially of) three motifs, such as said motifs of i), ii), and iii).

[0085] In a particular embodiment, the carbohydrate-binding module comprised in the detergent composition of present invention comprises two or three of the motifs as listed above. In a preferred embodiment, the carbohydrate-binding module comprises the following two motifs GX1X2QLX3 and TGKB1. In an even further preferred embodiment, the carbohydrate-binding module comprises the following three motifs GX1X2QLX3, TGKB1 and GIYQZ1.

[0086] In a particular embodiment, the carbohydrate-binding module comprised in the detergent composition of present invention consists essentially of two or three of the motifs as listed above. In a preferred embodiment, the carbohydrate-binding module consists essentially of the following two motifs GX1X2QLX3 and TGKB1. In an even further preferred embodiment, the carbohydrate-binding module consisting essentially of the following three motifs GX1X2QLX3, TGKB1 and GIYQZ1.

[0087] Accordingly, in a preferred embodiment, the carbohydrate-binding module comprised in the detergent composition of present invention comprises the motifs selected from the group consisting of: GIYQL+TGKN+GIFQLS, GIYQL+TGKN+GIYQLS, GIYQL+TGKN+GIFQLQ, GIYQL+TGKN+GIYQLQ, GIYQL+TGKN+GTFQLS, GIYQL+TGKN+GTYQLS, GIYQL+TGKN+GTFQLQ, GIYQL+TGKN+GTYQLQ, GIYQL+TGKN+GKFQLS, GIYQL+TGKN+GTFQLQ, GIYQL+TGKN+GKYQLS, GIYQL+TGKN+GKYQLQ, GIYQL+TGKH+GIFQLS, GIYQL+TGKH+GIYQLS, GIYQL+TGKH+GIFQLQ, GIYQL+TGKH+GIYQLQ, GIYQL+TGKH+GTFQLS, GIYQL+TGKH+GTYQLS, GIYQL+TGKH+GTFQLQ, GIYQL+TGKH+GTYQLQ, GIYQL+TGKH+GKFQLS, GIYQL+TGKH+GTFQLQ, GIYQL+TGKH+GKYQLS, GIYQL+TGKH+GKYQLQ, GIYQL+TGKS+GIFQLS, GIYQL+TGKS+GIYQLS, GIYQL+TGKS+GIFQLQ, GIYQL+TGKS+GIYQLQ, GIYQL+TGKS+GTFQLS, GIYQL+TGKS+GTYQLS, GIYQL+TGKS+GTFQLQ, GIYQL+TGKS+GTYQLQ, GIYQL+TGKS+GKFQLS, GIYQL+TGKS+GTFQLQ, GIYQL+TGKS+GKYQLS, GIYQL+TGKS+GKYQLQ, GIYQF+TGKN+GIFQLS, GIYQF+TGKN+GIYQLS, GIYQF+TGKN+GIFQLQ, GIYQF+TGKN+GIYQLQ, GIYQF+TGKN+GTFQLS, GIYQF+TGKN+GTYQLS, GIYQF+TGKN+GTYQLQ, GIYQF+TGKN+GKFQLS, GIYQF+TGKN+GTFQLQ, GIYQF+TGKN+GKYQLS, GIYQF+TGKN+GKYQLQ, GIYQF+TGKH+GIFQLS, GIYQF+TGKH+GIYQLS, GIYQF+TGKH+GIFQLQ, GIYQF+TGKH+GIYQLQ, GIYQF+TGKH+GTFQLS, GIYQF+TGKH+GTYQLS, GIYQF+TGKH+GTYQLQ, GIYQF+TGKH+GKFQLS, GIYQF+TGKH+GTFQLQ, GIYQF+TGKH+GKYQLS, GIYQF+TGKH+GKYQLQ, GIYQF+TGKS+GIFQLS, GIYQF+TGKS+GIYQLS, GIYQF+TGKS+GIFQLQ, GIYQF+TGKS+GIYQLQ, GIYQF+TGKS+GTFQLS, GIYQF+TGKS+GTYQLS, GIYQF+TGKS+GTFQLQ, GIYQF+TGKS+GKFQLS, GIYQF+TGKS+GKYQLS, and GIYQF+TGKS+GKYQLQ.

[0088] It is to be understood that the carbohydrate-binding module may be linked to other domains, polypeptides, or any other essentially biological part that would benefit from a carbohydrate-binding module. Thus, in one embodiment, the carbohydrate -binding module is operably linked to a catalytic domain, wherein said catalytic domain is obtained from a xanthan lyase or an endoglucanase.

[0089] The polypeptide comprised in the detergent composition of present invention may be any polypeptide having an enzymatic activity, preferably the enzymatic activity is degradation of the xanthan gum that is bound by the carbohydrate-binding module. Thus, the present invention also relates to detergent compositions comprising polypeptides having the ability of removing or processing xanthan gum, wherein the polypeptides comprises the carbohydrate-binding module as described herein.

[0090] In one particular embodiment, the polypeptide is selected from the group consisting of: SEQ ID NO: 13 and SEQ ID NO: 14.

[0091] The polypeptides comprising the carbohydrate-binding module, may be variants of a parent polypeptide. Accordingly, the present invention also relates to detergent compositions comprising variants of a polypeptide comprising

the carbohydrate-binding module. The variants may comprise one or more modifications at one or more (*e.g.*, several) other positions.

[0092] The amino acid changes may be of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of 1-30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

[0093] Examples of conservative substitutions are within the groups of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, In, The Proteins, Academic Press, New York. Common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

[0094] Alternatively, the amino acid changes are of such a nature that the physico-chemical properties of the polypeptides are altered. For example, amino acid changes may improve the thermal stability of the polypeptide, alter the substrate specificity, change the pH optimum, and the like.

[0095] Essential amino acids in a polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis. In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules can be tested for enzyme activity to identify amino acid residues that are critical to the activity of the molecule. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. The identity of essential amino acids can also be inferred from an alignment with a related polypeptide.

[0096] In one embodiment, the present invention relates to a detergent composition comprising a polypeptide variant comprising the carbohydrate-binding module, having a total number of modifications compared to SEQ ID NO: 13 or 14 between 1 and 30, such as between 1 and 20, such as between 1 and 10 or between 1 and 5, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 modifications.

[0097] In one embodiment, the present invention relates to a detergent composition comprising a polypeptide variant comprising the carbohydrate-binding module, having an activity on xanthan gum, preferably said activity on xanthan gum is a xanthan gum degrading activity, further preferably said xanthan gum degrading activity is EC 4.2.2.12 activity.

[0098] In an embodiment, polypeptide variant comprising the carbohydrate-binding module has an improved stability in a detergent composition compared to a parent enzyme (e.g., SEQ ID NO: 13 or 14).

[0099] In one embodiment, the present invention relates to a detergent composition comprising a polypeptide variant comprising the carbohydrate-binding module, having an improved stability in a detergent composition compared to the parent polypeptide (*e.g.*, with SEQ ID NO: 13 or 14); preferably said detergent composition comprises a chelator; further preferably said chelator is EDTA or citrate.

[0100] In one embodiment, the present invention relates to a detergent composition comprising a polypeptide variant comprising the carbohydrate-binding module of the invention, having a half-life improvement factor (HIF) of $\geq$1.0; preferably having a half-life improvement factor (HIF) of >1.0.

Parent

[0101] The parent polypeptide may be a polypeptide having at least 60% sequence identity to the polypeptide of SEQ ID NO: 13; (b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 17, or (ii) the full-length complement of (i); or (c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 17.

[0102] In an aspect, the parent has a sequence identity to the polypeptide of SEQ ID NO: 13 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, which have endoglucanase activity. In one aspect, the amino acid sequence of the parent differs by up to 10 amino acids, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from the mature polypeptide of SEQ ID NO: 13.

[0103] In another aspect, the parent comprises or consists of the amino acid sequence of SEQ ID NO: 13. In another aspect, the parent comprises or consists of the polypeptide of SEQ ID NO: 13. In another aspect, the parent is a fragment of the mature polypeptide of SEQ ID NO: 13 containing at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% or 95% of the number of amino acids of SEQ ID NO: 13. In another embodiment, the parent is an allelic variant of the polypeptide of SEQ ID NO: 13.

[0104] The parent polypeptide may be a polypeptide having at least 60% sequence identity to the polypeptide of SEQ ID NO: 14; (b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 18, or (ii) the full-length complement of (i); or (c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 18.

[0105] In an aspect, the parent has a sequence identity to the polypeptide of SEQ ID NO: 14 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, which have xanthan lyase activity. In one aspect, the amino acid sequence of the parent differs by up to 10 amino acids, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from the mature polypeptide of SEQ ID NO: 14.

[0106] In another aspect, the parent comprises or consists of the amino acid sequence of SEQ ID NO: 14. In another aspect, the parent comprises or consists of the polypeptide of SEQ ID NO: 14. In another aspect, the parent is a fragment of the mature polypeptide of SEQ ID NO: 14 containing at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% or 95% of the number of amino acids of SEQ ID NO: 14. In another embodiment, the parent is an allelic variant of the polypeptide of SEQ ID NO: 14.

[0107] In another aspect, the parent is encoded by a polynucleotide that hybridizes under very low stringency conditions, low stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 17 or 18, or (ii) the full-length complement of (i).

[0108] The polynucleotide of SEQ ID NO: 17 or 18, or a subsequence thereof, as well as the polypeptide of SEQ ID NO: 13 and 14 or a fragment thereof, may be used to design nucleic acid probes to identify and clone DNA encoding a parent from strains of different genera or species according to methods well known in the art. In particular, such probes can be used for hybridization with the genomic DNA or cDNA of a cell of interest, following standard Southern blotting procedures, in order to identify and isolate the corresponding gene therein. Such probes can be considerably shorter than the entire sequence, but should be at least 15, e.g., at least 25, at least 35, or at least 70 nucleotides in length. Preferably, the nucleic acid probe is at least 100 nucleotides in length, e.g., at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 600 nucleotides, at least 700 nucleotides, at least 800 nucleotides, or at least 900 nucleotides in length. Both DNA and RNA probes can be used. The probes are typically labeled for detecting the corresponding gene (for example, with ^{32}P, ^{3}H, ^{35}S, biotin, or avidin). Such probes are encompassed by the present invention.

[0109] A genomic DNA or cDNA library prepared from such other strains may be screened for DNA that hybridizes with the probes described above and encodes a parent. Genomic or other DNA from such other strains may be separated by agarose or polyacrylamide gel electrophoresis, or other separation techniques. DNA from the libraries or the separated DNA may be transferred to and immobilized on nitrocellulose or other suitable carrier material. In order to identify a clone or DNA that hybridizes with SEQ ID NO: 17 or 18, or a subsequence thereof, the carrier material is used in a Southern blot.

[0110] Hybridization indicates that the polynucleotide hybridizes to a labeled nucleic acid probe corresponding to (i) SEQ ID NO: 18 or 19; (ii) the mature polypeptide coding sequence of SEQ ID NO: 17 or 18; (iii) the full-length complement thereof; or (iv) a subsequence thereof; under very low to very high stringency conditions. Molecules to which the nucleic acid probe hybridizes under these conditions can be detected using, for example, X-ray film or any other detection means known in the art.

[0111] The nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 17. In another aspect, the nucleic acid probe is a polynucleotide that encodes the polypeptide of SEQ ID NO: 13; the mature polypeptide thereof; or a fragment thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 17.

[0112] The parent can be encoded by a polynucleotide having a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 17 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

[0113] The nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 18. In another aspect, the nucleic acid probe is a polynucleotide that encodes the polypeptide of SEQ ID NO: 14; the mature polypeptide thereof; or a fragment thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 18.

[0114] The parent can be encoded by a polynucleotide having a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 20 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

[0115] The polypeptide may be a hybrid polypeptide in which a region of one polypeptide is fused at the N-terminus or the C-terminus of a region of another polypeptide.

[0116] The parent may be a fusion polypeptide or cleavable fusion polypeptide in which another polypeptide is fused at the N-terminus or the C-terminus of the polypeptide. A fusion polypeptide is produced by fusing a polynucleotide

encoding another polypeptide to a polynucleotide. Techniques for producing fusion polypeptides are known in the art, and include ligating the coding sequences encoding the polypeptides so that they are in frame and that expression of the fusion polypeptide is under control of the same promoter(s) and terminator. Fusion polypeptides may also be constructed using intein technology in which fusion polypeptides are created post-translationally.

[0117] A fusion polypeptide can further comprise a cleavage site between the two polypeptides. Upon secretion of the fusion protein, the site is cleaved releasing the two polypeptides.

[0118] The parent may be obtained from microorganisms of any genus. For purposes of the present invention, the term "obtained from" as used herein in connection with a given source shall mean that the parent encoded by a polynucleotide is produced by the source or by a strain in which the polynucleotide from the source has been inserted. In one aspect, the parent is secreted extracellularly.

[0119] The parent may be a bacterial enzyme. For example, the parent may be a Gram-positive bacterial polypeptide such as a *Bacillus, Clostridium, Enterococcus, Geobacillus, Lactobacillus, Lactococcus, Oceanobacillus, Staphylococcus, Streptococcus,* or *Streptomyces* enzyme, or a Gram-negative bacterial polypeptide such as a *Campylobacter, E. coli, Flavobacterium, Fusobacterium, Helicobacter, Ilyobacter, Neisseria, Pseudomonas, Salmonella,* or *Ureaplasma* enzyme.

[0120] In one aspect, the parent is a *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis,* or *Bacillus thuringiensis* enzyme.

[0121] In another aspect, the parent is a *Streptococcus equisimilis, Streptococcus pyogenes, Streptococcus uberis,* or *Streptococcus equi* subsp. *Zooepidemicus* enzyme.

[0122] In another aspect, the parent is a *Streptomyces achromogenes, Streptomyces avermitilis, Streptomyces coelicolor, Streptomyces griseus,* or *Streptomyces lividans* enzyme.

[0123] The parent may be a fungal enzyme. For example, the parent may be a yeast enzyme such as a *Candida, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces,* or *Yarrowia* enzyme; or a filamentous fungal enzyme such as an *Acremonium, Agaricus, Alternaria, Aspergillus, Aureobasidium, Botryospaeria, Ceriporiopsis, Chaetomidium, Chrysosporium, Claviceps, Cochliobolus, Coprinopsis, Coptotermes, Corynascus, Cryphonectria, Cryptococcus, Diplodia, Exidia, Filibasidium, Fusarium, Gibberella, Holomastigotoides, Humicola, Irpex, Lentinula, Leptospaeria, Magnaporthe, Melanocarpus, Meripilus, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Piromyces, Poitrasia, Pseudoplectania, Pseudotrichonympha, Rhizomucor, Schizophyllum, Scytalidium, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trichoderma, Trichophaea, Verticillium, Volvariella,* or *Xylaria* enzyme.

[0124] In another aspect, the parent is a *Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis,* or *Saccharomyces oviformis* enzyme.

[0125] In another aspect, the parent is an *Acremonium cellulolyticus, Aspergillus aculeatus, Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola grisea, Humicola insolens, Humicola lanuginosa, Irpex lacteus, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium funiculosum, Penicillium purpurogenum, Phanerochaete chrysosporium, Thielavia achromatica, Thielavia albomyces, Thielavia albopilosa, Thielavia australeinsis, Thielavia fimeti, Thielavia microspora, Thielavia ovispora, Thielavia peruviana, Thielavia setosa, Thielavia spededonium, Thielavia subthermophila, Thielavia terrestris, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei,* or *Trichoderma viride* enzyme.

[0126] In another aspect, the parent is a *Paenibacillus sp.* endoglucanase, e.g., the endoglucanase of SEQ ID NO: 13.

[0127] In another aspect, the parent is a *Paenibacillus sp.* xanthan lyase, e.g., the xanthan lyase of SEQ ID NO: 14.

[0128] It will be understood that for the aforementioned species, the invention encompasses both the perfect and imperfect states, and other taxonomic equivalents, e.g., anamorphs, regardless of the species name by which they are known. Those skilled in the art will readily recognize the identity of appropriate equivalents.

[0129] Strains of these species are readily accessible to the public in a number of culture collections, such as the American Type Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Centraalbureau Voor Schimmelcultures (CBS), and Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL).

[0130] The parent may be identified and obtained from other sources including microorganisms isolated from nature (e.g., soil, composts, water, etc.) or DNA samples obtained directly from natural materials (e.g., soil, composts, water,

etc.) using the above-mentioned probes. Techniques for isolating microorganisms and DNA directly from natural habitats are well known in the art. A polynucleotide encoding a parent may then be obtained by similarly screening a genomic DNA or cDNA library of another microorganism or mixed DNA sample. Once a polynucleotide encoding a parent has been detected with the probe(s), the polynucleotide can be isolated or cloned by utilizing techniques that are known to those of ordinary skill in the art.

Preparation of carbohydrate-binding module

[0131] Methods for obtaining a carbohydrate-binding module (CBM) able to bind xanthan gum, comprise: (a) introducing into a plasmid the carbohydrate-binding module gene, (b) expressing carbohydrate-binding module, and (b) recovering the carbohydrate-binding module.

[0132] The CBMs can be prepared using any mutagenesis procedure known in the art, such as standard PCR techniques, synthetic gene construction, semi-synthetic gene construction, random mutagenesis, shuffling, etc.

[0133] The standard PCR techniques can be accomplished *in vitro* by PCR involving the use of oligonucleotide primers directed to the gene of the CBM.

[0134] A polypeptide comprising the carbohydrate-binding module may be a variant of a parent polypeptide. Methods for obtaining such polypeptide variants comprise the steps of (a) introducing one or more modifications in the polypeptide, and (b) recovering the polypeptide variant.

[0135] The polypeptide variants may be prepared using any mutagenesis procedure known in the art, such as site-directed mutagenesis, synthetic gene construction, semi-synthetic gene construction, random mutagenesis, shuffling, etc.

[0136] Site-directed mutagenesis is a technique in which one or more (e.g., several) mutations are introduced at one or more defined sites in a polynucleotide encoding the parent polypeptide.

[0137] Site-directed mutagenesis may also be performed *in vitro* by cassette mutagenesis involving the cleavage by a restriction enzyme at a site in the plasmid comprising a polynucleotide encoding the parent and subsequent ligation of an oligonucleotide containing the mutation in the polynucleotide. Usually the restriction enzyme that digests the plasmid and the oligonucleotide is the same, permitting sticky ends of the plasmid and the insert to ligate to one another.

[0138] Site-directed mutagenesis can also be accomplished *in vivo* by methods known in the art.

[0139] Any site-directed mutagenesis procedure can be used in the present invention. There are many commercial kits available that can be used to prepare variants.

[0140] Synthetic gene construction entails *in vitro* synthesis of a designed polynucleotide molecule to encode a polypeptide of interest. Gene synthesis can be performed utilizing a number of techniques, such as the multiplex microchip-based technology and similar technologies wherein oligonucleotides are synthesized and assembled upon photo-programmable microfluidic chips.

[0141] Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure. Other methods that can be used include error-prone PCR, phage display (e.g., U.S. Patent No. 5,223,409; WO 92/06204) and region-directed mutagenesis.

[0142] Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides expressed by host cells. Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using standard methods in the art. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide.

[0143] Semi-synthetic gene construction is accomplished by combining aspects of synthetic gene construction, and/or site-directed mutagenesis, and/or random mutagenesis, and/or shuffling. Semi-synthetic construction is typified by a process utilizing polynucleotide fragments that are synthesized, in combination with PCR techniques. Defined regions of genes may thus be synthesized *de novo,* while other regions may be amplified using site-specific mutagenic primers, while yet other regions may be subjected to error-prone PCR or non-error prone PCR amplification. Polynucleotide subsequences may then be shuffled.

Polynucleotides

[0144] Polynucleotides encode a carbohydrate-binding module as comprised in the detergent compositions of the present invention. These polynucleotides may be isolated polynucleotides.

[0145] The techniques used to isolate or clone a polynucleotide are known in the art and include isolation from genomic DNA or cDNA, or a combination thereof. The cloning of the polynucleotides from genomic DNA can be effected, *e.g.*, by using the well-known polymerase chain reaction (PCR) or antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. Other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligation activated transcription (LAT) and polynucleotide-based amplification (NASBA) may be used. The poly-

nucleotides may be cloned in a strain of *Bacillus subtilis* or *E. coli,* or a related organism and thus, for example, may be an allelic or species variant of the polypeptide encoding region of the polynucleotide.

[0146] Modification of a polynucleotide encoding a polypeptide may be necessary for synthesizing polypeptides substantially similar to the polypeptide. The term "substantially similar" to the polypeptide refers to non-naturally occurring forms of the polypeptide. These polypeptides may differ in some engineered way from the polypeptide isolated from its native source, e.g., variants that differ in specific activity, thermostability, pH optimum, or the like. The variants may be constructed on the basis of the polynucleotide presented as the mature polypeptide coding sequence of SEQ ID NO: 1, *e.g.,* a subsequence thereof, and/or by introduction of nucleotide substitutions that do not result in a change in the amino acid sequence of the polypeptide, but which correspond to the codon usage of the host organism intended for production of the enzyme, or by introduction of nucleotide substitutions that may give rise to a different amino acid sequence.

Nucleic acid constructs

[0147] Nucleic acid constructs comprise a polynucleotide encoding a carbohydrate-binding module operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences. Nucleic acid constructs comprise a polynucleotide encoding a polypeptide comprising a carbohydrate-binding module operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

[0148] A polynucleotide may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the polynucleotide prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides utilizing recombinant DNA methods are well known in the art.

[0149] The control sequence may be a promoter, a polynucleotide that is recognized by a host cell for expression of a polynucleotide encoding a polypeptide of the present invention. The promoter contains transcriptional control sequences that mediate the expression of the polypeptide. The promoter may be any polynucleotide that shows transcriptional activity in the host cell including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

[0150] Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a bacterial host cell are the promoters obtained from the *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus licheniformis* penicillinase gene *(penP), Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus subtilis* levansucrase gene *(sacB), Bacillus subtilis xylA* and *xylB* genes, *Bacillus thuringiensis cryIIIA* gene, *E. coli lac* operon, *E. coli trc* promoter, *Streptomyces coelicolor* agarase gene (*dagA*), and prokaryotic beta-lactamase gene, as well as the *tac* promoter. Examples of tandem promoters are disclosed in WO 99/43835.

[0151] Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*), *Aspergillus oryzae* TAKA amylase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Fusarium oxysporum* trypsin-like protease (WO 96/00787), *Fusarium venenatum* amyloglucosidase (WO 00/56900), *Fusarium venenatum* Daria (WO 00/56900), *Fusarium venenatum* Quinn (WO 00/56900), *Rhizomucor miehei* lipase, *Rhizomucor miehei* aspartic proteinase, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei* cellobiohydrolase I, *Trichoderma reesei cellobiohydrolase* I *Trichoderma reesei xanthan* lyase I, *Trichoderma reesei xanthan* lyase II, *Trichoderma reesei* xanthan lyase III, *Trichoderma reesei* xanthan lyase IV, *Trichoderma reesei* xanthan lyase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* beta-xylosidase, as well as the NA2-tpi promoter (a modified promoter from an *Aspergillus* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus* triose phosphate isomerase gene; non-limiting examples include modified promoters from an *Aspergillus niger* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus nidulans* or *Aspergillus oryzae* triose phosphate isomerase gene); and mutant, truncated, and hybrid promoters thereof.

[0152] In a yeast host, useful promoters are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* galactokinase (GAL1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH1, ADH2/GAP), *Saccharomyces cerevisiae* triose phosphate isomerase (TPI), *Saccharomyces cerevisiae* metallothionein (CUP1), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase.

[0153] The control sequence may also be a transcription terminator, which is recognized by a host cell to terminate transcription. The terminator is operably linked to the 3'-terminus of the polynucleotide encoding the polypeptide. Any terminator that is functional in the host cell may be used in the present invention.

[0154] Preferred terminators for bacterial host cells are obtained from the genes for *Bacillus clausii* alkaline protease (*aprH*), *Bacillus licheniformis* alpha-amylase (*amyL*), and *Escherichia coli* ribosomal RNA (*rrnB*).

[0155] Preferred terminators for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans*

anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase, *Aspergillus oryzae* TAKA amylase, and *Fusarium oxysporum* trypsin-like protease.

[0156] Preferred terminators for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase.

[0157] The control sequence may also be an mRNA stabilizer region downstream of a promoter and upstream of the coding sequence of a gene which increases expression of the gene.

[0158] Examples of suitable mRNA stabilizer regions are obtained from a *Bacillus thuringiensis cryIIIA* gene (WO 94/25612) and a *Bacillus subtilis* SP82 gene.

[0159] The control sequence may also be a leader, a nontranslated region of an mRNA that is important for translation by the host cell. The leader is operably linked to the 5'-terminus of the polynucleotide encoding the polypeptide. Any leader that is functional in the host cell may be used.

[0160] Preferred leaders for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase and *Aspergillus nidulans* triose phosphate isomerase.

[0161] Suitable leaders for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP).

[0162] The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3'-terminus of the polynucleotide and, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence that is functional in the host cell may be used.

[0163] Preferred polyadenylation sequences for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase *Aspergillus oryzae* TAKA amylase, and *Fusarium oxysporum* trypsin-like protease.

[0164] The control sequence may also be a signal peptide coding region that encodes a signal peptide linked to the N-terminus of a polypeptide and directs the polypeptide into the cell's secretory pathway. The 5'-end of the coding sequence of the polynucleotide may inherently contain a signal peptide coding sequence naturally linked in translation reading frame with the segment of the coding sequence that encodes the polypeptide. Alternatively, the 5'-end of the coding sequence may contain a signal peptide coding sequence that is foreign to the coding sequence. A foreign signal peptide coding sequence may be required where the coding sequence does not naturally contain a signal peptide coding sequence. Alternatively, a foreign signal peptide coding sequence may simply replace the natural signal peptide coding sequence in order to enhance secretion of the polypeptide. However, any signal peptide coding sequence that directs the expressed polypeptide into the secretory pathway of a host cell may be used.

[0165] Effective signal peptide coding sequences for bacterial host cells are the signal peptide coding sequences obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* beta-lactamase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus stearothermophilus* neutral proteases (*nprT, nprS, nprM*), and *Bacillus subtilis prsA.*

[0166] Effective signal peptide coding sequences for filamentous fungal host cells are the signal peptide coding sequences obtained from the genes for *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Aspergillus oryzae* TAKA amylase, *Humicola insolens* cellulase, *Humicola insolens* xanthan lyase V, *Humicola lanuginosa* lipase, and *Rhizomucor miehei* aspartic proteinase.

[0167] Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase.

[0168] The control sequence may also be a propeptide coding sequence that encodes a propeptide positioned at the N-terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to an active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding sequence may be obtained from the genes for *Bacillus subtilis* alkaline protease (*aprE*), *Bacillus subtilis* neutral protease (*nprT*), *Myceliophthora thermophila* laccase (WO 95/33836), *Rhizomucor miehei* aspartic proteinase, and *Saccharomyces cerevisiae* alpha-factor.

[0169] Where both signal peptide and propeptide sequences are present, the propeptide sequence is positioned next to the N-terminus of a polypeptide and the signal peptide sequence is positioned next to the N-terminus of the propeptide sequence.

[0170] It may also be desirable to add regulatory sequences that regulate expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those that cause expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems include the *lac, tac,* and *trp* operator systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the *Aspergillus niger* glucoamylase promoter, *Aspergillus oryzae* TAKA alpha-amylase promoter, and *Aspergillus oryzae* glucoamylase promoter may be used. Other examples of regulatory sequences are

those that allow for gene amplification. In eukaryotic systems, these regulatory sequences include the dihydrofolate reductase gene that is amplified in the presence of methotrexate, and the metallothionein genes that are amplified with heavy metals. In these cases, the polynucleotide encoding the polypeptide would be operably linked with the regulatory sequence.

Expression vectors

[0171] Recombinant expression vectors comprise a polynucleotide encoding a carbohydrate-binding module, a promoter, and transcriptional and translational stop signals. The various nucleotide and control sequences may be joined together to produce a recombinant expression vector that may include one or more convenient restriction sites to allow for insertion or substitution of the polynucleotide encoding the polypeptide at such sites. Alternatively, the polynucleotide may be expressed by inserting the polynucleotide or a nucleic acid construct comprising the polynucleotide into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

[0172] The recombinant expression vector may be any vector (*e.g.*, a plasmid or virus) that can be conveniently subjected to recombinant DNA procedures and can bring about expression of the polynucleotide. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may be a linear or closed circular plasmid.

[0173] The vector may be an autonomously replicating vector, i.e., a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one that, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids that together contain the total DNA to be introduced into the genome of the host cell, or a transposon, may be used.

[0174] The vector preferably contains one or more selectable markers that permit easy selection of transformed, transfected, transduced, or the like cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

[0175] Examples of bacterial selectable markers are *Bacillus licheniformis* or *Bacillus subtilis dal* genes, or markers that confer antibiotic resistance such as ampicillin, chloramphenicol, kanamycin, neomycin, spectinomycin, or tetracycline resistance. Suitable markers for yeast host cells include, but are not limited to, ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus* cell are *Aspergillus nidulans* or *Aspergillus oryzae amdS* and *pyrG* genes and a *Streptomyces hygroscopicus bar* gene.

[0176] The vector preferably contains an element(s) that permits integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

[0177] For integration into the host cell genome, the vector may rely on the polynucleotide's sequence encoding the polypeptide or any other element of the vector for integration into the genome by homologous or non-homologous recombination. Alternatively, the vector may contain additional polynucleotides for directing integration by homologous recombination into the genome of the host cell at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should contain a sufficient number of nucleic acids, such as 100 to 10,000 base pairs, 400 to 10,000 base pairs, and 800 to 10,000 base pairs, which have a high degree of sequence identity to the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding polynucleotides. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

[0178] For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. The origin of replication may be any plasmid replicator mediating autonomous replication that functions in a cell. The term "origin of replication" or "plasmid replicator" means a polynucleotide that enables a plasmid or vector to replicate *in vivo.*

[0179] Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in *E. coli,* and pUB110, pE194, pTA1060, and pAMß1 permitting replication in *Bacillus.*

[0180] Examples of origins of replication for use in a yeast host cell are the 2 micron origin of replication, ARS1, ARS4, the combination of ARS1 and CEN3, and the combination of ARS4 and CEN6.

[0181] Examples of origins of replication useful in a filamentous fungal cell are AMA1 and ANS1. Isolation of the AMA1

gene and construction of plasmids or vectors comprising the gene can be accomplished according to the methods disclosed in WO 00/24883.

[0182] More than one copy of a polynucleotide of the present invention may be inserted into a host cell to increase production of a polypeptide. An increase in the copy number of the polynucleotide can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the polynucleotide where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the polynucleotide, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

[0183] The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art.

Host cells

[0184] Recombinant host cells comprise a polynucleotide encoding a carbohydrate-binding module as described herein operably linked to one or more control sequences that direct the production of a carbohydrate-binding module. Recombinant host cells can further comprise a polynucleotide encoding a polypeptide comprising a carbohydrate-binding module as described herein operably linked to one or more control sequences that direct the production of a carbohydrate-binding module. A construct or vector comprising a polynucleotide is introduced into a host cell so that the construct or vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector as described earlier. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication. The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source.

[0185] The host cell may be any cell useful in the recombinant production of a polypeptide of the present invention, e.g., a prokaryote or a eukaryote.

[0186] The prokaryotic host cell may be any Gram-positive or Gram-negative bacterium. Gram-positive bacteria include, but are not limited to, *Bacillus, Clostridium, Enterococcus, Geobacillus, Lactobacillus, Lactococcus, Oceanobacillus, Staphylococcus, Streptococcus,* and *Streptomyces.* Gram-negative bacteria include, but are not limited to, *Campylobacter, E. coli, Flavobacterium, Fusobacterium, Helicobacter, Ilyobacter, Neisseria, Pseudomonas, Salmonella,* and *Ureaplasma.*

[0187] The bacterial host cell may be any Bacillus cell including, but not limited to, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, and Bacillus thuringiensis cells.

[0188] The bacterial host cell may also be any Streptococcus cell including, but not limited to, Streptococcus equisimilis, Streptococcus pyogenes, Streptococcus uberis, and Streptococcus equi subsp. Zooepidemicus cells.

[0189] The bacterial host cell may also be any Streptomyces cell including, but not limited to, Streptomyces achromogenes, Streptomyces avermitilis, Streptomyces coelicolor, Streptomyces griseus, and Streptomyces lividans cells.

[0190] The introduction of DNA into a *Bacillus* cell may be effected by protoplast transformation, competent cell transformation, electroporation, or conjugation. The introduction of DNA into an *E. coli* cell may be effected by protoplast transformation or electroporation. The introduction of DNA into a *Streptomyces* cell may be effected by protoplast transformation, electroporation, conjugation, or transduction. The introduction of DNA into a *Pseudomonas* cell may be effected by electroporation or conjugation. The introduction of DNA into a *Streptococcus* cell may be effected by natural competence, protoplast transformation, electroporation, or conjugation. However, any method known in the art for introducing DNA into a host cell can be used.

[0191] The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell.

[0192] The host cell may be a fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota as well as the Oomycota and all mitosporic fungi.

[0193] The fungal host cell may be a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (Endomycetales), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (Blastomycetes).

[0194] The yeast host cell may be a Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces, or Yarrowia cell, such as a Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis, or Yarrowia lipolytica cell.

[0195] The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision Eumycota and Oomycota. The filamentous fungi are generally characterized by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative.

[0196] The filamentous fungal host cell may be an Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceripori-

opsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes, or Trichoderma cell.

[0197] For example, the filamentous fungal host cell may be an Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, or Trichoderma viride cell.

[0198] Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known *per se.* Suitable procedures for transformation of *Aspergillus* and *Trichoderma* host cells are described for example in EP 238023. Suitable methods for transforming *Fusarium* species are described by WO 96/00787.

Methods of production

[0199] Methods of producing (*e.g.*, in vitro or ex vivo methods) a carbohydrate-binding module comprise: (a) cultivating a host cell under conditions suitable for expression of the carbohydrate-binding module; and (b) recovering the carbohydrate-binding module. The present invention also relates to methods of producing (*e.g.*, in vitro or ex vivo methods) a polypeptide comprising a carbohydrate-binding module, comprising: (a) cultivating a host cell under conditions suitable for expression of the carbohydrate-binding module; and (b) recovering the polypeptide.

[0200] Methods of producing (*e.g.*, in vitro or ex vivo methods) a carbohydrate-binding module as described herein comprise (a) cultivating a cell, which in its wild-type form produces the polypeptide, under conditions conducive for production of the polypeptide; and (b) recovering the carbohydrate-binding module. In a preferred aspect, the cell is a *Paenibacillus* cell, or a *Microbacterium* cell.

[0201] Methods of producing (*e.g.*, in vitro or ex vivo methods) a carbohydrate-binding module as described herein compris (a) cultivating a recombinant host cell of the present invention under conditions conducive for production of the carbohydrate-binding module; and (b) recovering the carbohydrate-binding module.

[0202] The host cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, or small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

[0203] The carbohydrate-binding module may be detected using methods known in the art that are specific for the carbohydrate-binding modules such as methods for determining endoglucanase or xanthan lyase activity. These detection methods include, but are not limited to, use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the polypeptide.

[0204] The carbohydrate-binding module may be recovered using methods known in the art. For example, the carbohydrate-binding module may be recovered from the nutrient medium by conventional procedures including, but not limited to, collection, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

[0205] The carbohydrate-binding module may be purified by a variety of procedures known in the art including, but not limited to, chromatography (*e.g.*, ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (*e.g.*, preparative isoelectric focusing), differential solubility (*e.g.*, ammonium sulfate precipitation), SDS-PAGE, or extraction to obtain substantially pure polypeptides.

[0206] In an alternative aspect, the carbohydrate-binding module is not recovered, but rather a host cell of the present invention expressing the carbohydrate-binding module is used as a source of the carbohydrate-binding module.

Compositions

[0207] The carbohydrate-binding modules comprised in the detergent compositions according to the invention have the ability to bind and/or facilitate binding to xanthan gum. Accordingly, inclusion of the carbohydrate-binding modules in such detergent composition is advantageous. In particular, such detergent compositions may be used for cleaning a surface, such as a textile or hard surface, such as a plate.

[0208] Besides the carbohydrate-binding module, the detergent compositions comprise additional components, such as e.g. enzymes. The choice of further additional components is within the skill of the artisan and includes conventional ingredients, including the exemplary non-limiting components set forth below. The choice of components may include, for fabric care, the consideration of the type of fabric to be cleaned, the type and/or degree of soiling, the temperature at which cleaning is to take place, and the formulation of the detergent product. Although components mentioned below are categorized by general header according to a particular functionality, this is not to be construed as a limitation, as a component may comprise additional functionalities as will be appreciated by the skilled artisan.

[0209] The detergent composition may be suitable for the laundering of textiles such as e.g. fabrics, cloths or linen, or for cleaning hard surfaces such as e.g. floors, tables, or dish wash.

Detergent compositions

[0210] In one embodiment, a carbohydrate-binding module of the present invention may be added to a detergent composition in an amount corresponding to 0.0001-200 mg of protein, such as 0.0005-100 mg of protein, preferably 0.001-30 mg of protein, more preferably 0.005-8 mg of protein, even more preferably 0.01-2 mg of protein per litre of wash liquor.

[0211] A composition for use in automatic dishwash (ADW), for example, may include 0.0001 %-50%, such as 0.001 %-20%, such as 0.01%-10%, such as 0.05-5% of protein by weight of the composition.

[0212] A composition for use in laundry granulation, for example, may include 0.0001 %-50%, such as 0.001 %-20%, such as 0.01%-10%, such as 0.05%-5% of enzyme protein by weight of the composition.

[0213] A composition for use in laundry liquid, for example, may include 0.0001%-10%, such as 0.001-7%, such as 0.1 %-5% of protein by weight of the composition.

[0214] The polypeptide(s) of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in, for example, WO92/19709 and WO92/19708.

[0215] In certain markets different wash conditions and, as such, different types of detergents are used. This is disclosed in e.g. EP 1 025 240. For example, In Asia (Japan) a low detergent concentration system is used, while the United States uses a medium detergent concentration system, and Europe uses a high detergent concentration system.

[0216] A low detergent concentration system includes detergents where less than about 800 ppm of detergent components are present in the wash water. Japanese detergents are typically considered low detergent concentration system as they have approximately 667 ppm of detergent components present in the wash water.

[0217] A medium detergent concentration includes detergents where between about 800 ppm and about 2000ppm of detergent components are present in the wash water. North American detergents are generally considered to be medium detergent concentration systems as they have approximately 975 ppm of detergent components present in the wash water.

[0218] A high detergent concentration system includes detergents where greater than about 2000 ppm of detergent components are present in the wash water. European detergents are generally considered to be high detergent concentration systems as they have approximately 4500-5000 ppm of detergent components in the wash water.

[0219] Latin American detergents are generally high suds phosphate builder detergents and the range of detergents used in Latin America can fall in both the medium and high detergent concentrations as they range from 1500 ppm to 6000 ppm of detergent components in the wash water. Such detergent compositions are all embodiments of the invention.

[0220] A polypeptide of the present invention may also be incorporated in the detergent formulations disclosed in WO97/07202, which is hereby incorporated by reference.

Surfactants

[0221] The detergent composition may comprise one or more surfactants, which may be anionic and/or cationic and/or non-ionic and/or semi-polar and/or zwitterionic, or a mixture thereof. In a particular embodiment, the detergent composition includes a mixture of one or more nonionic surfactants and one or more anionic surfactants. The surfactant(s) is typically present at a level of from about 0.1 % to 60% by weight, such as about 1% to about 40%, or about 3% to about 20%, or about 3% to about 10%. The surfactant(s) is chosen based on the desired cleaning application, and includes

any conventional surfactant(s) known in the art. Any surfactant known in the art for use in detergents may be utilized.

[0222] When included therein the detergent will usually comprise from about 1 % to about 40% by weight, such as from about 5% to about 30%, including from about 5% to about 15%, or from about 20% to about 25% of an anionic surfactant. Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, linear alkylbenzenesulfonates (LAS), isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS), primary alcohol sulfates (PAS), alcohol ethersulfates (AES or AEOS or FES, also known as alcohol ethoxysulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenyl/tetradecenyl succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or soap, and combinations thereof.

[0223] When included therein the detergent will usually comprise from about 0% to about 10% by weight of a cationic surfactant. Non-limiting examples of cationic surfactants include alkldimethylethanolamine quat (ADMEAQ), cetyltrimethylammonium bromide (CTAB), dimethyldistearylammonium chloride (DSDMAC), and alkylbenzyldimethylammonium, alkyl quaternary ammonium compounds, alkoxylated quaternary ammonium (AQA) compounds, and combinations thereof.

[0224] When included therein the detergent will usually comprise from about 0.2% to about 40% by weight of a non-ionic surfactant, for example from about 0.5% to about 30%, in particular from about 1% to about 20%, from about 3% to about 10%, such as from about 3% to about 5%, or from about 8% to about 12%. Non-limiting examples of non-ionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxylated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxylated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxy alkyl fatty acid amides, or *N*-acyl *N*-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamide, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof.

[0225] When included therein the detergent will usually comprise from about 0% to about 10% by weight of a semipolar surfactant. Non-limiting examples of semipolar surfactants include amine oxides (AO) such as alkyldimethylamineoxide, *N*-(coco alkyl)-*N,N*-dimethylamine oxide and *N*-(tallow-alkyl)-*N,N*-bis(2-hydroxyethyl)amine oxide, fatty acid alkanolamides and ethoxylated fatty acid alkanolamides, and combinations thereof.

[0226] When included therein the detergent will usually comprise from about 0% to about 10% by weight of a zwitterionic surfactant. Non-limiting examples of zwitterionic surfactants include betaine, alkyldimethylbetaine, sulfobetaine, and combinations thereof.

Hydrotropes

[0227] A hydrotrope is a compound that solubilises hydrophobic compounds in aqueous solutions (or oppositely, polar substances in a non-polar environment). Typically, hydrotropes have both hydrophilic and a hydrophobic character (so-called amphiphilic properties as known from surfactants); however, the molecular structure of hydrotropes generally do not favor spontaneous self-aggregation, see e.g. review by Hodgdon and Kaler (2007), Current Opinion in Colloid & Interface Science 12: 121-128. Hydrotropes do not display a critical concentration above which self-aggregation occurs as found for surfactants and lipids forming miceller, lamellar or other well defined meso-phases. Instead, many hydrotropes show a continuous-type aggregation process where the sizes of aggregates grow as concentration increases. However, many hydrotropes alter the phase behaviour, stability, and colloidal properties of systems containing substances of polar and non-polar character, including mixtures of water, oil, surfactants, and polymers. Hydrotropes are classically used across industries from pharma, personal care, food, to technical applications. Use of hydrotropes in detergent compositions allow for example more concentrated formulations of surfactants (as in the process of compacting liquid detergents by removing water) without inducing undesired phenomena such as phase separation or high viscosity.

[0228] The detergent may contain 0-5% by weight, such as about 0.5 to about 5%, or about 3% to about 5%, of a hydrotrope. Any hydrotrope known in the art for use in detergents may be utilized. Non-limiting examples of hydrotropes include sodium benzene sulfonate, sodium p-toluene sulfonate (STS), sodium xylene sulfonate (SXS), sodium cumene sulfonate (SCS), sodium cymene sulfonate, amine oxides, alcohols and polyglycolethers, sodium hydroxynaphthoate, sodium hydroxynaphthalene sulfonate, sodium ethylhexyl sulfate, and combinations thereof.

Builders and Co-Builders

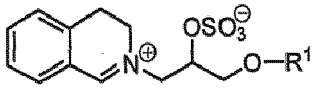
[0229] The detergent composition may contain about 0-65% by weight, such as about 5% to about 45% of a detergent

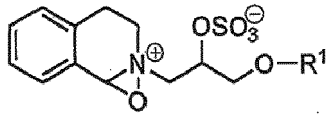
builder or co-builder, or a mixture thereof. In a dish wash deteregent, the level of builder is typically 40-65%, particularly 50-65%. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in laundry detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (e.g., SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as iminodiethanol), triethanolamine (TEA, also known as 2,2',2"-nitrilotriethanol), and carboxymethyl inulin (CMI), and combinations thereof.

[0230] The detergent composition may also contain 0-20% by weight, such as about 5% to about 10%, of a detergent co-builder, or a mixture thereof. The detergent composition may include include a co-builder alone, or in combination with a builder, for example a zeolite builder. Non-limiting examples of co-builders include homopolymers of polyacrylates or copolymers thereof, such as poly(acrylic acid) (PAA) or copoly(acrylic acid/maleic acid) (PAA/PMA). Further non-limiting examples include citrate, chelators such as aminocarboxylates, aminopolycarboxylates and phosphonates, and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2"-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-*N*,*N*'-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-N,N-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra-(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis(methylenephosphonic acid) (DTPMPA or DTMPA), *N*-(*2*-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-N-monoacetic acid (ASMA), aspartic acid-*N*,*N*-diacetic acid (ASDA), aspartic acid-*N*-monopropionic acid (ASMP), iminodisuccinic acid (IDA), *N*-(2-sulfomethyl)-aspartic acid (SMAS), *N*-(2-sulfoethyl)-aspartic acid (SEAS), *N*-(2-sulfomethyl)-glutamic acid (SMGL), *N*-(*2*-sulfoethyl)-glutamic acid (SEGL), *N*-methyliminodiacetic acid (MIDA), α-alanine-*N*,*N*-diacetic acid (α-ALDA), serine-*N*, *N*-diacetic acid (SEDA), isoserine-*N*, *N*-diacetic acid (ISDA), phenylalanine-*N*,*N*-diacetic acid (PHDA), anthranilic acid-N, N-diacetic acid (ANDA), sulfanilic acid-N, N-diacetic acid (SLDA), taurine-N, N-diacetic acid (TUDA) and sulfomethyl-*N*, *N*-diacetic acid (SMDA), *N*-(2-hydroxyethyl)-ethylidenediamine-*N*, *N',N'*-triacetate (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053

Bleaching Systems

[0231] The detergent may comprise 0-50% by weight, such as about 0.1% to about 25%, of a bleaching system. Any bleaching system known in the art for use in laundry detergents may be utilized. Suitable bleaching system components include bleaching catalysts, photobleaches, bleach activators, sources of hydrogen peroxide such as sodium percarbonate and sodium perborates, preformed peracids and mixtures thereof. Suitable preformed peracids include, but are not limited to, peroxycarboxylic acids and salts, percarbonic acids and salts, perimidic acids and salts, peroxymonosulfuric acids and salts, for example, Oxone (R), and mixtures thereof. Non-limiting examples of bleaching systems include peroxide-based bleaching systems, which may comprise, for example, an inorganic salt, including alkali metal salts such as sodium salts of perborate (usually mono- or tetra-hydrate), percarbonate, persulfate, perphosphate, persilicate salts, in combination with a peracid-forming bleach activator. The term bleach activator is meant herein as a compound which reacts with peroxygen bleach like hydrogen peroxide to form a peracid. The peracid thus formed constitutes the activated bleach. Suitable bleach activators to be used herein include those belonging to the class of esters amides, imides or anhydrides. Suitable examples are tetracetylethylene diamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene sulfonate (ISONOBS), diperoxy dodecanoic acid, 4-(dodecanoyloxy)benzenesulfonate (LOBS), 4-(decanoyloxy)benzenesulfonate, 4-(decanoyloxy)benzoate (DOBS), 4-(nonanoyloxy)-benzenesulfonate (NOBS), and/or those disclosed in WO98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particulary preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that it is environmental friendly as it eventually degrades into citric acid and alcohol. Furthermore acetyl triethyl citrate and triacetin has a good hydrolytical stability in the product upon storage and it is an efficient bleach activator. Finally ATC provides a good building capacity to the laundry additive. Alternatively, the bleaching system may comprise peroxyacids of, for example, the amide, imide, or sulfone type. The bleaching system may also comprise peracids such as 6-(phthalimido)peroxyhexanoic acid (PAP). The bleaching system may also include a bleach catalyst. In some embodiments the bleach component may be an organic catalyst selected from the group consisting of organic catalysts having the following formulae:

(i) 

(ii) 

(iii) and mixtures thereof; wherein each R^1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R^1 is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R^1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, iso-nonyl, iso-decyl, iso-tridecyl and iso-pentadecyl. Other exemplary bleaching systems are described, e.g. in WO2007/087258, WO2007/087244, WO2007/087259 and WO2007/087242. Suitable photobleaches may for example be sulfonated zinc phthalocyanine

Polymers

[0232] The detergent may comprise 0-10% by weight, such as 0.5-5%, 2-5%, 0.5-2% or 0.2-1 % of a polymer. Any polymer known in the art for use in detergents may be utilized. The polymer may function as a co-builder as mentioned above, or may provide antiredeposition, fiber protection, soil release, dye transfer inhibition, grease cleaning and/or anti-foaming properties. Some polymers may have more than one of the above-mentioned properties and/or more than one of the below-mentioned motifs. Exemplary polymers include (carboxymethyl)cellulose (CMC), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) or poly(ethylene oxide) (PEG), ethoxylated poly(ethyleneimine), carboxymethyl inulin (CMI), and polycarboxylates such as PAA, PAA/PMA, poly-aspartic acid, and lauryl methacrylate/acrylic acid copolymers , hydrophobically modified CMC (HM-CMC) and silicones, copolymers of terephthalic acid and oligomeric glycols, copolymers of poly(ethylene terephthalate) and poly(oxyethene terephthalate) (PET-POET), PVP, poly(vinylimidazole) (PVI), poly(vinylpyridine-*N*-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Further exemplary polymers include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated.

Fabric hueing agents

[0233] The detergent compositions of the present invention may also comprise fabric hueing agents such as dyes or pigments, which when formulated in detergent compositions can deposit onto a fabric when said fabric is contacted with a wash liquor comprising said detergent compositions and thus altering the tint of said fabric through absorption/reflection of visible light. Fluorescent whitening agents emit at least some visible light. In contrast, fabric hueing agents alter the tint of a surface as they absorb at least a portion of the visible light spectrum. Suitable fabric hueing agents include dyes and dye-clay conjugates, and may also include pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct Blue, Direct Red, Direct Violet, Acid Blue, Acid Red, Acid Violet, Basic Blue, Basic Violet and Basic Red, or mixtures thereof, for example as described in WO2005/03274, WO2005/03275, WO2005/03276 and EP1876226 (hereby incorporated by reference). The detergent composition preferably comprises from about 0.00003 wt% to about 0.2 wt%, from about 0.00008 wt% to about 0.05 wt%, or even from about 0.0001 wt% to about 0.04 wt% fabric hueing agent. The composition may comprise from 0.0001 wt% to 0.2 wt% fabric hueing agent, this may be especially preferred when the composition is in the form of a unit dose pouch. Suitable hueing agents are also disclosed in, e.g. WO 2007/087257 and WO2007/087243.

Additional Enzymes

[0234] The detergent additive as well as the detergent composition may comprise one or more [additional] enzymes such as a xanthan lyase, protease, lipase, cutinase, an amylase, lichenase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, xylanase, oxidase, e.g., a laccase, xanthan endoglucanase, and/or peroxidase.

[0235] In general, the properties of the selected enzyme(s) should be compatible with the selected detergent, (i.e., pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

[0236] Cellulases: Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, e.g.,* the fungal cellulases produced from *Humicola insolens, Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO

89/09259.

[0237] Especially suitable cellulases are the alkaline or neutral cellulases having color care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

[0238] Example of cellulases exhibiting endo-beta-1,4-glucanase activity (EC 3.2.1.4) are those having described in WO02/099091.

[0239] Other examples of cellulases include the family 45 cellulases described in WO96/29397, and especially variants thereof having substitution, insertion and/or deletion at one or more of the positions corresponding to the following positions in SEQ ID NO: 8 of WO 02/099091: 2, 4, 7, 8, 10, 13, 15, 19, 20, 21, 25, 26, 29, 32, 33, 34, 35, 37, 40, 42, 42a, 43, 44, 48, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 70, 72, 76, 79, 80, 82, 84, 86, 88, 90, 91, 93, 95, 95d, 95h, 95j, 97, 100, 101, 102, 103, 113, 114, 117, 119, 121, 133, 136, 137, 138, 139, 140a, 141, 143a, 145, 146, 147, 150e, 150j, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160c, 160e, 160k, 161, 162, 164, 165, 168, 170, 171, 172, 173, 175, 176, 178, 181, 183, 184, 185, 186, 188, 191, 192, 195, 196, 200, and/or 20, preferably selected among P19A, G20K, Q44K, N48E, Q119H or Q146 R.

[0240] Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

[0241] Proteases: The additional enzyme may be another protease or protease variant. The protease may be of animal, vegetable or microbial origin, including chemically or genetically modified mutants. Microbial origin is preferred. It may be an alkaline protease, such as a serine protease or a metalloprotease. A serine protease may for example be of the S1 family, such as trypsin, or the S8 family such as subtilisin. A metalloproteases protease may for example be a thermolysin from e.g. family M4, M5, M7 or M8.

[0242] The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 sub-divisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family. In one aspect of the invention the protease may be a subtilase, such as a subtilisin or a variant hereof. Further the subtilases (and the serine proteases) are characterised by having two active site amino acid residues apart from the serine, namely a histidine and an aspartic acid residue.

[0243] Examples of subtilisins are those derived from Bacillus such as subtilisin lentus, Bacillus lentus, subtilisin Novo, subtilisin Carlsberg, Bacillus licheniformis, subtilisin BPN', subtilisin 309, subtilisin 147 and subtilisin 168 described in WO 89/06279 and protease PD138 (WO 93/18140). Additional serine protease examples are described in WO 98/020115, WO 01/44452, WO 01/58275, WO 01/58276, WO 03/006602 and WO 04/099401. An example of a subtilase variants may be those having mutations in any of the positions: 3, 4, 9, 15, 27, 36, 68, 76, 87, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 106, 118, 120, 123, 128, 129, 130, 160, 167, 170, 194, 195, 199, 205, 217, 218, 222, 232, 235, 236, 245, 248, 252 and 274 using the BPN' numbering. More preferred the subtilase variants may comprise the mutations: S3T, V4I, S9R, A15T, K27R, *36D, V68A, N76D, N87S,R, *97E, A98S, S99G,D,A, S99AD, S101G,M,R S103A, V104I,Y,N, S106A, G118V,R, H120D,N, N123S, S128L, P129Q, S130A, G160D, Y167A, R170S, A194P, G195E, V199M, V205I, L217D, N218D, M222S, A232V, K235L, Q236H, Q245R, N252K, T274A (using BPN' numbering). A further preferred protease is the alkaline protease from Bacillus lentus DSM 5483, as described for example in WO 95/23221, and variants thereof which are described in WO 92/21760, WO 95/23221, EP 1921147 and EP 1921148.

[0244] Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the Fusarium protease described in WO 89/06270 and WO 94/25583. Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235, and 274.

[0245] Examples of metalloproteases are the neutral metalloprotease as described in WO 07/044993.

[0246] Preferred commercially available protease enzymes include Alcalase™, Coronase™, Duralase™, Ourazym™, Esperase™, Everlase™, Kannase™, Liquanase™, Liquanase Ultra™, Ovozyme™, Polarzyme™, Primase™, Relase™, Savinase™ and Savinase Ultra™, (Novozymes A/S), Axapem™ (Gist-Brocases N.V.), BLAP and BLAP X (Henkel AG & Co. KGaA), Excellase™, FN2™, FN3™, FN4™, Maxaca™, Maxapem™, Maxatase™, Properase™, Purafast™, Purafect™, Purafect OxP™, Purafect Prime™ and Puramax™ (Genencor int.).

[0247] Lipases and Cutinases: Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutant enzymes are included. Examples include lipase from *Thermomyces,* e.g. from *T. lanuginosus* (previously named *Humicola lanuginosa)* as described in EP258068 and EP305216, cutinase from *Humicola,* e.g. *H. insolens* (WO96/13580), lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g. *P. alcaligenes* or *P. pseudoalcaligenes* (EP218272), P. *cepacia* (EP331376), *P. sp.* strain SD705 (WO95/06720 & WO96/27002), *P. wisconsinensis* (WO96/12012), GDSL-type *Streptomyces* lipases (WO10/065455), cutinase from *Magnaporthe grisea* (WO10/107560), cutinase from *Pseudomonas mendocina* (US5,389,536), lipase

from *Thermobifida fusca* (WO11/084412), *Geobacillus stearothermophilus* lipase (WO11/084417), lipase from *Bacillus subtilis* (WO11/084599), and lipase from *Streptomyces griseus* (WO11/150157) and S. *pristinaespiralis* (WO12/137147).

[0248] Further examples are lipases sometimes referred to as acyltransferases or perhydrolases, e.g. acyltransferases with homology to *Candida antarctica* lipase A (WO10/111143), acyltransferase from *Mycobacterium smegmatis* (WO05/56782), perhydrolases from the CE 7 family (WO09/67279), and variants of the M. *smegmatis* perhydrolase in particular the S54V variant used in the commercial product Gentle Power Bleach from Huntsman Textile Effects Pte Ltd (WO10/100028).

[0249] Other examples are lipase variants such as those described in EP407225, WO92/05249, WO94/01541, WO94/25578, WO95/14783, WO95/30744, WO95/35381, WO95/22615, WO96/00292, WO97/04079, WO97/07202, WO00/34450, WO00/60063, WO01/92502, WO07/87508 and WO09/109500.

[0250] Preferred commercial lipase products include include Lipolase™, Lipex™; Lipolex™ and Lipoclean™ (Novozymes A/S), Lumafast (originally from Genencor) and Lipomax (originally from Gist-Brocades).

Amylases

[0251] The amylase may be an alpha-amylase, a beta-amylase or a glucoamylase and may be of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus, e.g.,* a special strain of *Bacillus licheniformis,* described in more detail in GB 1,296,839.

[0252] Examples of amylases are those having SEQ ID NO: 3 in WO 95/10603 or variants having 90% sequence identity to SEQ ID NO: 3 thereof. Preferred variants are described in WO 94/02597, WO 94/18314, WO 97/43424 and SEQ ID NO: 4 of WO 99/019467, such as variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and 444 of SEQ ID NO: 3 in WO 95/10603.

[0253] Further amylases which can be used are amylases having SEQ ID NO: 6 in WO 02/010355 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a deletion in positions 181 and 182 and a substitution in position 193.

[0254] Other amylase examples are hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from B. *amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of the B. *licheniformis* alpha-amylase shown in SEQ ID NO: 4 of WO 2006/066594 or variants having 90% sequence identity thereof. Preferred variants of this hybrid alpha-amylase are those having a substitution, a deletion or an insertion in one of more of the following positions: G48, T49, G107, H156, A181, N190, M197, I201, A209 and Q264. Most preferred variants of the hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 are those having the substitutions:

M197T;

H156Y+A181T+N190F+A209V+Q264S;

or

G48+T49+G107+H156+A181+N190+I201+A209+Q264.

[0255] Further amylase examples are amylases having SEQ ID NO: 6 in WO 99/019467 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a substitution, a deletion or an insertion in one or more of the following positions: R181, G182, H183, G184, N195, I206, E212, E216and K269. Particularly preferred amylases are those having deletion in positions G182 and H183 or positions H183 and G184.

[0256] Additional amylases are those having SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873 or variants thereof having 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 are those having a substitution, a deletion or an insertion in one or more of the following positions: 140, 181, 182, 183, 184, 195, 206, 212, 243, 260, 269, 304 and 476. More preferred variants are those having a deletion in positions 182 and 183 or positions 183 and 184. Most preferred amylase variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 are those having a deletion in positions 183 and 184 and a substitution in positions 140, 195, 206, 243, 260, 304 and 476.

[0257] Other amylases which can be used are amylases having SEQ ID NO: 2 of WO 08/153815, SEQ ID NO: 10 in WO 01/66712 or variants thereof having 90% sequence identity to SEQ ID NO: 2 of WO 08/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 01/66712. Preferred variants of SEQ ID NO: 10 in WO 01/66712 are those having a

substitution, a deletion or an insertion in one of more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264.

[0258] Further amylases which can be used are amylases having SEQ ID NO: 2 of WO 09/061380 or variants thereof having 90% sequence identity to SEQ ID NO: 2. Preferred variants of SEQ ID NO: 2 are those having a substitution, a deletion or an insertion in one of more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320, Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one of more of the following positions: Q87E,R, Q98R, S125A, N128C, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:

N128C+K178L+T182G+Y305R+G475K;

N128C+K178L+T182G+F202Y+Y305R+D319T+G475K;

S125A+N128C+K178L+T182G+Y305R+G475K;

or

[0259] S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K wherein the variants are C-terminally truncated and optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

[0260] Further suitable amylases are amylases having SEQ ID NO: 1 of WO13184577 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: N126, E132, K176, R178, G179, T180, G181, E187, N192, M199, I203, S241, Y303 R458, T459, D460, G476 and G477. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: N126Y, E132HY, K176L, E187P, N192FYH, M199L, I203YF, S241QADN, Y303DN, R458N, T459S, D460T, G476Kand G477K and/or deletion in position R178 and/or S179 or of T180 and/or G181. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

E187P+I203Y+G476K

E187P+I203Y+R458N+T459S+D460T+G476K

N126Y+T180D+E187P+I203Y+Y303D+G476T

N126Y+E132H+T180D+E187P+I203Y+Y303D+G476T+G477E

N126Y+F153W+T180H+I203Y+S239Q

[0261] Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO01/66712 or a variant having at least 90%, such as at least 95%, sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one of more of the following positions of SEQ ID NO: 12 in WO01/66712: R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, N484. Particular preferred amylases include variants having a deletion of D183 and G184 and having the substitutions R118K, N195F, R320K and R458K, and a variant additionally having substitutions in one or more position selected from the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions.

[0262] Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™, Stainzyme™, Stainzyme Plus™, Natalase™ and BAN™ (Novozymes A/S), Rapidase™ and Purastar™ (from Genencor International Inc.).

[0263] Peroxidases/Oxidases: Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from

Coprinus, e.g., from *C. cinereus,* and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

[0264] Commercially available peroxidases include Guardzyme™ (Novozymes A/S).

[0265] The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e., a separate additive or a combined additive, can be formulated, for example, as a granulate, liquid, slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

[0266] Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono-and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

Adjunct materials

[0267] Any detergent components known in the art for use in laundry detergents may also be utilized. Other optional detergent components include anti-corrosion agents, anti-shrink agents, anti-soil redeposition agents, anti-wrinkling agents, bactericides, binders, corrosion inhibitors, disintegrants/disintegration agents, dyes, enzyme stabilizers (including boric acid, borates, CMC, and/or polyols such as propylene glycol), fabric conditioners including clays, fillers/processing aids, fluorescent whitening agents/optical brighteners, foam boosters, foam (suds) regulators, perfumes, soil-suspending agents, softeners, suds suppressors, tarnish inhibitors, and wicking agents, either alone or in combination. Any ingredient known in the art for use in laundry detergents may be utilized. The choice of such ingredients is well within the skill of the artisan.

[0268] Dispersants: The detergent compositions of the present invention can also contain dispersants. In particular powdered detergents may comprise dispersants. Suitable water-soluble organic materials include the homo- or copolymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms. Suitable dispersants are for example described in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc.

[0269] Dye Transfer Inhibiting Agents: The detergent compositions of the present invention may also include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. When present in a subject composition, the dye transfer inhibiting agents may be present at levels from about 0.0001 % to about 10%, from about 0.01% to about 5% or even from about 0.1 % to about 3% by weight of the composition.

[0270] Fluorescent whitening agent: The detergent compositions of the present invention will preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agent or optical brighteners. Where present the brightener is preferably at a level of about 0,01% to about 0,5%.. Any fluorescent whitening agent suitable for use in a laundry detergent composition may be used in the composition of the present invention. The most commonly used fluorescent whitening agents are those belonging to the classes of diaminostilbene-sulphonic acid derivatives, diarylpyrazoline derivatives and bisphenyl-distyryl derivatives. Examples of the diaminostilbene-sulphonic acid derivative type of fluorescent whitening agents include the sodium salts of: 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate; 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino) stilbene-2.2'-disulphonate; 4,4'-bis-(2-anilino-4(N-methyl-N-2-hydroxy-ethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulphonate, 4,4'-bis-(4-phenyl-2,1,3-triazol-2-yl)stilbene-2,2'-disulphonate; 4,4'-bis-(2-anilino-4(1-methyl-2-hydroxy-ethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulphonate and 2-(stilbyl-4"-naptho-1.,2':4,5)-1,2,3-trizole-2"-sulphonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt of 4,4'-bis-(2-morpholino-4 anilino-s-triazin-6-ylamino) stilbene disulphonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenyl-styryl) disulphonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins. Suitable fluorescent brightener levels include lower levels of from about 0.01, from 0.05, from about 0.1 or even from about 0.2 wt % to upper levels of 0.5 or even 0.75 wt%.

[0271] Soil release polymers: The detergent compositions of the present invention may also include one or more soil

release polymers which aid the removal of soils from fabrics such as cotton and polyester based fabrics, in particular the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalte based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides see for example Chapter 7 in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc. Another type of soil release polymers is amphiphilic alkoxylated grease cleaning polymers comprising a core structure and a plurality of alkoxylate groups attached to that core structure. The core structure may comprise a polyalkylenimine structure or a polyalkanolamine structure as described in detail in WO 2009/087523 (hereby incorporated by reference). Furthermore, random graft co-polymers are suitable soil release polymers Suitable graft co-polymers are described in more detail in WO 2007/138054, WO 2006/108856 and WO 2006/113314 (hereby incorporated by reference). Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose deriviatives such as those described in EP 1867808 or WO 2003/040279 (both are hereby incorporated by reference). Suitable cellulosic polymers include cellulose, cellulose ethers, cellulose esters, cellulose amides and mixtures thereof. Suitable cellulosic polymers include anionically modified cellulose, nonionically modified cellulose, cationically modified cellulose, zwitterionically modified cellulose, and mixtures thereof. Suitable cellulosic polymers include methyl cellulose, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, ester carboxy methyl cellulose, and mixtures thereof.

[0272] Anti-redeposition agents: The detergent compositions of the present invention may also include one or more anti-redeposition agents such as carboxymethylcellulose (CMC), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), polyoxyethylene and/or polyethyleneglycol (PEG), homopolymers of acrylic acid, copolymers of acrylic acid and maleic acid, and ethoxylated polyethyleneimines. The cellulose based polymers described under soil release polymers above may also function as anti-redeposition agents.

[0273] Other suitable adjunct materials include, but are not limited to, anti-shrink agents, anti-wrinkling agents, bactericides, binders, carriers, dyes, enzyme stabilizers, fabric softeners, fillers, foam regulators, hydrotropes, perfumes, pigments, sod suppressors, solvents, and structurants for liquid detergents and/or structure elasticizing agents.

Formulation of detergent products

[0274] The detergent composition may be in any convenient form, e.g., a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid. There are a number of detergent formulation forms such as layers (same or different phases), pouches, as well as forms for machine dosing unit.

[0275] Pouches can be configured as single or multi-compartments. It can be of any form, shape and material which is suitable for hold the composition, e.g. without allowing the release of the composition from the pouch prior to water contact. The pouch is made from water soluble film which encloses an inner volume. Said inner volume can be divided into compartments of the pouch. Preferred films are polymeric materials preferably polymers which are formed into a film or sheet. Preferred polymers, copolymers or derivatives thereof are selected polyacrylates, and water soluble acrylate copolymers, methyl cellulose, carboxy methyl cellulose, sodium dextrin, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, malto dextrin, poly methacrylates, most preferably polyvinyl alcohol copolymers and, hydroxyprpyl methyl cellulose (HPMC). Preferably the level of polymer in the film for example PVA is at least about 60%. Preferred average molecular weight will typically be about 20,000 to about 150,000. Films can also be of blend compositions comprising hydrolytically degradable and water soluble polymer blends such as polyactide and polyvinyl alcohol (known under the Trade reference M8630 as sold by Chris Craft In. Prod. Of Gary, Ind., US) plus plasticisers like glycerol, ethylene glycerol, Propylene glycol, sorbitol and mixtures thereof. The pouches can comprise a solid laundry cleaning composition or part components and/or a liquid cleaning composition or part components separated by the water soluble film. The compartment for liquid components can be different in composition than compartments containing solids. Ref: (US2009/0011970 A1).

[0276] Detergent ingredients can be separated physically from each other by compartments in water dissolvable pouches or in different layers of tablets. Thereby negative storage interaction between components can be avoided. Different dissolution profiles of each of the compartments can also give rise to delayed dissolution of selected components in the wash solution.

[0277] A liquid or gel detergent, which is not unit dosed, may be aqueous, typically containing at least 20% by weight and up to 95% water, such as up to about 70% water, up to about 65% water, up to about 55% water, up to about 45% water, up to about 35% water. Other types of liquids, including without limitation, alkanols, amines, diols, ethers and polyols may be included in an aqueous liquid or gel. An aqueous liquid or gel detergent may contain from 0-30% organic solvent. A liquid or gel detergent may be non-aqueous.

Uses

[0278] The present invention is also directed to methods for using the detergent compositions according to the invention.

[0279] Accordingly, the present invention relates to use of the carbohydrate-binding module comprised in the detergent composition of present invention for one or more of the following:

i) binding or facilitating binding to xanthan gum in a cleaning process;
ii) facilitating or improving degradation of xanthan gum in a cleaning process;
iii) facilitating or improving binding affinity and/or specificity and/or adhesion of a polypeptide to xanthan gum in a cleaning process;
iv) facilitating or improving adhesion of a composition (e.g., according to claim 6) to a textile in a cleaning process;
v) washing or cleaning a textile and/or a hard surface such as dish wash including Automatic Dish Wash (ADW);
vi) in a cleaning process such as laundry or hard surface cleaning including dish wash including Automatic Dish Wash (ADW) and industrial cleaning;
vii) laundering and/or hard surface cleaning including dish wash including Automatic Dish Wash (ADW);
viii) facilitating or improving removal of xanthan gum containing soil, e.g., in a cleaning process;
ix) facilitating or improving removal of xanthan gum containing soil in the presence of xanthan lyase and/or endoglucanase activity, e.g., in a cleaning process.

Use to degrade xanthan gum

[0280] Xanthan gum has been used as an ingredient in many consumer products including foods and cosmetics and has found use in the oil industry. Therefore, the degradation of xanthan gum can result in improved cleaning processes, such as the easier removal of stains containing gums, such as xanthan gum. Thus, the present invention is directed to the use of the carbohydrate-binding module comprised in the detergent composition of the invention to degrade xanthan gum. Degradation of xanthan gum can preferably be measured using a viscosity reduction assay (e.g., ViPr assay).

[0281] GH9 endoglucanase activity may alternatively be measured by assessment of reducing ends on xanthan gum pre-treated with xanthan lyase using the colorimetric assay developed by Lever (1972), Anal. Biochem. 47: 273-279, 1972. A preferred embodiment is the use of 0.1% xanthan gum pre-treated with xanthan lyase. Degradation of xanthan gum pre-treated with xanthan lyase may be determined by calculating difference between blank and sample wherein a difference of more than 0.5 mAU, preferably more than 0.6 mAU, more preferably more than 0.7 mAU or even more preferably more than 0.8 mAU shows degradation of xanthan gum pre-treated with xanthan lyase.

[0282] Xanthan lyase activity may alternatively be measured by assessment of reducing ends on xanthan gum using the colorimetric assay developed by Lever (1972), Anal. Biochem. 47: 273-279, 1972. A preferred embodiment is the use of 0.1% xanthan gum. Degradation of xanthan gum may be determined by calculating difference between blank and sample, wherein a difference of more than 0.1 mAU, preferably more than 0.15 mAU, more preferably more than 0.2 mAU or even more preferably more than 0.25 mAU, shows degradation of xanthan gum.

[0283] Xanthan lyase (e.g. polypeptides comprising the carbohydrate-binding module of the present invention) and endoglucanase (e.g. polypeptides comprising the carbohydrate-binding module of the present invention) activity may alternatively be measured by assessment of reducing ends on xanthan gum using the colorimetric assay developed by Lever (1972), Anal. Biochem. 47: 273-279, 1972. A preferred embodiment is the use of 0.1 % xanthan gum. Degradation of xanthan gum may be determined by calculating difference between blank and sample wherein a difference of more than 0.4 mAU, preferably more than 0.5 mAU, more preferably more than 0.6 mAU or even more preferably more than 0.8 mAU shows degradation of xanthan gum.

Use in detergents

[0284] The present invention *inter alia* relates to the use of detergent compositions comprising carbohydrate-binding module in cleaning processes such as the laundering of textiles and fabrics (e.g., household laundry washing and industrial laundry washing), as well as household and industrial hard surface cleaning, such as dish wash.

[0285] In some aspects to the detergent compositions comprising carbohydrate-binding modules may be added an endoglucanase(s) or xanthan lyase(s). The invention relates to the use of such detergent compositions in cleaning processes such as the laundering of textiles and fabrics (e.g. household laundry washing and industrial laundry washing), as well as household and industrial hard surface cleaning, such as dish wash.

[0286] The detergent composition may be formulated, for example, as a hand or machine laundry detergent composition for both household and industrial laundry cleaning, including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household or industrial hard surface cleaning operations, or be formulated for hand or machine (both household

and industrial) dishwashing operations.

[0287] The invention also relates to methods for degrading xanthan gum on the surface of a textile or hard surface, such as dish wash, comprising applying a detergent compositions comprising one or more carbohydrate-binding modules to xanthan gum. In some aspects the invention relates to a method for degrading xanthan gum on the surface of a textile or hard surface, such as dish wash, comprising applying a detergent compositions comprising one or more carbohydrate-binding modules together with one or more endoglucanases and/or xanthan lyases to xanthan gum. Use of carbohydrate-binding modules may have a detergency benefit.

[0288] It has been contemplated that the use of a carbohydrate-binding module of the invention in the detergent composition of present invention alone gives a detergency benefit, preferably an enzyme detergency benefit on xanthan gum.

[0289] In some aspects the invention relates to the use of a detergent composition comprising one or more detergent components and an isolated carbohydrate-binding module as described herein together with a GH9 endoglucanase and/or a xanthan lyase. In some aspects the invention relates to the use of a detergent composition comprising one or more detergent components and an isolated carbohydrate-binding module together with a GH9 endoglucanase and/or a xanthan lyase.

Methods

Method of degrading xanthan gum in a laundering process

[0290] Xanthan gum, as described elsewhere herein, is found in many consumer products, such as foods and cosmetics. Thus, textile and/or fabrics in any form may be soiled by xanthan gum originating from foods or cosmetics. Accordingly, in one aspect, the present invention relates to a method of cleaning a textile or fabric by using of a composition comprising a CBM domain of the invention (e.g. polypeptides comprising the CBM of the present invention).

[0291] The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

EXAMPLES

Example 1: Cloning and expression of the CBM domain of an endoglucanase in *Bacillus subtilis* with N-terminal His tag

[0292] The gene fragment (SEQ ID 16) of the GH9 gene (SEQ ID NO:2 of WO2013167581) encoding for the CBM#2 polypeptide domain (SEQ ID 12) was amplified by standard PCR techniques using specific forward (D2003, SEQ ID NO: 19) and reverse (D2004, SEQ ID NO: 20) primers (see Table 1 for sequence details). The primers contain overhang to cloning vector, which is a derivative of the plasmid C6221 (described in WO2012/025577), modified by introducing a poly histidine tag (HHHHHHPR SEQ ID NO: 22) after the secretion signal.

Table 1. Primers used for PCR amplification

Amplification of CBM domain of endoglucanase gene	**Forward primer** (SEQ ID NO: 19)	**Reverse primer** (SEQ ID NO: 20)
CBM domain	D2003: 5'TCACCATCATCCTAGGAATAAAC TGGAGGCGGAA3'	D2004: 5'TTATTGATTAACGCGTTTACGGAA CTGGAACAAGCTG3'

[0293] The PCR fragment was cloned in the plasmid digested with AvrII and MluI enzymes using the In-Fusion HD cloning kit (Clontech product number 639648) following the instructions from the manufacturer (PT5162-1).

[0294] The CBM domain of the GH9 polypeptide was expressed with the secretion signal of *Bacillus clausii* with the following amino acid sequence (MKKPLGKIVASTALLISVAFSSSIASA - SEQ ID NO: 22) followed by a poly histidine tag (SEQ ID NO: 21) to easy the purification process.

[0295] The In-Fusion reaction was transformed in *E. coli* and ampicillin resistant transformants were selected and cultivated for subsequently DNA plasmid preparation. Three clones of each construct were analysed by DNA sequencing to verify the correct DNA sequence of the constructs.

[0296] One clone with the correct recombinant gene sequence was selected and the corresponding plasmid was integrated by homologous recombination into the *Bacillus subtilis* host cell genome and the gene construct was expressed under the control of a triple promoter system as described in WO99/43835. The gene coding for chloramphenicol acetyltransferase was used as a marker (as described in Diderichsen et a/., 1993, Plasmid 30:312-315).

[0297] Chloramphenicol resistant transformants were analysed by PCR to verify the correct size of the amplified fragment. A recombinant B. *subtilis* clone containing the integrated expression construct was selected and grown in liquid culture. The CBM polypeptide domain containing supernatant was harvested and was purified as described in Examples 2 and 3.

Example 2: Purification of carbohydrate-binding modules

[0298] Broth was filtered through 0.2 μm rapid PES bottle top filters (Thermo Scientific, Waltham, MA). The filtered culture broth was mixed with equal volume of 2.8 M ammoniumsulfate buffered in 40 mM TrisHCl, pH 7.5. The sample was left at room temperature for 20 min with continuous stirring. Precipitated protein was removed by filtration through a Rapid PES 0.2 μm bottle-top filter. The filtered sample was loaded on a Phenyl Sepharose High Performance column (50 mL, GE Healthcare, Uppsala, Sweden) pre-equilibrated with 20 mM Tris-HCl, pH 7.5 + 1.4 M ammoniumsulfate. The column was washed with the equilibration buffer until a stable UV-baseline was obtained. Bound protein was eluted by a linear ammonium sulphate gradient from 1.4 M to 0.0 M lasting over 10 column volumes. SDS-PAGE analysis (4-12 % NuPAGE, Thermo Scientific) was carried out according to the manufacturer's instruction on the flow-through and on peak fractions. The target protein was found in the flow-through fraction. The flow-through fraction was concentrated and buffer exchanged to 20 mM Tris-HCl, pH 7.5 by ultra-filtration using a 5 kDa cut-off membrane. The desalted protein sample was loaded on a 20 mL Source 15S column (GE Healthcare) equilibrated with 20 mM Tris-HCl, pH 7.5. The column was washed with equilibration buffer until a stable UV baseline was obtained. Elution was done by a linear NaCl gradient from 0.0 to 0.250 M over 5 column volumes. Fractions were evaluated for purify by SDS-PAGE (4-12 % NuPAGE). Fractions showing a single band with the expected molecular weight were pooled. The identity of the purified protein was confirmed by mass spectrometry.

Example 3: Purification of *Hypocrea jecorina* endoglucanase (GH12)

[0299] Fermentation broths containing *Hypocrea jecorina* endoglucanase[1] were filtered through PES Bottle top filter with a 0.22 μm) cut-off. Ammonium sulphate was added to the filtered fermentation broths to make a 350 mM solution. The *Hypocrea jecorina* endoglucanase was purified by HIC/affinity chromatography followed by IEX/affinity chromatography.

[0300] In the HIC/affinity chromatographic step, the fermentation broths were applied to a 200 ml Phenyl SEPHAROSE® 6 Fast Flow column (high sub) (GE Healthcare, Piscataway, NJ, USA) which had been pre-equilibrated with 350 mM ammonium sulphate, 25 mM MES pH 6.0. After applying the sample, the column was washed with 2 CV (Column Volume) 350 mM M ammonium sulphate followed by 1 CV 25 mM MES, pH 6, 5 % Ethanol. The bound proteins were batch eluted with 25 mM MES, pH 6, 50 % Ethanol.

[0301] The elution of the protein was monitored at 280 nm. Fractions with high 280 nm absorbance were analyzed on SDS-PAGE using 12-well NUPAGE® 4-12% Bis-Tris gel (GE Healthcare, Piscataway, NJ, USA) for their *Hypocrea jecorina* endoglucanase content. Fractions with high content of this protein were pooled and collected for further purification. The pooled fractions were desalted on a SEPHADEX™ G-25 (medium) column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 12.5 mM Acetate pH 5.0. The elution of the protein was monitored at 280 nm and fractions with high absorbance at 280 nm were chosen for the second chromatographic step.

[0302] The pooled fractions were applied to the 60 ml RESOURCE™ S column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 12.5 mM Acetate pH 5.0 and bound proteins were eluted with a linear 0-300 mM sodium chloride gradient for 3 CV. The elution of the protein was monitored at 280 nm and fractions with high absorbance at 280 nm were analyzed on SDS-PAGE. Fractions with high content of *Hypocrea jecorina* endoglucanase were pooled.

Example 4: Measuring Xanthan gum bound carbohydrate-binding modules of the invention (CBM) and *Hypocrea jecorina* endoglucanase (GH12)

[0303] The fraction of bound *Hypocrea jecorina* endoglucanase (GH12) and carbohydrate-binding modules of the invention to Xanthan gum were estimated by measuring protein intrinsic fluorescence at λ_{EX} ~ 280 nm λ_{EM} ~ 303 nm (CBM X320) and λ_{EX} ~ 280 nm λ_{EM} ~ 345 nm *(Hypocrea jecorina* endoglucanase)

[0304] 200 μl Xanthan gum (2 mg/ml - 0.004 mg/ml) was added to each well in a 96 well filter plate (Millipore MultiScreen$_{HTS}$ Durapore™ 96-Well Filter Plate). 50 μl *Hypocrea jecorina* endoglucanase (GH12) (0.03 mg/ml) and CBM (0.2 mg/ml) was added to the wells and the plate was transferred to a thermomixeer and was incubated for 30 min (25

°C, 900 rpm). Following the filter plate was placed on top of a 96 well plate and was centrifuged for 1 h 25 °C 4000 G. After centrifugation 130 μl of the flow through was transferred to a 96 greiner bio one plate, and the concentration of the proteins were measured at emission at at λ_{EX} ~ 280 nm λ_{EM} ~ 303 nm (CBM) and λ_{EX} ~ 280 nm λ_{EM} ~ 345 nm *(Hypocrea jecorina* endoglucanase). For all the measurement, the blank measurement (without protein) was subtracted and all experiments were carried out in triplicates.

[0305] The Intensities (λ_{EX} ~ 280 nm λ_{EM} ~ 303 nm or λ_{EM} ~ 334) measured for the *Hypocrea jecorina* endoglucanase (GH12) and CBM in the flow through are normalized to enzyme concentration (mg/ml) by using a standard curve of CBM in varying concentrations. The standards were CBM diluted in 50 mM sodium acetate pH 5 to a concentration of 0.2, 0.1, 0,05, 0.03, 0.013, 0.0063, 0.003 and 0 mg/ml and *Hypocrea jecorina* endoglucanase diluted in 50 mM sodium acetate pH 5 to a concentration of 0.032, 0.016, 0,008, 0.004, 0.002, 0.001, 0.0005 and 0 mg/ml. The Xanthan bound *Hypocrea jecorina* endoglucanase (GH12) and CBM ([Protein]bound) were calculated by assuming $[Protein]_{total}$ = [Protein]bound +[Protein]free, where $[Protein]_{total}$ is the total amount of added enzyme and $[Protein]_{free}$ is the concentration of protein in the flow through. Fraction bound CBM and *Hypocrea jecorina* endoglucanase was calculated as $[Protein]_{bound}/[Protein]_{total}$.

[0306] The results as shown in Table 2 demonstrated that around 90 % of the CBM was bound to xanthan gum at 2 gL^{-1}- 0.13 gL^{-1}, -60 % at 0.063 gL^{-1}, -20 % at 0.031 gL^{-1} and -0 % at 0.015-0.004 % Xanthan gum. Data for *Hypocrea jecorina* endoglucanase showed that 0 % was bound to Xanthan gum at all loads.

Table 2: Bound Xanthan gum

Xanthan gum (g/L)	Fraction bound CBM	Fraction bound *Hypocrea jecorina* endoglucanase
2	0.95 ± 0.02	0.09 ± 0.05
1	0.90 ± 0.01	0.05 ± 0.02
0.5	0.92 ± 0.02	0.06 ± 0.03
0.25	0.90 ± 0.03	0.03 ± 0.02
0.125	0.92 ± 0.02	-0.01 ± 0.05
0.0625	0.58 ± 0.02	-0.01± 0.04
0.03125	0.18 ± 0.07	-0.04± 0.03
0.01563	0.04 ± 0.01	0.00 ± 0.02
0.00781	-0.03 ± 0.02	0.00 ± 0.04
0.00391	-0.07 ± 0.05	-0.05 ± 0.05

Reference:

[0307]

1) Murphy, L., Cruys-Bagger, N., Damgaard, H. D., Baumann, M. J., Olsen, S. N., Borch, K., Lassen, S. F., Sweeney, M., Tatsumi, H., and Westh, P. (2012) Origin of Initial Burst in Activity for Trichoderma Reesei Endo-Glucanases Hydrolyzing Insoluble Cellulose. The Journal of biological chemistry 287, 1252-1260

Example 5: Stability and Wash performance of detergent compositions of present invention

[0308] The wash performance of detergent compositions comprising a polypeptide comprising a carbohydrate-binding module (CBM) according to the invention was determined by using a liquid washing agent with the following composition as base formulation (all values in weight percent):

	% Active Matter raw material	Active Matter in Formula
Water demin.	100	ad 100%
Protease Stabilisator	100	0,8-1,2%
Citric acid	100	2,5-4%
Antifoaming agent	t.q.	0,02-0,05%

(continued)

	% Active Matter raw material	Active Matter in Formula
FAEOS	70	7,5-9,5%
FAEO, Nonionic surf.	100	5,5-7,5%
LAS	96	6-8%
palm kernel oil fatty acid	30	2,5-4,5%
NaOH	50	2,5-3,5%
1,2-Propandiol	100	4-7%
DTPMP-xNa	40	0,5-1%
Soil repellent	70	1-1,8%
Ethanol	93	1-4%
Optical brightener	90	0,05-2%
Enzyme Mix	100	1,4-1,8%
Perfume	100	0,5-1,4%
Dye	tq	0,001-0,004%

[0309] The pH of the detergent composition was between 8,2-8,6.

[0310] The polypeptides comprising the CBM were added on top in a concentration of 2,6 mg active enzyme per Washload. The dosing ratio of the liquid washing agent was 73 ml per wash load and the washing procedure was performed for 60 minutes at a temperature of 40°C, the water having a water hardness between 15.5 and 16.5° (German degrees of hardness).

[0311] The whiteness, i.e. the brightening of the stains, was determined photometrically as an indication of wash performance. A Minolta CM508d spectrometer device was used, which was calibrated beforehand using a white standard provided with the unit.

SEQUENCE LISTING

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<120> DETERGENT COMPOSITIONS COMPRISING POLYPEPTIDE COMPRISING CARBOHYDRATE-BINDING DOMAIN AND POLYNUCLEOTIDES ENCODING THE SAME

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Asp Ser Thr Ser Ile Thr Thr Thr Tyr Arg His Leu Tyr Asp Met Ala
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Leu Ala Tyr Thr Thr Tyr Gly Ser Ser Leu Gln Gly Asn Ala Ala Leu
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Lys Ala Asp Ile Ile Ser Gly Leu Asp Trp Met Asn Ala Asn Gln Phe
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Tyr Asn Gly Cys Ser Gln Tyr Gln Asn Trp Trp His Trp Gln Ile Gly
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Gly Pro Met Ala Leu Asn Asp Ile Val Ala Leu Met Tyr Thr Glu Leu
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Thr Ala Thr Gln Ile Ser Asn Tyr Met Ala Ala Ile Tyr Tyr Thr Gln
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Phe Ile Leu Asn Gly Ser Ser Trp Gln Val Thr Asp Pro Asn Lys Asn
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Asn Val Tyr Arg Trp Ile Tyr Asp Ser Tyr Glu Pro Phe Ile Tyr Lys
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Gly Asn Leu Met Asp Met Val Arg Gly Arg Glu Ile Ser Arg His Gly
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Leu Gln Asp Asp Lys Ala Ala Val Thr Val Met Ala Ser Ile Ile Arg
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Leu Ser Gln Thr Ala Ala Ser Ala Asp Ala Thr Ala Phe Lys Arg Met
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Val Lys Tyr Trp Leu Leu Leu Asp Thr Asp Lys Thr Phe Leu Lys Ala
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Val Ser Ile Asp Leu Ile Ile Ala Ala Asn Gln Leu Val Asn Asp Ser
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Met Phe Ser Ser Arg Ile Gly Asn Tyr Glu Ser Ile Asn Ala Glu Asn
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Asn Lys Gly Trp His Thr Gly Asp Gly Met Thr Tyr Leu Tyr Asn Thr
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Asp Leu Ser Gln Phe Asn Asp His Phe Trp Ala Thr Val Asp Asn Tyr
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Ala Lys Lys Ser Trp Phe Met Phe Asp Asp Glu Ile Val Ala Leu Gly
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Ser Gly Ile Ala Ser Thr Asp Gly Ile Ala Thr Glu Thr Ile Val Glu
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Asn Arg Lys Leu Asn Ser Ser Gly Asn Asn Ala Leu Ile Val Asn Gly
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Thr Ala Lys Pro Gly Ser Leu Gly Trp Ser Glu Thr Met Thr Gly Thr
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Asn Tyr Ile His Leu Ala Gly Ser Val Pro Gly Ser Asp Ile Gly Tyr
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Leu His Thr Arg Asn Phe Met Thr Leu Trp Phe Asp His Gly Met Asn
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Pro Thr Asn Gly Ser Tyr Ser Tyr Val Leu Leu Pro Asn Lys Thr Ser
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Ser Ala Val Ala Ser Tyr Ala Ala Thr Pro Gln Ile Ser Ile Leu Glu
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Lys Asn Val Ser Ser Ser Gly Tyr Lys Leu Met Leu Asp Asn Phe Ser
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Leu Val Ser Thr Gly Ile Asp Thr Thr Val Ile Val Asp Asn Ala Asp
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Gly Thr Lys Ser Val Thr Phe Thr Pro Asn Val Pro Ile Ser Gly Thr
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Claims

1. A detergent composition comprising a polypeptide comprising a carbohydrate-binding module (CBM), wherein the carbohydrate-binding module is selected from the group consisting of:

a) a polypeptide comprising (or consisting essentially of) one or more of the following motifs:

i) GIYQZ1 (SEQ ID NO: 1), wherein Z1 is independently selected from the group of amino acids L and F; preferably said GIYQZ1 motif is either GIYQL (SEQ ID NO: 2) or GIYQF (SEQ ID NO: 3);
ii) TGKB1 (SEQ ID NO: 4), wherein B1 is independently selected from the group of amino acids N, H and S; preferably said TGKB1 motif is either TGKN (SEQ ID NO: 5) or TGKH (SEQ ID NO: 6) or TGKS (SEQ ID NO: 7);
iii) GX1X2QLX3 (SEQ ID NO: 8), wherein X1 is independently selected from the group consisting of amino acids I, T and K; wherein X2 is independently selected from the group consisting of amino acids F and Y; wherein X3 is independently selected from the group consisting of amino acids S and Q; preferably said GX1X2QLX3 motif is either GIYQLQ (SEQ ID NO: 9) or GIYQLS (SEQ ID NO: 10);

b) a polypeptide having at least 34% (e.g., at least 35%, at least 40%, at least 45%, at least 46%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100%) sequence identity to the carbohydrate-binding module having either

i') SEQ ID NO: 11, preferably said polypeptide has at least 34% sequence identity to the SEQ ID NO: 11; or
ii') SEQ ID NO: 12, preferably said polypeptide has at least 46% sequence identity to the SEQ ID NO: 12;

c) the polypeptide according to (b), wherein said polypeptide comprises one or more of motifs according to (a);
d) preferably the carbohydrate-binding module does not have an enzymatic activity (e.g., does not have xanthan lyase or endoglucanase activity).

2. The detergent composition of claim 1, wherein said carbohydrate-binding module comprises (or consisting essentially of) two or three of said motifs; preferably said carbohydrate-binding module comprises (or consisting essentially of) two motifs, such as said motif of ii) and iii); further preferably said carbohydrate-binding module comprises (or consisting essentially of) three motifs, such as said motifs of i), ii), and iii).

3. The detergent composition of claim 1 or 2, wherein said carbohydrate-binding module is operably linked to a catalytic domain, wherein said catalytic domain is obtained from a xanthan lyase or an endoglucanase.

4. The detergent composition according to claim 3, wherein the polypeptide is selected from the group consisting of: SEQ ID NO: 13 and SEQ ID NO: 14.

5. The detergent composition according to any one of claims 1 - 4, wherein the composition is in form of a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

6. The detergent composition of any one of claims 1-5, the composition further comprising one or more additional enzymes selected among protease, lipase, cutinase, amylase, carbohydrase, cellulase, pectinase, pectatlyase, mannanase, arabinase, galactanase, xylanase, oxidase, xanthanase, laccase, and/or peroxidase.

7. The detergent composition of any one of claims 1-6, wherein the composition is a laundry detergent composition or a dishwashing composition, preferably a machine dishwashing composition.

8. Use of a detergent composition of any of claims 1-7 for degrading xanthan gum.

9. Use of a detergent composition according to any one of claims 1-8 in a cleaning process, preferably in laundry or hard surface cleaning such as dish wash.

10. A method for removing a stain from a surface, which comprises contacting the surface with a detergent composition according to any one of claims 1 - 7.

11. A method for degrading xanthan gum comprising applying a detergent composition according to any of claims 1 to 7 to xanthan gum.

12. The method of claim 11, wherein the xanthan gum is on the surface of a textile or of a hard surface, such as in dish wash.

13. A method for facilitating or improving binding affinity and/or specificity and/or adhesion of a polypeptide to xanthan gum, said method comprising fusing said polypeptide with the carbohydrate-binding module according to any one of the preceding claims.

14. Use of the carbohydrate-binding module comprised in the detergent composition according to any one of the preceding claims for one or more of the following:

x) binding or facilitating binding to xanthan gum in a cleaning process;
xi) facilitating or improving degradation of xanthan gum in a cleaning process;
xii) facilitating or improving binding affinity and/or specificity and/or adhesion of a polypeptide to xanthan gum in a cleaning process;
xiii) facilitating or improving adhesion of a composition (e.g., according to claim 6) to a textile in a cleaning process;
xiv) washing or cleaning a textile and/or a hard surface such as dish wash including Automatic Dish Wash (ADW);
xv) in a cleaning process such as laundry or hard surface cleaning including dish wash including Automatic Dish Wash (ADW) and industrial cleaning;
xvi) laundering and/or hard surface cleaning including dish wash including Automatic Dish Wash (ADW);
xvii) facilitating or improving removal of xanthan gum containing soil, e.g., in a cleaning process;
xviii) facilitating or improving removal of xanthan gum containing soil in the presence of xanthan lyase and/or endoglucanase activity, e.g., in a cleaning process.

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EUROPEAN SEARCH REPORT

Application Number
EP 17 16 9957

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C11D

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	31 October 2017	van Klompenburg, Wim

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