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(54) **CLEANING COMPOSITIONS INCLUDING ENZYMES**

(57) Cleaning compositions that include an amylase enzyme and a glycosyl hydrolase enzyme. Methods of making and using cleaning compositions that include a glycosyl hydrolase enzyme. Use of a glycosyl hydrolase enzyme.

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**Description**

## REFERENCE TO A SEQUENCE LISTING

5 **[0001]** This application contains a Sequence Listing in computer readable form, which is incorporated herein by reference.

## FIELD OF THE INVENTION

10 **[0002]** The present disclosure relates to cleaning compositions comprising a specific glycoside hydrolase enzyme. The present disclosure also relates to methods of making and using such cleaning compositions. The present disclosure also relates to the use of the glycoside hydrolase enzyme.

## BACKGROUND OF THE INVENTION

15 **[0003]** The detergent formulator is constantly aiming to improve the performance of detergent compositions. One particular challenge is the removal of certain soils of microbial origin from surfaces such as textiles. Such soils can be sticky and difficult to remove. Furthermore, because they are sticky they tend to adhere body soils and/or particulate soils to the surface, making soil removal difficult and having a tendency to build up over time. This may be particularly noticeable for example on collars and cuffs where incomplete cleaning may occur.

20 **[0004]** There is a need for improved cleaning compositions which provide cleaning of such soils. The present inventors have found that this problem may be ameliorated by cleaning compositions comprising certain glycoside hydrolases. Glycosyl hydrolases are enzymes that catalyze the hydrolysis of the glycosyl bond to release smaller sugars. There are over 100 classes of glycosyl hydrolase and many different enzymes fall within the class of glycosyl hydrolases, for example cellulases and xyloglucanases which can be used in cleaning compositions. Surprisingly, certain specific gly-

25 **[0005]** Glycoside hydrolases are described by Coutinho, P.M. and Henrissat, B., 1999, Carbohydrate-active enzymes: an integrated database approach, in "Recent Advances In Carbohydrate Bioengineering", H.J. Gilbert, G. Davies, B. Henrissat and B. Svensson eds., The Royal Society of Chemistry, Cambridge, pp. 3-12.

30 **[0006]** The present invention provides a cleaning and/or treatment composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity, said glycosyl hydrolase enzyme being selected from the endo-alpha-1,4-polygalactosminidase class (EC 3.2.1.109) of enzymes.

35 **[0007]** A preferred glycoside hydrolase enzyme having glycoside hydrolase activity is a variant having at least 60% identity or at least 65% or at least 70% or at least 75% or at least 80% or at least 85% or at least 90% or at least 95% identity less than or up to 100% identity with SEQ ID NO:1.

40 **[0008]** Preferably the composition comprises from 1 to 80 wt% of a surfactant system, preferably comprising an anionic surfactant. The present invention provides a method of cleaning a surface, such as a textile, that comprises mixing a cleaning composition as described herein with water to form an aqueous liquor and contacting a surface with the aqueous liquor, in a laundering step. Preferably the glycoside hydrolase enzyme is present in the aqueous wash liquor in an amount of from 0.01ppm to 1000 ppm enzyme, based on active protein.

45 **[0009]** The present invention also relates to the use of a composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity selected from the endo-alpha-1,4-polygalactosminidase class (EC 3.2.1.109) of enzymes and preferably having at least 60% or at least 65% or at least 70% or at least 75% or at least 80% or at least 85% or at least 90% or at least 95% identity to 100% identity with SEQ ID NO:1, to enhance soil and/or stain removal and/or for malodour reduction, in particular for body soil removal.

50 **[0010]** A preferred composition comprises a second glycosyl hydrolase enzyme selected from glycoside hydrolase family 39.

## DETAILED DESCRIPTION OF THE INVENTION

55 **[0011]** The components of the compositions and processes of the present disclosure are described in more detail below.

**[0012]** As used herein, the articles "a" and "an" when used in a claim, are understood to mean one or more of what is claimed or described. As used herein, the terms "include," "includes," and "including" are meant to be non-limiting. The compositions of the present disclosure can comprise, consist essentially of, or consist of, the components of the present disclosure.

**[0013]** The terms "substantially free of" or "substantially free from" may be used herein. This means that the indicated material is at the very minimum not deliberately added to the composition to form part of it, or, preferably, is not present at analytically detectable levels. It is meant to include compositions whereby the indicated material is present only as an impurity in one of the other materials deliberately included. The indicated material may be present, if at all, at a level of less than 1%, or less than 0.1%, or less than 0.01%, or even 0%, by weight of the composition.

**[0014]** As used herein, the term "etheramine" includes the term "polyetheramine" and includes amines that have one or more ether groups.

**[0015]** Unless otherwise noted, all component or composition levels are in reference to the active portion of that component or composition, and are exclusive of impurities, for example, residual solvents or by-products, which may be present in commercially available sources of such components or compositions.

**[0016]** All temperatures herein are in degrees Celsius (°C) unless otherwise indicated. Unless otherwise specified, all measurements herein are conducted at 20°C and under atmospheric pressure.

**[0017]** In all embodiments of the present disclosure, all percentages are by weight of the total composition, unless specifically stated otherwise. All ratios are weight ratios, unless specifically stated otherwise.

**[0018]** It should be understood that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

**[0019]** As used herein, the term "alkoxy" is intended to include C1-C8 alkoxy and C1-C8 alkoxy derivatives of polyols having repeating units such as butylene oxide, glycidol oxide, ethylene oxide or propylene oxide.

**[0020]** As used herein, unless otherwise specified, the terms "alkyl" and "alkyl capped" are intended to include C1-C18 alkyl groups, or even C1-C6 alkyl groups.

**[0021]** As used herein, unless otherwise specified, the term "aryl" is intended to include C3-12 aryl groups.

**[0022]** As used herein, unless otherwise specified, the term "arylalkyl" and "alkaryl" are equivalent and are each intended to include groups comprising an alkyl moiety bound to an aromatic moiety, typically having C1-C18 alkyl groups and, in one aspect, C1-C6 alkyl groups.

**[0023]** The terms "ethylene oxide," "propylene oxide" and "butylene oxide" may be shown herein by their typical designation of "EO," "PO" and "BO," respectively.

**[0024]** As used herein, the term "cleaning and/or treatment composition" includes, unless otherwise indicated, granular, powder, liquid, gel, paste, unit dose, bar form and/or flake type washing agents and/or fabric treatment compositions, including but not limited to products for laundering fabrics, fabric softening compositions, fabric enhancing compositions, fabric freshening compositions, and other products for the care and maintenance of fabrics, and combinations thereof. Such compositions may be pre-treatment compositions for use prior to a washing step or may be rinse added compositions, as well as cleaning auxiliaries, such as bleach additives and/or "stain-stick" or pre-treat compositions or substrate-laden products such as dryer added sheets.

**[0025]** As used herein, "cellulosic substrates" are intended to include any substrate which comprises cellulose, either 100% by weight cellulose or at least 20% by weight, or at least 30 % by weight or at least 40 or at least 50 % by weight or even at least 60 % by weight cellulose. Cellulose may be found in wood, cotton, linen, jute, and hemp. Cellulosic substrates may be in the form of powders, fibers, pulp and articles formed from powders, fibers and pulp. Cellulosic fibers, include, without limitation, cotton, rayon (regenerated cellulose), acetate (cellulose acetate), triacetate (cellulose triacetate), and mixtures thereof. Typically cellulosic substrates comprise cotton. Articles formed from cellulosic fibers include textile articles such as fabrics. Articles formed from pulp include paper.

**[0026]** As used herein, the term "maximum extinction coefficient" is intended to describe the molar extinction coefficient at the wavelength of maximum absorption (also referred to herein as the maximum wavelength), in the range of 400 nanometers to 750 nanometers.

**[0027]** As used herein "average molecular weight" is reported as a weight average molecular weight, as determined by its molecular weight distribution; as a consequence of their manufacturing process, polymers disclosed herein may contain a distribution of repeating units in their polymeric moiety.

**[0028]** As used herein the term "variant" refers to a polypeptide that contains an amino acid sequence that differs from a wild type or reference sequence. A variant polypeptide can differ from the wild type or reference sequence due to a deletion, insertion, or substitution of a nucleotide(s) relative to said reference or wild type nucleotide sequence. The reference or wild type sequence can be a full-length native polypeptide sequence or any other fragment of a full-length polypeptide sequence. A polypeptide variant generally has at least about 70% amino acid sequence identity with the reference sequence, but may include 75% amino acid sequence identity within the reference sequence, 80% amino acid sequence identity within the reference sequence, 85% amino acid sequence identity with the reference sequence, 86% amino acid sequence identity with the reference sequence, 87% amino acid sequence identity with the reference

sequence, 88% amino acid sequence identity with the reference sequence, 89% amino acid sequence identity with the reference sequence, 90% amino acid sequence identity with the reference sequence, 91% amino acid sequence identity with the reference sequence, 92% amino acid sequence identity with the reference sequence, 93% amino acid sequence identity with the reference sequence, 94% amino acid sequence identity with the reference sequence, 95% amino acid sequence identity with the reference sequence, 96% amino acid sequence identity with the reference sequence, 97% amino acid sequence identity with the reference sequence, 98% amino acid sequence identity with the reference sequence, 98.5% amino acid sequence identity with the reference sequence or 99% amino acid sequence identity with the reference sequence.

**[0029]** As used herein, the term "solid" includes granular, powder, bar and tablet product forms.

**[0030]** As used herein, the term "fluid" includes liquid, gel, paste, and gas product forms.

#### Cleaning Composition

**[0031]** The present disclosure relates to cleaning and/or treatment compositions. The cleaning composition may be selected from the group of light duty liquid detergents compositions, heavy duty liquid detergent compositions, solid, for example powder detergent, hard surface cleaning compositions, detergent gels commonly used for laundry, bleaching compositions, laundry additives, fabric enhancer compositions, shampoos, body washes, other personal care compositions, and mixtures thereof. The cleaning composition may be a hard surface cleaning composition (such as a dish-washing composition) or a laundry composition (such as a heavy duty liquid detergent composition).

**[0032]** The cleaning compositions may be in any suitable form. The composition can be selected from a liquid, solid, or combination thereof. As used herein, "liquid" includes free-flowing liquids, as well as pastes, gels, foams and mousses. Non-limiting examples of liquids include light duty and heavy duty liquid detergent compositions, fabric enhancers, detergent gels commonly used for laundry, bleach and laundry additives. Gases, e.g., suspended bubbles, or solids, e.g. particles, may be included within the liquids. A "solid" as used herein includes, but is not limited to, powders, agglomerates, and mixtures thereof. Non-limiting examples of solids include: granules, microcapsules, beads, noodles, and pearlised balls. Solid compositions may provide a technical benefit including, but not limited to, through-the-wash benefits, pre-treatment benefits, and/or aesthetic effects.

**[0033]** The cleaning composition may be in the form of a unitized dose article, such as a tablet or in the form of a pouch. Such pouches typically include a water-soluble film, such as a polyvinyl alcohol water-soluble film, that at least partially encapsulates a composition. Suitable films are available from MonoSol, LLC (Indiana, USA). The composition can be encapsulated in a single or multi-compartment pouch. A multi-compartment pouch may have at least two, at least three, or at least four compartments. A multi-compartmented pouch may include compartments that are side-by-side and/or superposed. The composition contained in the pouch may be liquid, solid (such as powders), or combinations thereof.

#### Glycoside Hydrolase Enzyme

**[0034]** The enzyme essential to the present invention comprises glycoside hydrolase activity belonging to the endo-alpha-1,4-polygalactosaminidase class (EC 3.2.1.109) of enzymes, preferably having at least 60% or 65% or more preferably at least 70% or 75% or 80% or 85% or 90% or 95% up to 100% identity to SEQ ID NO:1.

**[0035]** Preferably the glycoside hydrolase is from GH family 114.

**[0036]** Preferably, the glycoside hydrolase enzyme is a microbial enzyme. The glycoside hydrolase enzyme may be fungal or bacterial in origin. Bacterial glycoside hydrolases may be most preferred. Fungal glycoside hydrolases may be most preferred.

**[0037]** The glycoside hydrolase may be obtainable from *Pseudomonas*, such as a *Pseudomonas aeruginosa*. Suitable examples from class EC 3.2.1.109 are described in Baker et al., (2016) Sci Adv, 2, such as the mature polypeptide SEQ ID NO: 1 of the present invention from *Pseudomonas aeruginosa*. Preferably the glycoside hydrolase in the cleaning composition of the invention is PelAh, optionally in addition to further glycoside hydrolases.

**[0038]** Preferably the glycoside hydrolase is an isolated glycoside hydrolase.

**[0039]** Preferably the glycoside hydrolase enzyme is present in the cleaning composition in an amount from 0.001 to 1 wt% based on active protein in the composition, or from 0.005 to 0.5 wt% or from 0.01 to 0.25 wt% based on weight of the composition.

**[0040]** Preferably the glycoside hydrolase enzyme is present in the laundering aqueous liquor in an amount of from 0.01ppm to 1000 ppm enzyme, based on active protein or from 0.05 or from 0.1ppm to 750 or 500ppm.

**[0041]** The glycoside hydrolases described herein may also give rise to biofilm-disrupting effects or soil anti-redeposition effects.

## Amylase Enzyme

**[0042]** The composition comprises an amylase enzyme. Suitable alpha-amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants (variants) are included. A preferred alkaline alpha-amylase is derived from a strain of *Bacillus*, such as *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or other *Bacillus* sp., such as *Bacillus* sp. NCIB 12289, NCIB 12512, NCIB 12513, DSM 9375 (USP 7,153,818) DSM 12368, DSMZ no. 12649, KSM AP1378 (WO 97/00324), KSM K36 or KSM K38 (EP 1,022,334). Preferred amylases include:

(a) the variants described in WO 94/02597, WO 94/18314, WO96/23874 and WO 97/43424, especially the variants with substitutions in one or more of the following positions versus the enzyme listed as SEQ ID No.2 in WO 96/23874 (SEQ ID NO: 2 herein): 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

(b) the variants described in USP 5,856,164 and WO99/23211, WO 96/23873, WO00/60060 and WO 06/002643, especially the variants with one or more substitutions in the following positions versus the AA560 enzyme listed as SEQ ID No. 12 in WO 06/002643 (SEQ ID NO: 3 herein):

26, 30, 33, 82, 37, 106, 118, 128, 133, 149, 150, 160, 178, 182, 186, 193, 203, 214, 231, 256, 257, 258, 269, 270, 272, 283, 295, 296, 298, 299, 303, 304, 305, 311, 314, 315, 318, 319, 339, 345, 361, 378, 383, 419, 421, 437, 441, 444, 445, 446, 447, 450, 461, 471, 482, 484, preferably that also contain the deletions of D183\* and G184\*.

(c) variants exhibiting at least 90% identity with SEQ ID No. 4 in WO06/002643 (SEQ ID NO: 4 herein), the wild-type enzyme from *Bacillus* SP722, especially variants with deletions in the 183 and 184 positions and variants described in WO 00/60060, which is incorporated herein by reference.

(d) variants exhibiting at least 95% identity with the wild-type enzyme from *Bacillus* sp.707 (SEQ ID NO:7 in US 6,093, 562) (SEQ ID NO: 5 herein), especially those comprising one or more of the following mutations M202, M208, S255, R172, and/or M261. Preferably said amylase comprises one or more of M202L, M202V, M202S, M202T, M202I, M202Q, M202W, S255N and/or R172Q. Particularly preferred are those comprising the M202L or M202T mutations.

(e) variants described in WO 09/149130, preferably those exhibiting at least 90% identity with SEQ ID NO: 1 or SEQ ID NO:2 in WO 09/149130 (SEQ ID NO:6 and SEQ ID NO: 7, respectively herein), the wild-type enzyme from *Geobacillus Stearothermophilus* or a truncated version thereof;

(f) variants as described in EP2540825 and EP2357220, EP2534233;

(g) variants as described in WO2009100102 and WO2010115028;

(h) variants exhibiting at least 89% identity with SEQ ID NO:1 in WO2016091688 (SEQ ID NO: 8 herein), especially those comprising deletions at positions H183+G184 and additionally one or more mutations at positions 405, 421, 422 and/or 428.

(i) variants exhibiting at least 60% amino acid sequence identity with the "PcuAmyl  $\alpha$ -amylase" from *Paenibacillus curdlanolyticus* YK9 (SEQ ID NO:3 in WO2014099523), (SEQ ID NO: 9 herein).

(j) variants exhibiting at least 60% amino acid sequence identity with the "CspAmy2 amylase" from *Cytophaga* sp. (SEQ ID NO:1 in WO2014164777, (SEQ ID NO: 10 herein)).

(k) variants exhibiting at least 85% identity with AmyE from *Bacillus subtilis* (SEQ ID NO:1 in WO2009149271, (SEQ ID NO: 11 herein)).

(l) Variants exhibiting at least 90% identity variant with the wild-type amylase from *Bacillus* sp. KSM-K38 with accession number AB051102.

**[0043]** Suitable commercially available alpha-amylases include DURAMYL®, LIQUEZYME®, TERMAMYL®, TERMAMYL ULTRA®, NATALASE®, SUPRAMYL®, STAINZYME®, STAINZYME PLUS®, FUNGAMYL® and BAN® (Novozymes A/S, Bagsvaerd, Denmark), KEMZYM® AT 9000 Biozym Biotech Trading GmbH Wehlstrasse 27b A-1200 Wien Austria, RAPIDASE®, PURASTAR®, ENZYSE®, OPTISIZE HT PLUS®, POWERASE® and PURASTAR OX-AM® (Genencor International Inc., Palo Alto, California) and KAM® (Kao, 14-10 Nihonbashi Kayabacho, 1-chome, Chuo-ku Tokyo 103-8210, Japan). In one aspect, suitable amylases include NATALASE®, STAINZYME® and STAINZYME PLUS® and mixtures thereof. The amylase is preferably present in an amount from about 0.00001% to about 2%, from about 0.0001% to about 1% or even from about 0.001% to about 0.5% enzyme protein by weight of the composition.

Optional Second Glycosyl Hydrolase Enzyme

**[0044]** A preferred composition comprises a second glycosyl hydrolase enzyme, preferably selected from GH family 39. A preferred second glycosyl hydrolase comprises glycoside hydrolase enzyme having at least 60% or at least 65% or at least 70% or at least 75% or at least 80% or at least 85% or at least 90% or at least 95%, and less than or up to 100% identity to SEQ ID NO:13. Preferably, the second glycoside hydrolase enzyme comprises a microbial enzyme, which, may be fungal or bacterial in origin.

**[0045]** The second glycoside hydrolase may be obtainable from *Pseudomonas*, such as a *Pseudomonas aeruginosa*. Suitable examples are described in Baker et al., (2016) Sci Adv, 2, such as the mature polypeptide SEQ ID NO: 12 herein from *Pseudomonas aeruginosa*. A preferred second glycoside hydrolase in the cleaning composition of the invention is PslGh. When present in the composition, the aforementioned second glycosyl hydrolase may be present at levels from about 0.00001% to about 2%, from about 0.0001% to about 1% or even from about 0.001% to about 0.5% enzyme protein by weight of the composition.

Adjuncts

**[0046]** The cleaning compositions described herein may optionally include other adjunct components, for example fabric care benefit agent; additional enzyme; surfactant system; fabric shading dye; deposition aid; rheology modifier; builder; chelant; bleach; bleach activator, bleaching agent; bleach precursor; bleach booster; bleach catalyst; perfume and/or perfume microcapsules; perfume loaded zeolite; starch encapsulated accord; polyglycerol esters; whitening agent; pearlescent agent; enzyme stabilizing systems; scavenging agents including fixing agents for anionic dyes, complexing agents for anionic surfactants, and mixtures thereof; optical brighteners or fluoresters; polymer including but not limited to soil release polymer and/or soil suspension polymer; dispersants; antifoam agents; non-aqueous solvent; fatty acid; suds suppressors, e.g., silicone suds suppressors; cationic starches; scum dispersants; substantive dyes; colorants; opacifier; antioxidant; hydrotropes such as toluenesulfonates, cumenesulfonates and naphthalenesulfonates; color speckles; colored beads, spheres or extrudates; clay softening agents; anti-bacterial agents, quaternary ammonium compounds. In particular quaternary ammonium compounds may be present in particular for fabric enhancer compositions, such as fabric softeners, and comprise quaternary ammonium cations that are positively charged polyatomic ions of the structure  $NR_4^+$ , where R is an alkyl group or an aryl group.

Additional Enzymes

**[0047]** Preferably the composition of the invention comprises additional enzyme, for example selected from lipases, proteases, nucleases, galactanases, mannanases, pectate lyases, cellulases, cutinases, and mixtures thereof. The cleaning compositions preferably comprise one or more additional enzymes from the group selected from nucleases, galactanases, mannanases and mixtures thereof. The cleaning composition preferably comprises one or more additional enzymes selected from the group nucleases, galactanases, mannanases and mixtures thereof. Preferably in addition, the cleaning compositions comprise one or more additional enzymes selected from proteases. Preferably the cleaning composition comprises one or more additional enzymes selected from lipases. The composition may also comprise hemicellulases, peroxidases, xylanases, pectinases, keratinases, reductases, oxidases, phenoloxidases, lipoxigenases, ligninases, pullulanases, tannases, pentosanases, malanases,  $\beta$ -glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase and mixtures thereof. When present in the composition, the aforementioned additional enzymes may be present at levels from about 0.00001% to about 2%, from about 0.0001% to about 1% or even from about 0.001% to about 0.5% enzyme protein by weight of the composition.

Nucleases

**[0048]** In a preferred composition, the composition additionally comprises a nuclease enzyme. The nuclease enzyme is an enzyme capable of cleaving the phosphodiester bonds between the nucleotide sub-units of nucleic acids. Suitable nuclease enzymes may be deoxyribonuclease or ribonuclease enzyme or a functional fragment thereof. By functional fragment or part is meant the portion of the nuclease enzyme that catalyzes the cleavage of phosphodiester linkages in the DNA backbone and so is a region of said nuclease protein that retains catalytic activity. Thus it includes truncated, but functional versions, of the enzyme and/or variants and/or derivatives and/or homologues whose functionality is maintained.

**[0049]** Preferably the nuclease enzyme is a deoxyribonuclease, preferably selected from any of the classes E.C. 3.1.21.x, where x=1, 2, 3, 4, 5, 6, 7, 8 or 9, E.C. 3.1.22.y where y=1, 2, 4 or 5, E.C. 3.1.30.z where z= 1 or 2, E.C. 3.1.31.1 and mixtures thereof. Nuclease enzymes from class E.C. 3.1.21.x and especially where x=1 are particularly preferred. Nucleases in class E.C. 3.1.22.y cleave at the 5' hydroxyl to liberate 3' phosphomonoesters. Enzymes in class E.C.

3.1.30.z may be preferred as they act on both DNA and RNA and liberate 5'-phosphomonoesters. Suitable examples from class E.C. 3.1.31.2 are described in US2012/0135498A, such as SEQ ID NO:3 therein. Such enzymes are commercially available as DENARASE® enzyme from c-LECTA. Nuclease enzymes from class E.C. 3.1.31.1 produce 3'-phosphomonoesters.

**[0050]** Preferably, the nuclease enzyme comprises a microbial enzyme. The nuclease enzyme may be fungal or bacterial in origin. Bacterial nucleases may be most preferred. Fungal nucleases may be most preferred.

**[0051]** The microbial nuclease is obtainable from *Bacillus*, such as a *Bacillus licheniformis* or *Bacillus subtilis* bacterial nucleases. A preferred nuclease is obtainable from *Bacillus licheniformis*, preferably from strain EI-34-6. A preferred deoxyribonuclease is a variant of *Bacillus licheniformis*, from strain EI-34-6 nucB deoxyribonuclease defined in SEQ ID NO:14 herein, or variant thereof, for example having at least 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto. Other suitable nucleases are defined in SEQ ID NO:15 herein, or variant thereof, for example having at least 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto. Other suitable nucleases are defined in SEQ ID NO:16 herein, or variant thereof, for example having at least 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto.

**[0052]** A fungal nuclease is obtainable from *Aspergillus*, for example *Aspergillus oryzae*. A preferred nuclease is obtainable from *Aspergillus oryzae* defined in SEQ ID NO:17 herein, or variant thereof, for example having at least 60% or 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto.

**[0053]** Another suitable fungal nuclease is obtainable from *Trichoderma*, for example *Trichoderma harzianum*. A preferred nuclease is obtainable from *Trichoderma harzianum* defined in SEQ ID NO: 18 herein, or variant thereof, for example having at least 60% or 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto.

**[0054]** Other fungal nucleases include those encoded by the DNA sequences of *Aspergillus oryzae* RIB40, *Aspergillus oryzae* 3.042, *Aspergillus flavus* NRRL3357, *Aspergillus parasiticus* SU-1, *Aspergillus nomius* NRRL13137, *Trichoderma reesei* QM6a, *Trichoderma virens* Gv29-8, *Oidiodendron maius* Zn, *Metarhizium guizhouense* ARSEF 977, *Metarhizium majus* ARSEF 297, *Metarhizium robertsii* ARSEF 23, *Metarhizium acridum* CQMa 102, *Metarhizium brunneum* ARSEF 3297, *Metarhizium anisopliae*, *Colletotrichum fiorinae* PJ7, *Colletotrichum sublineola*, *Trichoderma atroviride* IMI 206040, *Tolypocladium ophioglossoides* CBS 100239, *Beauveria bassiana* ARSEF 2860, *Colletotrichum higginsianum*, *Hirsutella minnesotensis* 3608, *Scedosporium apiospermum*, *Phaeomoniella chlamydospora*, *Fusarium verticillioides* 7600, *Fusarium oxysporum* f. sp. cubense race 4, *Colletotrichum graminicola* M1.001, *Fusarium oxysporum* FOSC 3-a, *Fusarium avenaceum*, *Fusarium langsethiae*, *Grosmannia clavigera* kw1407, *Claviceps purpurea* 20.1, *Verticillium longisporum*, *Fusarium oxysporum* f. sp. cubense race 1, *Magnaporthe oryzae* 70-15, *Beauveria bassiana* D1-5, *Fusarium pseudograminearum* CS3096, *Neonectria ditissima*, *Magnaportheopsis poae* ATCC 64411, *Cordyceps militaris* CM01, *Marssonina brunnea* f. sp. 'multigermtubi' MB\_m1, *Diaporthe ampelina*, *Metarhizium album* ARSEF 1941, *Colletotrichum gloeosporioides* Nara gc5, *Madurella mycetomatis*, *Metarhizium brunneum* ARSEF 3297, *Verticillium alfalfae* VaMs.102, *Gaeumannomyces graminis* var. tritici R3-111a-1, *Nectria haematococca* mpVI 77-13-4, *Verticillium longisporum*, *Verticillium dahliae* VdLs.17, *Torribiella hemipterigena*, *Verticillium longisporum*, *Verticillium dahliae* VdLs.17, *Botrytis cinerea* B05.10, *Chaetomium globosum* CBS 148.51, *Metarhizium anisopliae*, *Stemphylium lycopersici*, *Sclerotinia borealis* F-4157, *Metarhizium robertsii* ARSEF 23, *Myceliophthora thermophila* ATCC 42464, *Phaeosphaeria nodorum* SN15, *Phialophora attae*, *Ustilagoidea virens*, *Diplodia seriata*, *Ophiostoma piceae* UAMH 11346, *Pseudogymnoascus pan-norum* VKM F-4515 (FW-2607), *Bipolaris oryzae* ATCC 44560, *Metarhizium guizhouense* ARSEF 977, *Chaetomium thermophilum* var. thermophilum DSM 1495, *Pestalotiopsis fici* W106-1, *Bipolaris zeicola* 26-R-13, *Setosphaeria turcica* Et28A, *Arthroderma otae* CBS 113480 and *Pyrenophora tritici-repentis* Pt-1C-BFP.

**[0055]** Preferably the nuclease is an isolated nuclease.

**[0056]** Preferably the nuclease enzyme is present in the aqueous solution in an amount from 0.01ppm to 1000 ppm of the nuclease enzyme, or from 0.05 or from 0.1ppm to 750 or 500ppm.

#### Galactanases

**[0057]** Preferably as an additional enzyme, the composition comprises a galactanase. Particularly preferred are the endo-beta-1,6-galactanase extracellular polymer-degrading enzyme. The term "endo-beta-1,6-galactanase" or "a polypeptide having endo-beta-1,6-galactanase activity" means an endo-beta-1,6-galactanase (EC 3.2.1.164) from the glycoside hydrolase family 30 that catalyzes the hydrolytic cleavage of 1,6-3-D-galactooligosaccharides with a degree of polymerization (DP) higher than 3, and their acidic derivatives with 4-O-methylglucosyluronate or glucosyluronate groups at the non-reducing terminals. For purposes of the present disclosure, endo-beta-1,6-galactanase activity is determined according to the procedure described in WO 2015185689 in Assay I. Suitable examples from class EC 3.2.1.164 are described in WO 2015185689, such as the mature polypeptide SEQ ID NO: 2 described therein.

**[0058]** Preferably the galactanase enzyme is selected from Glycoside Hydrolase Family 30.

**[0059]** Preferably, the endo-beta-1,6-galactanase is a microbial enzyme. The endo-beta-1,6-galactanase may be fungal or bacterial in origin. Bacterial endo-beta-1,6-galactanase may be most preferred. Fungal endo-beta-1,6-galac-

tanase may be most preferred.

**[0060]** A bacterial endo-beta-1,6-galactanase is obtainable from *Streptomyces*, for example *Streptomyces davawensis*. A preferred endo-beta-1,6-galactanase is obtainable from *Streptomyces davawensis* JCM 4913 defined in SEQ ID NO: 19 herein, or variant thereof, for example having at least 40% or 50% or 60% or 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto.

**[0061]** Other bacterial endo-beta-1,6-galactanase include those encoded by the DNA sequences of *Streptomyces avermitilis* MA-4680 with amino acid sequence defined in SEQ ID NO: 20 herein, or variant thereof, for example having at least 40% or 50% or 60% or 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto.

**[0062]** A fungal endo-beta-1,6-galactanase is obtainable from *Trichoderma*, for example *Trichoderma harzianum*. A preferred endo-beta-1,6-galactanase is obtainable from *Trichoderma harzianum* defined in SEQ ID NO: 21 herein, or variant thereof, for example having at least 40% or 50% or 60% or 70% or 75% or 80% or 85% or 90% or 95%, 96%, 97%, 98%, 99% or 100% identical thereto.

**[0063]** Other fungal endo-beta-1,6-galactanase include those encoded by the DNA sequences of *Ceratocystis fimbriata* f. sp. Platani, *Muscodora strobilii* WG-2009a, *Oculimacula yallundae*, *Trichoderma viride* GD36A, *Thermomyces stellatus*, *Myceliophthora thermophila*.

Preferably the galactanase has an amino acid sequence having at least 60%, or at least 80%, or at least 90% or at least 95% identity with the amino acid sequence shown in SEQ ID NO: 19, SEQ ID NO: 20 or SEQ ID NO: 21.

**[0064]** Preferably the galactanase is an isolated galactanase.

**[0065]** Preferably the galactanase enzyme is present in the composition in an amount from 0.001 to 1 wt% based on active protein in the composition, or from 0.005 to 0.5 wt% or from 0.01 to 0.25 wt% based on the weight of the composition. Preferably the galactanase enzyme is present in the laundering aqueous solution in an amount of from 0.01 ppm to 1000 ppm of the galactanase enzyme, or from 0.05 or from 0.1 ppm to 750 or 500 ppm.

#### Mannanases

**[0066]** Preferably the composition comprises a mannanase enzyme. Mannanase enzymes are polypeptides having mannan endo-1,4- beta-mannosidase activity (EC 3.2.1.78) from the glycoside hydrolase family 26 that catalyzes the hydrolysis of 1,4-3-D-mannosidic linkages in mannans, galactomannans and glucomannans. Alternative names of mannan endo-1,4-beta-mannosidase are 1,4-3-D-mannan mannanohydrolase; endo-1,4-3-mannanase; endo-  $\beta$ -1,4-mannase;  $\beta$ -mannanase B; 3-1,4-mannan 4-mannanohydrolase; endo-3-mannanase; and  $\beta$ -D-mannanase. Preferred mannanases are members of the glycoside hydrolase family 26.

**[0067]** For purposes of the present disclosure, mannanase activity may be determined using the Reducing End Assay as described in the experimental section of WO 2015040159.

Suitable examples from class EC 3.2.1.78 are described in WO 2015040159, such as the mature polypeptide SEQ ID NO: 2 described therein.

**[0068]** Preferred mannanases are variants having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81 %, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 mature polypeptide SEQ ID NO: 22 from *Ascobolus stictoides*;

**[0069]** Preferred mannanases are variants having at least 81 %, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 mature polypeptide SEQ ID NO: 23 from *Chaetomium*

#### *virescens*.

**[0070]** Preferred mannanases are variants having at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 mature polypeptide SEQ ID NO: 24 from *Preussia aemulans*.

**[0071]** Preferred mannanases are variants having at least at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 mature polypeptide SEQ ID NO: 25 from *Yunnania penicillata*.

**[0072]** Preferred mannanases are variants having at least at least 75%, at least 76%, at least 77%, at least 78%, at



least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 mature polypeptide SEQ ID NO: 26 from *Myrothecium roridum*.

**[0073]** Preferably the mannanase is an isolated mannanase.

**[0074]** Preferably the mannanase enzyme is present in the cleaning compositions in an amount from 0.001 to 1 wt% based on active protein in the composition, or from 0.005 to 0.5 wt% or from 0.01 to 0.25 wt%. Preferably the mannanase enzyme is present in a the laundering aqueous solution in an amount of from 0.01ppm to 1000 ppm of the mannanase enzyme, or from 0.05 or from 0.1ppm to 750 or 500ppm.

The mannanases may also give rise to biofilm-disrupting effects. Xanthan-degrading enzyme

The composition preferably comprises a xanthan-degrading enzyme. Xanthan gum is a polysaccharide secreted by the bacterium *Xanthomonas campestris*. Xanthan is composed of pentasaccharide subunits, forming a cellulose backbone with trisaccharide side chains composed of mannose-(beta 1, 4)-glucuronic-acid-(beta 1, 2)-mannose attached to alternate glucose residues in the backbone by alpha1 ,3 linkages. The cleaning composition preferably includes a xanthan degrading polypeptide having xanthan lyase activity and/or endo-beta-1,4-glucanase activity. Xanthan lyases are enzymes that cleave the beta-D-mannosylalpha-beta-D-1 ,4-glucuronosyl bond of xanthan, preferably xanthan lyases isolated from *Paenibacillus alginolyticus* XL-1. Preferred xanthan-degrading enzymes are selected from the glycosyl hydrolase family 5 (GH5).

#### Acetylglucosaminidases

**[0075]** In a preferred composition, the composition may additionally comprise an acetylglucosaminidase enzyme, preferably a  $\beta$ -N-acetylglucosaminidase enzyme from E.C. 3.2.1.52, preferably an enzyme having at least 70%, or at least 75% or at least 80% or at least 85% or at least 90% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or at least or 100% identity to SEQ ID NO:27.

#### Proteases

**[0076]** Preferably the composition comprises one or more proteases. Suitable proteases include metalloproteases and serine proteases, including neutral or alkaline microbial serine proteases, such as subtilisins (EC 3.4.21.62). Suitable proteases include those of animal, vegetable or microbial origin. In one aspect, such suitable protease may be of microbial origin. The suitable proteases include chemically or genetically modified mutants of the aforementioned suitable proteases. In one aspect, the suitable protease may be a serine protease, such as an alkaline microbial protease or/and a trypsin-type protease. Examples of suitable neutral or alkaline proteases include:

(a) subtilisins (EC 3.4.21.62), preferably those derived from *Bacillus* sp., such as *B. lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *B. pumilus* and *B. gibsonii* and *B. akibaii* described in WO2004067737, WO2015091989, WO2015091990, WO2015024739, WO2015143360, US 6,312,936 B1, US 5,679,630, US 4,760,025, US7,262,042 and WO09/021867, DE102006022216A1, DE102006022224A1, WO2015089447, WO2015089441, WO2016066756, WO2016066757, WO2016069557, WO2016069563, WO2016069569.

(b) trypsin-type or chymotrypsin-type proteases, such as trypsin (e.g., of porcine or bovine origin), including the *Fusarium* protease described in WO 89/06270 and the chymotrypsin proteases derived from *Cellulomonas* described in WO 05/052161 and WO 05/052146.

(c) metalloproteases, preferably those derived from *Bacillus amyloliquefaciens* described in WO 07/044993A2; from *Bacillus*, *Brevibacillus*, *Thermoactinomyces*, *Geobacillus*, *Paenibacillus*, *Lysinibacillus* or *Streptomyces* spp. Described in WO2014194032, WO2014194054 and WO2014194117; from *Kribella alluminosa* described in WO2015193488; and from *Streptomyces* and *Lysobacter* described in WO2016075078.

(d) Protease having at least 90% identity to the subtilase from *Bacillus* sp. TY145, NCIMB 40339, described in WO92/17577 (Novozymes A/S), including the variants of this *Bacillus* sp TY145 subtilase described in WO2015024739, and WO2016066757.

**[0077]** Preferred proteases include those derived from *Bacillus gibsonii* or *Bacillus Lentus*.

**[0078]** Suitable commercially available protease enzymes include those sold under the trade names Alcalase®, Savinase®, Primase®, Durazym®, Polarzyme®, Kannase®, Liquanase®, Liquanase Ultra®, Savinase Ultra®, Ovozime®, Neutrase®, Everlase® and Esperase® by Novozymes A/S (Denmark), those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Properase®, Purafect®, Purafect Prime®, Purafect Ox®, FN3®, FN4®, Excellase® and Purafect OXP® by Genencor International, those sold under the tradename Opticlean® and Optimase® by Solvay Enzymes, those available from Henkel/ Kemira, namely BLAP (sequence shown in Figure 29 of US 5,352,604 with the following

mutations S99D + S101 R + S103A + V104I + G159S, hereinafter referred to as BLAP), BLAP R (BLAP with S3T + V4I + V199M + V205I + L217D), BLAP X (BLAP with S3T + V4I + V205I) and BLAP F49 (BLAP with S3T + V4I + A194P + V199M + V205I + L217D) - all from Henkel/Kemira; and KAP (*Bacillus alkalophilus* subtilisin with mutations A230V + S256G + S259N) from Kao, or as disclosed in WO2009/149144, WO2009/149145, WO2010/56653, WO2010/56640, WO2011/072117, US2011/0237487, WO2011/140316, WO2012/151480, EP2510092, EP2566960 OR EP2705145.

#### Lipases

**[0079]** Preferably the composition comprises one or more lipases, including "first cycle lipases" such as those described in U.S. Patent 6,939,702 B1 and US PA 2009/0217464. Preferred lipases are first-wash lipases. In one embodiment of the invention the composition comprises a first wash lipase. First wash lipases includes a lipase which is a polypeptide having an amino acid sequence which: (a) has at least 90% identity with the wild-type lipase derived from *Humicola lanuginosa* strain DSM 4109; (b) compared to said wild-type lipase, comprises a substitution of an electrically neutral or negatively charged amino acid at the surface of the three-dimensional structure within 15A of E1 or Q249 with a positively charged amino acid; and (c) comprises a peptide addition at the C-terminal; and/or (d) comprises a peptide addition at the N-terminal and/or (e) meets the following limitations: i) comprises a negative amino acid in position E210 of said wild-type lipase; ii) comprises a negatively charged amino acid in the region corresponding to positions 90-101 of said wild-type lipase; and iii) comprises a neutral or negative amino acid at a position corresponding to N94 or said wild-type lipase and/or has a negative or neutral net electric charge in the region corresponding to positions 90-101 of said wild-type lipase. Preferred are variants of the wild-type lipase from *Thermomyces lanuginosus* comprising one or more of the T231R and N233R mutations. The wild-type sequence is the 269 amino acids (amino acids 23 - 291) of the Swissprot accession number Swiss-Prot 059952 (derived from *Thermomyces lanuginosus* (*Humicola lanuginosa*)). Preferred lipases would include those sold under the tradenames Lipex® and Lipolex® and Lipoclean®. Other suitable lipases include those described in European Patent Application No. 12001034.3 or EP2623586.

#### Endoglucanases

**[0080]** Other preferred enzymes include microbial-derived endoglucanases exhibiting endo-beta-1,4-glucanase activity (E.C. 3.2.1.4), including a bacterial polypeptide endogenous to a member of the genus *Bacillus* which has a sequence of at least 90%, 94%, 97% and even 99% identity to the amino acid sequence SEQ ID NO:2 in US7,141,403B2) and mixtures thereof. Suitable endoglucanases are sold under the tradenames Celluclean® and Whitezyme® (Novozymes A/S, Bagsvaerd, Denmark).

#### Pectate Lyases

**[0081]** Other preferred enzymes include pectate lyases sold under the tradenames Pectawash®, Pectaway®, Xpect® and mannanases sold under the tradenames Mannaway® (all from Novozymes A/S, Bagsvaerd, Denmark), and Purabrite® (Genencor International Inc., Palo Alto, California).

#### Surfactant system

**[0082]** The cleaning composition may comprise a surfactant system. The cleaning composition may comprise from about 1% to about 80%, or from 1% to about 60%, preferably from about 5% to about 50% more preferably from about 8% to about 40%, by weight of the cleaning composition, of a surfactant system.

**[0083]** Surfactants suitable for use in the surfactant system may be derived from natural and/or renewable sources.

**[0084]** The surfactant system may comprise an anionic surfactant, more preferably an anionic surfactant selected from the group consisting of alkyl benzene sulfonate, alkyl sulfate, alkyl alkoxy sulfate. Alkyl ethoxy sulfate, paraffin sulfonate and mixtures thereof may be preferred, however, alkyl benzene sulfonates are particularly preferred. The surfactant system may further comprise a surfactant selected from the group consisting of nonionic surfactant, cationic surfactant, amphoteric surfactant, zwitterionic surfactant, and mixtures thereof. The surfactant system preferably comprises a nonionic surfactant, for example an ethoxylated nonionic surfactant. The surfactant system may comprise an amphoteric surfactant, for example an amine oxide surfactant, such as an alkyl dimethyl amine oxide. The surfactant system may comprise a zwitterionic surfactant, such as a betaine.

**[0085]** The most preferred surfactant system for the detergent composition of the present invention comprises from 1% to 40%, preferably 6% to 35%, more preferably 8% to 30% weight of the total composition of an anionic surfactant, preferably comprising an alkyl benzene sulphonate. The preferred surfactant system may optionally in addition comprise an alkyl alkoxy sulfate surfactant, more preferably an alkyl ethoxy sulfate, optionally combined with 0.5% to 15%, preferably from 1% to 12%, more preferably from 2% to 10% by weight of the composition of amphoteric and/or zwitterionic

surfactant, more preferably an amphoteric and even more preferably an amine oxide surfactant, especially an alkyl dimethyl amine oxide.

**[0086]** Most preferably the surfactant system comprises an anionic and a nonionic surfactant, preferably the weight ratio of the anionic to nonionic surfactant is from 25:1 to 1:2.

#### Anionic surfactant

**[0087]** Anionic surfactants may be in salt form or acid form, typically in the form of a water-soluble sodium, potassium, ammonium, magnesium or mono-, di- or tri- C2-C3 alkanolammonium salt, with the sodium cation being the usual one chosen.

#### Sulfonate Surfactant

**[0088]** Suitable anionic sulfonate surfactants for use herein include water-soluble salts of C8-C18 alkyl or hydroxyalkyl sulfonates; C11-C18 alkyl benzene sulfonates (LAS), modified alkylbenzene sulfonate (MLAS) as discussed in WO 99/05243, WO 99/05242, WO 99/05244, WO 99/05082, WO 99/05084, WO 99/05241, WO 99/07656, WO 00/23549, and WO 00/23548; methyl ester sulfonate (MES); and alpha-olefin sulfonate (AOS). Those also include the paraffin sulfonates may be monosulfonates and/or disulfonates, obtained by sulfonating paraffins of 10 to 20 carbon atoms. The sulfonate surfactant may also include the alkyl glyceryl sulfonate surfactants.

#### Sulfated anionic surfactant

**[0089]** Preferably the sulfated anionic surfactant is alkoxyated, more preferably, an alkoxyated branched sulfated anionic surfactant having an alkoxylation degree of from about 0.2 to about 4, even more preferably from about 0.3 to about 3, even more preferably from about 0.4 to about 1.5 and especially from about 0.4 to about 1. Preferably, the alkoxy group is ethoxy. When the sulfated anionic surfactant is a mixture of sulfated anionic surfactants, the alkoxylation degree is the weight average alkoxylation degree of all the components of the mixture (weight average alkoxylation degree). In the weight average alkoxylation degree calculation the weight of sulfated anionic surfactant components not having alkoxyated groups should also be included.

$$\text{Weight average alkoxylation degree} = (x_1 * \text{alkoxylation degree of surfactant 1} + x_2 * \text{alkoxylation degree of surfactant 2} + \dots) / (x_1 + x_2 + \dots)$$

wherein  $x_1, x_2, \dots$  are the weights in grams of each sulfated anionic surfactant of the mixture and alkoxylation degree is the number of alkoxy groups in each sulfated anionic surfactant.

**[0090]** Preferably, the branching group is an alkyl. Typically, the alkyl is selected from methyl, ethyl, propyl, butyl, pentyl, cyclic alkyl groups and mixtures thereof. Single or multiple alkyl branches could be present on the main hydrocarbyl chain of the starting alcohol(s) used to produce the sulfated anionic surfactant used in the detergent of the invention. Most preferably the branched sulfated anionic surfactant is selected from alkyl sulfates, alkyl ethoxy sulfates, and mixtures thereof.

**[0091]** The branched sulfated anionic surfactant can be a single anionic surfactant or a mixture of anionic surfactants. In the case of a single surfactant the percentage of branching refers to the weight percentage of the hydrocarbyl chains that are branched in the original alcohol from which the surfactant is derived.

**[0092]** In the case of a surfactant mixture the percentage of branching is the weight average and it is defined according to the following formula:

$$\text{Weight average of branching (\%)} = [(x_1 * \text{wt\% branched alcohol 1 in alcohol 1} + x_2 * \text{wt\% branched alcohol 2 in alcohol 2} + \dots) / (x_1 + x_2 + \dots)] * 100$$

wherein  $x_1, x_2, \dots$  are the weight in grams of each alcohol in the total alcohol mixture of the alcohols which were used as starting material for the anionic surfactant for the detergent of the invention. In the weight average branching degree calculation the weight of anionic surfactant components not having branched groups should also be included.

**[0093]** Suitable sulfate surfactants for use herein include water-soluble salts of C8-C18 alkyl or hydroxyalkyl, sulfate and/or ether sulfate. Suitable counterions include alkali metal cation or ammonium or substituted ammonium, but pref-

erably sodium.

**[0094]** The sulfate surfactants may be selected from C8-C18 primary, branched chain and random alkyl sulfates (AS); C8-C18 secondary (2,3) alkyl sulfates; C8-C18 alkyl alkoxy sulfates (AExS) wherein preferably x is from 1-30 in which the alkoxy group could be selected from ethoxy, propoxy, butoxy or even higher alkoxy groups and mixtures thereof.

**[0095]** Alkyl sulfates and alkyl alkoxy sulfates are commercially available with a variety of chain lengths, ethoxylation and branching degrees. Commercially available sulfates include, those based on Neodol alcohols ex the Shell company, Lial - Isalchem and Safol ex the Sasol company, natural alcohols ex The Procter & Gamble Chemicals company.

**[0096]** Preferred alkyl sulfates are those in which the anionic surfactant is an alkyl ethoxy sulfate with a degree of ethoxylation of from about 0.2 to about 3, more preferably from about 0.3 to about 2, even more preferably from about 0.4 to about 1.5, and especially from about 0.4 to about 1. They are also preferred anionic surfactant having a level of branching of from about 5% to about 40%, even more preferably from about 10% to 35% and especially from about 20% to 30%.

#### Nonionic surfactant

**[0097]** Preferably the surfactant system comprises a nonionic surfactant, in an amount of from 0.1% to 40%, preferably 0.2% to 20%, most preferably 0.5% to 10% by weight of the composition. Suitable nonionic surfactants include the condensation products of aliphatic alcohols with from 1 to 25 moles of ethylene oxide. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from 8 to 22 carbon atoms. Particularly preferred are the condensation products of alcohols having an alkyl group containing from 10 to 18 carbon atoms, preferably from 10 to 15 carbon atoms with from 2 to 18 moles, preferably 2 to 15, more preferably 5-12 of ethylene oxide per mole of alcohol. Highly preferred nonionic surfactants are the condensation products of guerbet alcohols with from 2 to 18 moles, preferably 2 to 15, more preferably 5-12 of ethylene oxide per mole of alcohol.

**[0098]** Other suitable non-ionic surfactants for use herein include fatty alcohol polyglycol ethers, alkylpolyglucosides and fatty acid glucamides.

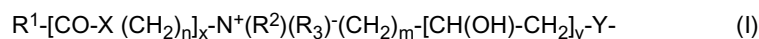
#### Amphoteric surfactant

**[0099]** The surfactant system may include amphoteric surfactant, such as amine oxide. Preferred amine oxides are alkyl dimethyl amine oxide or alkyl amido propyl dimethyl amine oxide, more preferably alkyl dimethyl amine oxide and especially coco dimethyl amino oxide. Amine oxide may have a linear or mid-branched alkyl moiety. Typical linear amine oxides include water-soluble amine oxides containing one R1 C8-18 alkyl moiety and 2 R2 and R3 moieties selected from the group consisting of C1-3 alkyl groups and C1-3 hydroxyalkyl groups. Preferably amine oxide is characterized by the formula  $R1 - N(R2)(R3)O$  wherein R1 is a C8-18 alkyl and R2 and R3 are selected from the group consisting of methyl, ethyl, propyl, isopropyl, 2-hydroxyethyl, 2-hydroxypropyl and 3-hydroxypropyl. The linear amine oxide surfactants in particular may include linear C10-C18 alkyl dimethyl amine oxides and linear C8-C12 alkoxy ethyl dihydroxy ethyl amine oxides. Preferred amine oxides include linear C10, linear C10-C12, and linear C12-C14 alkyl dimethyl amine oxides. As used herein "mid-branched" means that the amine oxide has one alkyl moiety having n1 carbon atoms with one alkyl branch on the alkyl moiety having n2 carbon atoms. The alkyl branch is located on the  $\alpha$  carbon from the nitrogen on the alkyl moiety. This type of branching for the amine oxide is also known in the art as an internal amine oxide. The total sum of n1 and n2 is from 10 to 24 carbon atoms, preferably from 12 to 20, and more preferably from 10 to 16. The number of carbon atoms for the one alkyl moiety (n1) should be approximately the same number of carbon atoms as the one alkyl branch (n2) such that the one alkyl moiety and the one alkyl branch are symmetric. As used herein "symmetric" means that  $|n1 - n2|$  is less than or equal to 5, preferably 4, most preferably from 0 to 4 carbon atoms in at least 50 wt%, more preferably at least 75 wt% to 100 wt% of the mid-branched amine oxides for use herein.

**[0100]** The amine oxide may further comprise two moieties, independently selected from a C1-3 alkyl, a C1-3 hydroxy-alkyl group, or a polyethylene oxide group containing an average of from about 1 to about 3 ethylene oxide groups. Preferably the two moieties are selected from a C1-3 alkyl, more preferably both are selected as a C1 alkyl.

#### Zwitterionic surfactant

**[0101]** Other suitable surfactants include betaines, such as alkyl betaines, alkylamidobetaine, amidazoliniumbetaine, sulfobetaine (INCI Sultaines) as well as the Phosphobetaine and preferably meets formula (I):



wherein

R<sup>1</sup> is a saturated or unsaturated C6-22 alkyl residue, preferably C8-18 alkyl residue, in particular a saturated C10-16 alkyl residue, for example a saturated C12-14 alkyl residue;

X is NH, NR<sup>4</sup> with C1-4 Alkyl residue R<sup>4</sup>, O or S,

n a number from 1 to 10, preferably 2 to 5, in particular 3,

x 0 or 1, preferably 1,

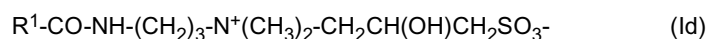
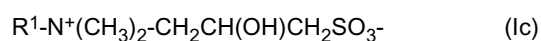
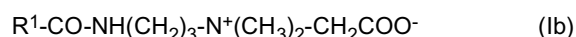
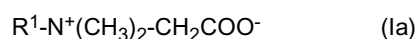
R<sup>2</sup>, R<sup>3</sup> are independently a C1-4 alkyl residue, potentially hydroxy substituted such as a hydroxyethyl, preferably a methyl.

m a number from 1 to 4, in particular 1, 2 or 3,

y 0 or 1 and

Y is COO, SO<sub>3</sub>, OPO(OR<sup>5</sup>)O or P(O)(OR<sup>5</sup>)O, whereby R<sup>5</sup> is a hydrogen atom H or a C1-4 alkyl residue.

**[0102]** Preferred betaines are the alkyl betaines of the formula (Ia), the alkyl amido propyl betaine of the formula (Ib), the Sulfo betaines of the formula (Ic) and the Amido sulfobetaine of the formula (Id);



in which R<sup>1</sup> has the same meaning as in formula I. Particularly preferred betaines are the Carbobetaine [wherein Y = COO<sup>-</sup>], in particular the Carbobetaine of the formula (Ia) and (Ib), more preferred are the Alkylamidobetaine of the formula (Ib).

**[0103]** Examples of suitable betaines and sulfobetaine are the following [designated in accordance with INCI]: Almondamidopropyl of betaines, Apricotamidopropyl betaines, Avocamidopropyl of betaines, Babassamidopropyl of betaines, Behenamidopropyl betaines, Behenyl of betaines, betaines, Canolamidopropyl betaines, Capryl/Capramidopropyl betaines, Carnitine, Cetyl of betaines, Cocamidoethyl of betaines, Cocamidopropyl betaines, Cocamidopropyl Hydroxysultaine, Coco betaines, Coco Hydroxysultaine, Coco/Oleamidopropyl betaines, Coco Sultaine, Decyl of betaines, Dihydroxyethyl Oleyl Glycinate, Dihydroxyethyl Soy Glycinate, Dihydroxyethyl Stearyl Glycinate, Dihydroxyethyl Tallow Glycinate, Dimethicone Propyl of PG-betaines, Erucamidopropyl Hydroxysultaine, Hydrogenated Tallow of betaines, Isostearamidopropyl betaines, Lauramidopropyl betaines, Lauryl of betaines, Lauryl Hydroxysultaine, Lauryl Sultaine, Milkamidopropyl betaines, Minkamidopropyl of betaines, Myristamidopropyl betaines, Myristyl of betaines, Oleamidopropyl betaines, Oleamidopropyl Hydroxysultaine, Oleyl of betaines, Olivamidopropyl of betaines, Palmamidopropyl betaines, Palm itamidopropyl betaines, Palmitoyl Carnitine, Palm Kernelamidopropyl betaines, Polytetrafluoroethylene Acetoxypentyl of betaines, Ricinoleamidopropyl betaines, Sesamidopropyl betaines, Soyamidopropyl betaines, Stearamidopropyl betaines, Stearyl of betaines, Tallowamidopropyl betaines, Tallowamidopropyl Hydroxysultaine, Tallow of betaines, Tallow Dihydroxyethyl of betaines, Undecylenamidopropyl betaines and Wheat Germamidopropyl betaines. A preferred betaine is, for example, Cocoamidopropylbetaine.

#### Fatty Acid

**[0104]** Especially when in liquid form, preferably, the detergent composition comprises between 1.5% and 20%, more preferably between 2% and 15%, even more preferably between 3% and 10%, most preferably between 4% and 8% by weight of the liquid detergent composition of soap, preferably a fatty acid salt, more preferably an amine neutralized fatty acid salt, wherein preferably the amine is an alkanolamine more preferably selected from monoethanolamine, diethanolamine, triethanolamine or a mixture thereof, more preferably monoethanolamine.

#### Perfume

**[0105]** Preferred compositions of the invention comprise perfume. Typically the composition comprises a perfume that comprises one or more perfume raw materials, selected from the group as described in WO08/87497. However, any perfume useful in a detergent may be used. A preferred method of incorporating perfume into the compositions of the invention is via an encapsulated perfume particle comprising either a water-soluble hydroxylic compound or melamine-formaldehyde or modified polyvinyl alcohol. In one aspect the encapsulate comprises (a) an at least partially water-soluble solid matrix comprising one or more water-soluble hydroxylic compounds, preferably starch; and (b) a perfume oil encapsulated by the solid matrix. In a further aspect the perfume may be pre-complexed with a polyamine, preferably

a polyethylenimine so as to form a Schiff base.

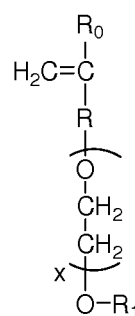
### Polymers

**[0106]** The detergent composition may comprise one or more polymers for example for cleaning and/or care. Examples are optionally modified carboxymethylcellulose, poly (ethylene glycol), poly(vinyl alcohol), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid co-polymers and carboxylate polymers.

**[0107]** Suitable carboxylate polymers include maleate/acrylate random copolymer or polyacrylate homopolymer. The carboxylate polymer may be a polyacrylate homopolymer having a molecular weight of from 4,000 Da to 9,000 Da, or from 6,000 Da to 9,000 Da. Other suitable carboxylate polymers are co-polymers of maleic acid and acrylic acid, and may have a molecular weight in the range of from 4,000 Da to 90,000 Da.

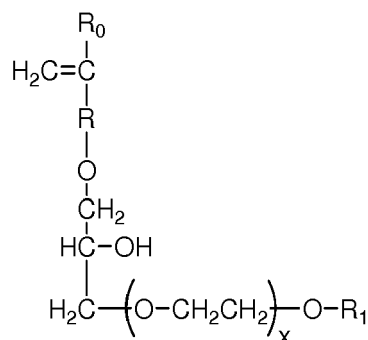
**[0108]** Other suitable carboxylate polymers are co-polymers comprising: (i) from 50 to less than 98 wt% structural units derived from one or more monomers comprising carboxyl groups; (ii) from 1 to less than 49 wt% structural units derived from one or more monomers comprising sulfonate moieties; and (iii) from 1 to 49 wt% structural units derived from one or more types of monomers selected from ether bond-containing monomers represented by formulas (I) and (II):

formula (I):



wherein in formula (I),  $R_0$  represents a hydrogen atom or  $CH_3$  group, R represents a  $CH_2$  group,  $CH_2CH_2$  group or single bond, X represents a number 0-5 provided X represents a number 1-5 when R is a single bond, and  $R_1$  is a hydrogen atom or C1 to C20 organic group;

formula (II)



in formula (II),  $R_0$  represents a hydrogen atom or  $CH_3$  group, R represents a  $CH_2$  group,  $CH_2CH_2$  group or single bond, X represents a number 0-5, and  $R_1$  is a hydrogen atom or C1 to C20 organic group.

**[0109]** The composition may comprise one or more amphiphilic cleaning polymers such as the compound having the following general structure:  $\text{bis}((C_2H_5O)(C_2H_4O)_n)(CH_3)-N^+-C_xH_{2x}-N^+-(CH_3)-\text{bis}((C_2H_5O)(C_2H_4O)_n)$ , wherein n = from 20 to 30, and x = from 3 to 8, or sulphated or sulphonated variants thereof. In one aspect, this polymer is sulphated or sulphonated to provide a zwitterionic soil suspension polymer.

**[0110]** The composition preferably comprises amphiphilic alkoxyated grease cleaning polymers which have balanced hydrophilic and properties such that they remove grease particles from fabrics and surfaces. Preferred amphiphilic alkoxyated grease cleaning polymers comprise a core structure and a plurality of alkoxyate groups attached to that

core structure. These may comprise alkoxyated polyalkylenimines, preferably having an inner polyethylene oxide block and an outer polypropylene oxide block. Typically these may be incorporated into the compositions of the invention in amounts of from 0.005 to 10 wt%, generally from 0.5 to 8 wt%.

[0111] Alkoxyated polycarboxylates such as those prepared from polyacrylates are useful herein to provide additional grease removal performance. Such materials are described in WO 91/08281 and PCT 90/01815. Chemically, these materials comprise polyacrylates having one ethoxy side-chain per every 7-8 acrylate units. The side-chains are of the formula  $-(CH_2CH_2O)_m(CH_2)_nCH_3$  wherein m is 2-3 and n is 6-12. The side-chains are ester-linked to the polyacrylate "backbone" to provide a "comb" polymer type structure. The molecular weight can vary, but is typically in the range of about 2000 to about 50,000. Such alkoxyated polycarboxylates can comprise from about 0.05% to about 10%, by weight, of the compositions herein.

[0112] The composition may comprise polyethylene glycol polymers and these may be particularly preferred in compositions comprising mixed surfactant systems. Suitable polyethylene glycol polymers include random graft co-polymers comprising: (i) hydrophilic backbone comprising polyethylene glycol; and (ii) side chain(s) selected from the group consisting of: C4-C25 alkyl group, polypropylene, polybutylene, vinyl ester of a saturated C1-C6 mono-carboxylic acid, C1-C6 alkyl ester of acrylic or methacrylic acid, and mixtures thereof. Suitable polyethylene glycol polymers have a polyethylene glycol backbone with random grafted polyvinyl acetate side chains. The average molecular weight of the polyethylene glycol backbone can be in the range of from 2,000 Da to 20,000 Da, or from 4,000 Da to 8,000 Da. The molecular weight ratio of the polyethylene glycol backbone to the polyvinyl acetate side chains can be in the range of from 1:1 to 1:5, or from 1:1.2 to 1:2. The average number of graft sites per ethylene oxide units can be less than 1, or less than 0.8, the average number of graft sites per ethylene oxide units can be in the range of from 0.5 to 0.9, or the average number of graft sites per ethylene oxide units can be in the range of from 0.1 to 0.5, or from 0.2 to 0.4. A suitable polyethylene glycol polymer is Sokalan HP22.

[0113] Typically these polymers when present are each incorporated into the compositions of the invention in amounts from 0.005 to 10 wt%, more usually from 0.05 to 8 wt%.

[0114] Preferably the composition comprises one or more carboxylate polymer, such as a maleate/acrylate random copolymer or polyacrylate homopolymer. In one aspect, the carboxylate polymer is a polyacrylate homopolymer having a molecular weight of from 4,000 Da to 9,000 Da, or from 6,000 Da to 9,000 Da. Typically these are incorporated into the compositions of the invention in amounts from 0.005 to 10 wt%, or from 0.05 to 8 wt%.

[0115] Preferably the composition comprises one or more soil release polymers.

[0116] Suitable soil release polymers are polyester soil release polymers such as Repel-o-tex polymers, including Repel-o-tex SF, SF-2 and SRP6 supplied by Rhodia. Other suitable soil release polymers include Texcare polymers, including Texcare SRA100, SRA300, SRN100, SRN170, SRN240, SRN260, SRN300 and SRN325 supplied by Clariant. Other suitable soil release polymers are Marloquest polymers, such as Marloquest SL supplied by Sasol.

[0117] Preferably the composition comprises one or more cellulosic polymer, including those selected from alkyl cellulose, alkyl alkoxyalkyl cellulose, carboxyalkyl cellulose, alkyl carboxyalkyl cellulose. Preferred cellulosic polymers are selected from the group comprising carboxymethyl cellulose, methyl cellulose, methyl hydroxyethyl cellulose, methyl carboxymethyl cellulose, and mixtures thereof. In one aspect, the carboxymethyl cellulose has a degree of carboxymethyl substitution from 0.5 to 0.9 and a molecular weight from 100,000 Da to 300,000 Da.

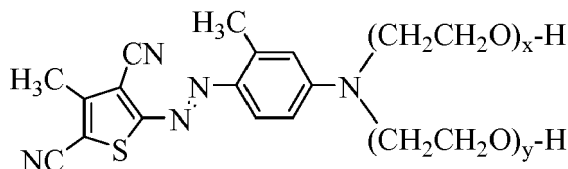
[0118] The composition preferably comprises a cationically-modified polysaccharide polymer. Preferably, the cationic polysaccharide polymer is selected from cationically modified hydroxyethyl cellulose, cationically modified hydroxypropyl cellulose, cationically and hydrophobically modified hydroxyethyl cellulose, cationically and hydrophobically modified hydroxypropyl cellulose, or a mixture thereof, more preferably cationically modified hydroxyethyl cellulose, cationically and hydrophobically modified hydroxyethyl cellulose, or a mixture thereof.

#### Amines

[0119] The cleaning compositions described herein may contain an amine. The cleaning compositions may include from about 0.1% to about 10%, or from about 0.2% to about 5%, or from about 0.5% to about 4%, or from about 0.1% to about 4%, or from about 0.1% to about 2%, by weight of the composition, of an amine. The amine can be subjected to protonation depending on the pH of the cleaning medium in which it is used. Non-limiting examples of amines include, but are not limited to, etheramines, cyclic amines, polyamines, oligoamines (e.g., triamines, diamines, pentamines, tetraamines), or combinations thereof. The compositions described herein may comprise an amine selected from the group consisting of oligoamines, etheramines, cyclic amines, and combinations thereof. In some aspects, the amine is not an alkanolamine. In some aspects, the amine is not a polyalkyleneimine. Examples of suitable oligoamines include tetraethylenepentamine, triethylenetetraamine, diethylenetriamine, and mixtures thereof. Etheramines and cyclic amines may be particularly preferred.

Fabric Shading Dye

**[0120]** The composition may comprise a fabric shading agent. Suitable fabric shading agents include dyes, dye-clay conjugates, and 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. Preferred dyes include alkoxyated azothiophenes, Solvent Violet 13, Acid Violet 50 and Direct Violet 9. Particularly preferred dyes are polymeric dyes, particularly comprising polyalkoxy, most preferably polyethoxy groups, for example:



wherein the index values x and y are independently selected from 1 to 10.

Dye Transfer Inhibitors

**[0121]** Suitable dye transfer inhibitors include polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylpyrrolidone, polyvinylloxazolidone, polyvinylimidazole and mixtures thereof. Preferred are poly(vinyl pyrrolidone), poly(vinylpyridine betaine), poly(vinylpyridine N-oxide), poly(vinyl pyrrolidone-vinyl imidazole) and mixtures thereof. Suitable commercially available dye transfer inhibitors include PVP-K15 and K30 (Ashland), Sokalan® HP165, HP50, HP53, HP59, HP56K, HP56, HP66 (BASF), Chromabond® S-400, S403E and S-100 (Ashland).

Chelant

**[0122]** The composition may comprise chelant for example selected from phosphonic, sulphonic, succinic and acetic chelants or mixtures thereof. Suitable examples include HEDP, DTPA, EDTA, MGDA, GLDA, EDDS and 4,5-dihydroxy-1,3-benzenedisulfonic acids and salts thereof.

Methods of Making the Composition

**[0123]** The present disclosure relates to methods of making the compositions described herein. The compositions of the invention may be solid (for example granules or tablets) or liquid form. It may be preferred for the compositions to be in liquid form. They may be made by any process chosen by the formulator, including by a batch process, a continuous loop process, or combinations thereof.

**[0124]** When in the form of a liquid, the compositions of the invention may be aqueous (typically above 2 wt% or even above 5 or 10 wt% total water, up to 90 or up to 80wt% or 70 wt% total water) or non-aqueous (typically below 2 wt% total water content). Typically the compositions of the invention will be in the form of an aqueous solution or uniform dispersion or suspension of optical brightener, DTI and optional additional adjunct materials, some of which may normally be in solid form, that have been combined with the normally liquid components of the composition, such as the liquid alcohol ethoxylate nonionic, the aqueous liquid carrier, and any other normally liquid optional ingredients. Such a solution, dispersion or suspension will be acceptably phase stable. When in the form of a liquid, the detergents of the invention preferably have viscosity from 1 to 1500 centipoises (1-1500 mPa\*s), more preferably from 100 to 1000 centipoises (100-1000 mPa\*s), and most preferably from 200 to 500 centipoises (200-500 mPa\*s) at 20s-1 and 21°C. Viscosity can be determined by conventional methods. Viscosity may be measured using an AR 550 rheometer from TA instruments using a plate steel spindle at 40 mm diameter and a gap size of 500 μm. The high shear viscosity at 20s-1 and low shear viscosity at 0.05-1 can be obtained from a logarithmic shear rate sweep from 0.1-1 to 25-1 in 3 minutes time at 21°C. The preferred rheology described therein may be achieved using internal existing structuring with detergent ingredients or by employing an external rheology modifier. More preferably the detergents, such as detergent liquid compositions have a high shear rate viscosity of from about 100 centipoise to 1500 centipoise, more preferably from 100 to 1000 cps. Unit Dose detergents, such as detergent liquid compositions have high shear rate viscosity of from 400 to 1000cps. Detergents such as laundry softening compositions typically have high shear rate viscosity of from 10 to 1000, more preferably from 10 to 800 cps, most preferably from 10 to 500 cps. Hand dishwashing compositions have high shear rate viscosity of from 300 to 4000 cps, more preferably 300 to 1000 cps.



**[0125]** The cleaning and/or treatment compositions in the form of a liquid herein can be prepared by combining the components thereof in any convenient order and by mixing, e.g., agitating, the resulting component combination to form a phase stable liquid detergent composition. In a process for preparing such compositions, a liquid matrix is formed containing at least a major proportion, or even substantially all, of the liquid components, e.g., nonionic surfactant, the non-surface active liquid carriers and other optional liquid components, with the liquid components being thoroughly admixed by imparting shear agitation to this liquid combination. For example, rapid stirring with a mechanical stirrer may usefully be employed. While shear agitation is maintained, substantially all of any anionic surfactants and the solid form ingredients can be added. Agitation of the mixture is continued, and if necessary, can be increased at this point to form a solution or a uniform dispersion of insoluble solid phase particulates within the liquid phase. After some or all of the solid-form materials have been added to this agitated mixture, particles of any enzyme material to be included, e.g., enzyme granulates, are incorporated. As a variation of the composition preparation procedure hereinbefore described, one or more of the solid components may be added to the agitated mixture as a solution or slurry of particles premixed with a minor portion of one or more of the liquid components. After addition of all of the composition components, agitation of the mixture is continued for a period of time sufficient to form compositions having the requisite viscosity and phase stability characteristics. Frequently this will involve agitation for a period of from about 30 to 60 minutes.

**[0126]** The adjunct ingredients in the compositions of this invention may be incorporated into the composition as the product of the synthesis generating such components, either with or without an intermediate purification step. Where there is no purification step, commonly the mixture used will comprise the desired component or mixtures thereof (and percentages given herein relate to the weight percent of the component itself unless otherwise specified) and in addition unreacted starting materials and impurities formed from side reactions and/or incomplete reaction. For example, for an ethoxylated or substituted component, the mixture will likely comprise different degrees of ethoxylation/substitution.

#### Method of Use

**[0127]** The present disclosure relates to a method of using the cleaning composition of the present disclosure to clean a surface, such as a textile. In general, the method includes mixing the cleaning composition as described herein with water to form an aqueous liquor and contacting a surface, preferably a textile, with the aqueous liquor in a laundering step. The target surface may include a greasy soil or body soil.

**[0128]** The present invention also provides use of a composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity belonging to the endo- $\alpha$ -1,4-polygalactosaminidase class (EC 3.2.1.109) of enzymes for enhanced stain removal from a surface, preferably a fabric surface, particularly greasy stain or body soil removal and/or for reducing malodour. Preferably the glycoside hydrolase enzyme is a variant having at least 60% or 65% or 70% or 75% or 80% or 85% or 90% or 95% identity to 100% SEQ ID NO:1.

**[0129]** The compositions of this invention, typically prepared as hereinbefore described, can be used to form aqueous (washing/treatment) liquor for use in the laundering/treatment of fabrics and/or hard surfaces. Generally, an effective amount of such a composition is added to water, for example in a conventional fabric automatic washing machine, to form such aqueous liquor. The aqueous liquor so formed is then contacted, typically under agitation, with the fabrics to be laundered/treated therewith. An effective amount of the cleaning composition herein added to water to form aqueous liquor can comprise amounts sufficient to form from about 500 to 25,000 ppm, or from 500 to 15,000 ppm of composition in the aqueous liquor, or from about 1,000 to 3,000 ppm of the cleaning composition herein will be provided in aqueous liquor.

**[0130]** Typically, the aqueous liquor is formed by contacting the cleaning composition with wash water in such an amount so that the concentration of the anionic surfactant in the wash liquor is from above 0.1g/l to 5g/l, or from 1g/l, and to 4.5g/l, or to 4.0g/l, or to 3.5g/l, or to 3.0g/l, or to 2.5g/l, or even to 2.0g/l, or even to 1.5g/l. The method of laundering fabric or textile may be carried out in a top-loading or front-loading automatic washing machine, or can be used in a hand-wash laundry application. In these applications, the aqueous liquor formed and concentration of cleaning composition in the wash liquor is that of the main wash cycle. Any input of water during any optional rinsing step(s) is not included when determining the volume of the aqueous liquor.

**[0131]** The aqueous liquor may comprise 40 litres or less of water, or 30 litres or less, or 20 litres or less, or 10 litres or less, or 8 litres or less, or even 6 litres or less of water. The aqueous liquor may comprise from above 0 to 15 litres, or from 2 litres, and to 12 litres, or even to 8 litres of water. Typically from 0.01kg to 2kg of fabric per litre of aqueous liquor is dosed into said aqueous liquor. Typically from 0.01kg, or from 0.05kg, or from 0.07kg, or from 0.10kg, or from 0.15kg, or from 0.20kg, or from 0.25kg fabric per litre of aqueous liquor is dosed into said aqueous liquor. Optionally, 50g or less, or 45g or less, or 40g or less, or 35g or less, or 30g or less, or 25g or less, or 20g or less, or even 15g or less, or even 10g or less of the composition is contacted to water to form the aqueous liquor. Such compositions are typically employed at concentrations of from about 500 ppm to about 15,000 ppm in solution. The water temperature typically ranges from about 5 °C to about 90 °C for example from 20 °C to 60 °C, preferably up to 40 °C or 30 °C and, when laundering fabric, the water to fabric ratio is typically from about 1:1 to about 30:1. Typically the aqueous liquor

comprising the cleaning composition of the invention has a pH of from 3 to 11.5, typically from 7 to 11, more usually 8 to 10.5.

**[0132]** In one aspect, such method comprises the steps of optionally washing and/or rinsing said surface or fabric, contacting said surface or fabric with any composition disclosed in this specification then optionally washing and/or rinsing said surface or fabric, with an optional drying step.

**[0133]** Drying of such surfaces or fabrics may be accomplished by any one of the common means employed either in domestic or industrial settings: machine drying or open-air drying. The fabric may comprise any fabric capable of being laundered in normal consumer or institutional use conditions, and the invention is particularly suitable for synthetic textiles such as polyester and nylon and especially for treatment of mixed fabrics and/or fibres comprising synthetic and cellulosic fabrics and/or fibres. As examples of synthetic fabrics are polyester, nylon, these may be present in mixtures with cellulosic fibres, for example, polycotton fabrics.

#### EXAMPLES

**[0134]** The following are illustrative examples of cleaning compositions according to the present disclosure and are not intended to be limiting.

Examples 1 to 18: Unit Dose Compositions.

**[0135]** These examples provide various formulations for unit dose laundry detergents and comprise double compartment unit dose products comprising one powder and one liquid compartment. The film used to encapsulate the compositions in PVA. Each example is prepared by combining a liquid compartment composition selected from compositions A-E with a powder compartment composition selected from compositions F-K.

Example	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
Liquid composition	20g A	25g A	20g A	15g A	20g B	20g B
Solid composition	15g F	12g G	12g H	12g I	15g J	15g K

Example	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>
Liquid composition	15g B	17g B	20g C	19g C	15g C	25g C
Solid composition	15g L	14g F	15g G	18g H	15g I	12g J

Example	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>
Liquid composition	20g D	18g D	22g D	32g E	32g E	27g E
Solid composition	20g K	13g L	15g F	17g G	12g H	18g I

Ingredients	A	B	C	D	E
	% weight of compartment				
LAS	19.09	16.76	8.59	6.56	3.44
AE3S	1.91	0.74	0.18	0.46	0.07
AE7	14.00	17.50	26.33	28.08	31.59
Citric Acid	0.6	0.6	0.6	0.6	0.6
C12-15 Fatty Acid	14.8	14.8	14.8	14.8	14.8
Polymer 3	4.0	4.0	4.0	4.0	4.0
Chelant 2	1.2	1.2	1.2	1.2	1.2

# EP 3 330 348 A1

(continued)

Ingredients	A	B	C	D	E
	% weight of compartment				
Optical Brightener 1	0.20	0.25	0.01	0.01	0.50
Optical Brightener 2	0.20	-	0.25	0.03	0.01
Optical Brightener 3	0.18	0.09	0.30	0.01	-
DTI 1	0.10	-	0.20	0.01	0.05
DTI 2	-	0.10	0.20	0.25	0.05
Glycerol	6.1	6.1	6.1	6.1	6.1
Monoethanol amine	8.0	8.0	8.0	8.0	8.0
Tri-isopropanol amine	-	-	2.0	-	-
Tri-ethanol amine	-	2.0	-	-	-
Cumene sulfonate	-	-	-	-	2.0
Protease	0.80	0.60	0.07	1.00	1.50
Mannanase	0.07	0.05	0.05	0.10	0.01
Amylase 1	0.20	0.11	0.30	0.50	0.05
Amylase 2	0.11	0.20	0.10	-	0.50
Hydrolase of SEQ ID NO:1 (active protein)	0.005	0.05	0.005	0.010	0.01
Second hydrolase of SEQ ID NO: 13 (active protein)	0.001	-	0.001	-	-
Polishing enzyme	0.005	0.05	-	-	-
Nuclease	0.005	-	-	-	0.005
Dispersin B	0.010	0.05	0.005	0.005	-
Cyclohexyl dimethanol	-	-	-	2.0	-
Acid violet 50	0.03	0.02			
Violet DD			0.01	0.05	0.02
Structurant	0.14	0.14	0.14	0.14	0.14
Perfume	1.9	1.9	1.9	1.9	1.9
Water, solvents and miscellaneous	To 100%				
pH	7.5-8.2				

Ingredient	F	G	H	I	J	K
	% weight					
Sodium carbonate	20.0	35.0	30.0	29.0	28.0	18.0
Carboxymethyl cellulose	2.0	1.0	-	-	2.5	0.6
Sodium silicate 2R	5.0	-	5.0	3.2	20.0	-
Tetraacetyl ethylenediamine	20.0	15.0	18.0	15.0	-	25.0
Sodium percarbonate	50.0	44.0	45.0	45.0	29.0	50.0
Polyetheramine	0.5	2	0.5	1	0.5	4
Sulfate/ Water & Miscellaneous	Balance					

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**[0136]** Based on total cleaning and/or treatment composition/compartment weight. Enzyme levels are reported as raw material.

### Examples 19 to 24

**[0137]** Granular laundry detergent compositions for hand washing or washing machines, typically top-loading washing machines.

Ingredient	19	20	21	22	23	24
	% weight					
LAS	11.33	10.81	7.04	4.20	3.92	2.29
Quaternary ammonium	0.70	0.20	1.00	0.60	-	-
AE3S	0.51	0.49	0.32	-	0.08	0.10
AE7	8.36	11.50	12.54	11.20	16.00	21.51
Sodium Tripolyphosphate	5.0	-	4.0	9.0	2.0	-
Zeolite A	-	1.0	-	1.0	4.0	1.0
Sodium silicate 1.6R	7.0	5.0	2.0	3.0	3.0	5.0
Sodium carbonate	20.0	17.0	23.0	14.0	14.0	16.0
Polyacrylate MW 4500	1.0	0.6	1.0	1.0	1.5	1.0
Polymer 6	0.1	0.2	-	-	0.1	-
Carboxymethyl cellulose	1.0	0.3	1.0	1.0	1.0	1.0
Acid Violet 50	0.05	-	0.02	-	0.04	-
Violet DD	-	0.03	-	0.03	-	0.03
Protease 2	0.10	0.10	0.10	0.10	-	0.10
Amylase	0.03	-	0.03	0.03	0.03	0.03
Lipase	0.03	0.07	0.30	0.10	0.07	0.40
Polishing enzyme	0.002	-	0.05	-	0.02	-
Hydrolase of SEQ ID NO: 1 (active protein)	0.001	0.001	0.01	0.05	0.002	0.02
Nuclease (as active protein)	0.001	-	-	-	0.001	-
Dispersin B	0.001	0.001	0.05	-	0.001	-
Optical Brightener 1	0.200	0.001	0.300	0.650	0.050	0.001
Optical Brightener 2	0.060	-	0.650	0.180	0.200	0.060
Optical Brightener 3	0.100	0.060	0.050	-	0.030	0.300
Chelant 1	0.60	0.80	0.60	0.25	0.60	0.60
DTI 1	0.32	0.15	0.15	-	0.10	0.10
DTI 2	0.32	0.15	0.30	0.30	0.10	0.20
Sodium Percarbonate	4.6	5.2	5.0	5.7	4.5	7.3
Nonanoyloxybenzenesulfonate	1.9	0.0	1.66	0.0	0.33	0.75
Tetraacetylenediamine	0.58	1.2	0.51	0.0	0.015	0.28
Photobleach	0.0030	0.0	0.0012	0.0030	0.0021	-
S-ACMC	0.1	0.0	0.0	0.0	0.06	0.0
Polyetheramine	0.5	2	0.5	1	0.5	4

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(continued)

Ingredient	19	20	21	22	23	24
	% weight					
Sulfate/Moisture	Balance					

## Examples 25-30

**[0138]** Granular laundry detergent compositions typically for front-loading automatic washing machines.

Ingredient	25	26	27	28	29	30
	% weight					
LAS	6.08	5.05	4.27	3.24	2.30	1.09
AE3S	-	0.90	0.21	0.18	-	0.06
AS	0.34	-	-	-	-	-
AE7	4.28	5.95	6.72	7.98	9.20	10.35
Quaternary ammonium	0.5	-	-	0.3	-	-
Crystalline layered silicate	4.1	-	4.8	-	-	-
Zeolite A	5.0	-	2.0	-	2.0	2.0
Citric acid	3.0	4.0	3.0	4.0	2.5	3.0
Sodium carbonate	11.0	17.0	12.0	15.0	18.0	18.0
Sodium silicate 2R	0.08	-	0.11	-	-	-
Optical Brightener 1	-	0.25	0.05	0.01	0.10	0.02
Optical Brightener 2	-	-	0.25	0.20	0.01	0.08
Optical Brightener 3	-	0.06	0.04	0.15	-	0.05
DTI 1	0.08	-	0.04	-	0.10	0.01
DTI 2	0.08	-	0.04	0.10	0.10	0.02
Soil release agent	0.75	0.72	0.71	0.72	-	-
Acrylic /maleic acid copolymer	1.1	3.7	1.0	3.7	2.6	3.8
Carboxymethyl cellulose	0.2	1.4	0.2	1.4	1.0	0.5
Protease 3	0.20	0.20	0.30	0.15	0.12	0.13
Amylase 3	0.20	0.15	0.20	0.30	0.15	0.15
Lipase	0.05	0.15	0.10	-	-	-
Amylase 2	0.03	0.07	-	-	0.05	0.05
Cellulase 2	-	-	-	-	0.10	0.10
Polishing enzyme	0.003	0.005	0.020	-	-	-
Hydrolase of SEQ ID NO:1 (active protein)	0.002	0.010	0.020	0.020	0.020	0.003
Nuclease	-	-	-	-	0.005	0.005
Dispersin B	0.002	-	0.020	0.020	-	-
Tetraacetylenediamine	3.6	4.0	3.6	4.0	2.2	1.4
Sodium percarbonate	13.0	13.2	13.0	13.2	16.0	14.0
Chelant 3	-	0.2	-	0.2	-	0.2

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(continued)

Ingredient	25	26	27	28	29	30
	% weight					
Chelant 2	0.2	-	0.2	-	0.2	0.2
MgSO <sub>4</sub>	-	0.42	-	0.42	-	0.4
Perfume	0.5	0.6	0.5	0.6	0.6	0.6
Suds suppressor agglomerate	0.05	0.10	0.05	0.10	0.06	0.05
Soap	0.45	0.45	0.45	0.45	-	-
Acid Violet 50	0.04	-	0.05	-	0.04	-
Violet DD	-	0.04	-	0.05	-	0.04
S-ACMC	0.01	0.01	-	0.01	-	-
Direct Violet 9 (active)	-	-	0.0001	0.0001	-	-
Polyetheramine	0.5	2	0.5	1	0.5	4
Sulfate/ Water & Miscellaneous	Balance					

Examples 31-37: Heavy Duty Liquid laundry detergent compositions.

[0139]

Ingredients	31	32	33	34	35	36	37
	% weight						
AE <sub>1.8</sub> S	6.77	5.16	1.36	1.30	-	-	-
AE <sub>3</sub> S	-	-	-	-	0.45	-	-
LAS	0.86	2.06	2.72	0.68	0.95	1.56	3.55
HSAS	1.85	2.63	1.02	-	-	-	-
AE9	6.32	9.85	10.20	7.92			
AE8							35.45
AE7					8.40	12.44	
C <sub>12-14</sub> dimethyl Amine Oxide	0.30	0.73	0.23	0.37	-	-	-
C <sub>12-18</sub> Fatty Acid	0.80	1.90	0.60	0.99	1.20	-	15.00
Citric Acid	2.50	3.96	1.88	1.98	0.90	2.50	0.60
Optical Brightener 1	1.00	0.80	0.10	0.30	0.05	0.50	0.001
Optical Brightener 3	0.001	0.05	0.01	0.20	0.50	-	1.00
Sodium formate	1.60	0.09	1.20	0.04	1.60	1.20	0.20
DTI 1	0.32	0.05	-	0.60	0.10	0.60	0.01
DTI 2	0.32	0.10	0.60	0.60	0.05	0.40	0.20
Sodium hydroxide	2.30	3.80	1.70	1.90	1.70	2.50	2.30
Monoethanolamine	1.40	1.49	1.00	0.70	-	-	-
Diethylene glycol	5.50	-	4.10	-	-	-	-
Chelant 1	0.15	0.15	0.11	0.07	0.50	0.11	0.80
4-formyl-phenylboronic acid	-	-	-	-	0.05	0.02	0.01

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(continued)

	Ingredients	31	32	33	34	35	36	37
		% weight						
5	Sodium tetraborate	1.43	1.50	1.10	0.75	-	1.07	-
	Ethanol	1.54	1.77	1.15	0.89	-	3.00	7.00
	Polymer 1	0.10	-	-	-	-	-	2.00
10	Polymer 2	0.30	0.33	0.23	0.17	-	-	-
	Polymer 3	-	-	-	-	-	-	0.80
	Polymer 4	0.80	0.81	0.60	0.40	1.00	1.00	-
15	1,2-Propanediol	-	6.60	-	3.30	0.50	2.00	8.00
	Structurant	0.10	-	-	-	-	-	0.10
	Perfume	1.60	1.10	1.00	0.80	0.90	1.50	1.60
	Perfume encapsulate	0.10	0.05	0.01	0.02	0.10	0.05	0.10
20	Protease	0.80	0.60	0.70	0.90	0.70	0.60	1.50
	Hydrolase of SEQ ID: No 1 (active protein)	0.07	0.05	0.045	0.06	0.04	0.045	0.10
	Amylase 1	0.30	-	0.30	0.10	-	0.40	0.10
25	Amylase 2	-	0.20	0.10	0.15	0.07	-	0.10
	Xyloglucanase	0.20	0.10	-	-	0.05	0.05	0.20
	Lipase	0.40	0.20	0.30	0.10	0.20	-	-
	Polishing enzyme	-	0.04	-	-	-	0.004	-
30	Nuclease	0.05	0.03	0.01	0.03	0.03	0.003	0.003
	Dispersin B	-	-	-	0.05	0.03	0.001	0.001
	Acid Violet 50	0.05	-	-	-	-	-	0.005
35	Direct Violet 9	-	-	-	-	-	0.05	-
	Violet DD	-	0.035	0.02	0.037	0.04	-	-
	Water insoluble plant fiber	0.2	-	-	-	1.2	-	-
	Dye control agent	-	0.3	-	0.5	-	0.3	-
40	Alkoxylated polyaryl/ polyalkyl phenol	-	-	1.2	-	-	-	3.1
	Water, dyes & minors	Balance						
	pH	8.2						

45 Based on total cleaning and/or treatment composition weight. Unless indicated otherwise, enzyme levels are reported as raw material.

AE1.8S is C<sub>12-15</sub> alkyl ethoxy sulfate with an average degree of ethoxylation of 1.8  
 AE3S is C<sub>12-15</sub> alkyl ethoxy sulfate with an av degree of ethoxylation of 3.0  
 AE7 is C<sub>12-13</sub> alcohol ethoxylate, with an average degree of ethoxylation of 7  
 AE8 is C<sub>12-13</sub> alcohol ethoxylate, with an average degree of ethoxylation of 8  
 AE9 is C<sub>12-13</sub> alcohol ethoxylate, with an average degree of ethoxylation of 9  
 Alkoxylated polyaryl / polyalkyl phenol is alkoxylated polyaryl/polyalkyl phenol for example Emulsogen® TS160, Hos-  
 tapal® BV conc., Sapogenat® T110 or Sapogenat® T139, all from Clariant  
 Amylase 1 is Stainzyme®, 15 mg active/g  
 Amylase 2 is Natalase®, 29 mg active/g  
 Amylase 3 is Stainzyme® Plus, 20 mg active/g,

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	AS	is C <sub>12-14</sub> alkylsulfate
	Cellulase 2	is Celluclean™, 15.6 mg active/g
	Xyloglucanase	is Whitezyme®, 20mg active/g
	Chelant 1	is diethylene triamine pentaacetic acid
5	Chelant 2	is 1-hydroxyethane 1,1-diphosphonic acid
	Chelant 3	is sodium salt of ethylenediamine-N,N'-disuccinic acid, (S,S) isomer (EDDS)
	Dispersin B	is a glycoside hydrolase, reported as 1000mg active/g
	DTI 1	is poly(4-vinylpyridine-1-oxide) (such as Chromabond S-403E®),
	DTI 2	is poly(1-vinylpyrrolidone-co-1-vinylimidazole) (such as Sokalan HP56®).
10	Dye Control Agent	is for example Suparex® O.IN (M1), Nylofixan® P (M2), Nylofixan® PM (M3), or Nylofixan® HF (M4)
	HSAS	is mid-branched alkyl sulfate as disclosed in US 6,020,303 and US6,060,443
	LAS	is linear alkylbenzenesulfonate having an average aliphatic carbon chain length C <sub>9</sub> -C <sub>15</sub> (HLAS is acid form).
15	Lipase	is Lipex®, 18 mg active/g
	Mannanase	is Mannaway®, 25 mg active/g
	Nuclease	is a Phosphodiesterase according to any of SEQ ID NOs: 2 to 6, preferably SEQ ID NO: 2, 3 or 4, reported as active protein
	Optical Brightener 1	is disodium 4,4'-bis[[4-anilino-6-morpholino-s-triazin-2-yl]-amino]-2,2'-stilbenedisulfonate
20	Optical Brightener 2	is disodium 4,4'-bis-(2-sulfostyryl)biphenyl (sodium salt)
	Optical Brightener 3	is Optiblanc SPL10® from 3V Sigma
	Perfume encapsulate	is a core-shell melamine formaldehyde perfume microcapsules
	Photobleach	is a sulfonated zinc phthalocyanine
25	Polishing enzyme	is Para-nitrobenzyl esterase, reported as 1000mg active/g
	Polyetheramine	as described in present disclosure.
	Polymer 1	is bis((C <sub>2</sub> H <sub>5</sub> O)(C <sub>2</sub> H <sub>4</sub> O) <sub>n</sub> )(CH <sub>3</sub> )-N <sup>+</sup> -C <sub>x</sub> H <sub>2x</sub> -N <sup>+</sup> -(CH <sub>3</sub> )-bis((C <sub>2</sub> H <sub>5</sub> O)(C <sub>2</sub> H <sub>4</sub> O) <sub>n</sub> ), wherein n = 20-30, x = 3 to 8 or sulphated or sulfonated variants thereof
	Polymer 2	is ethoxylated (EO <sub>15</sub> ) tetraethylene pentamine
30	Polymer 3	is ethoxylated polyethylenimine
	Polymer 4	is ethoxylated hexamethylene diamine
	Polymer 5	is Acusol 305, provided by Rohm&Haas
	Polymer 6	is a polyethylene glycol polymer grafted with vinyl acetate side chains, provided by BASF.
35	Protease 1	is Purafect Prime®, 40.6 mg active/g
	Protease 2	is Savinase®, 32.89 mg active/g
	Protease 3	is Purafect®, 84 mg active/g
	Quaternary ammonium	is C <sub>12-14</sub> Dimethylhydroxyethyl ammonium chloride
	S-ACMC	is Reactive Blue 19 Azo-CM-Cellulose provided by Megazyme
40	Soil release agent	is Repel-o-tex® SF2, supplied by Solvay
	Structurant	is Hydrogenated Castor Oil
	Violet DD	is a thiophene azo polymeric hueing dye provided by Milliken

**[0140]** The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as "40 mm" is intended to mean "about 40 mm."



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## SEQUENCE LISTING

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<120> CLEANING COMPOSITIONS INCLUDING ENZYMES

<130> CM04645FM

<160> 32

<170> PatentIn version 3.5

<210> 1

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<213> Pseudomonas aeruginosa

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Lys Pro Ala Asp Val Gly Tyr Leu Lys Glu Gln Gly Ser Thr Pro Phe  
35 40 45

Ala Tyr Leu Ser Val Gly Glu Phe Asp Gly Asp Ala Ala Ala Ile Ala  
50 55 60

Asp Ser Gly Leu Ala Arg Gly Lys Ser Ala Val Arg Asn Gln Ala Trp  
65 70 75 80

Asn Ser Gln Val Met Asp Leu Ala Ala Pro Ser Trp Arg Ala His Leu  
85 90 95

Leu Lys Arg Ala Ala Glu Leu Arg Lys Gln Gly Tyr Ala Gly Leu Phe  
100 105 110

Leu Asp Thr Leu Asp Ser Phe Gln Leu Gln Ala Glu Glu Arg Arg Glu  
115 120 125

Gly Gln Arg Arg Ala Leu Ala Ser Phe Leu Ala Gln Leu His Arg Gln  
130 135 140

Glu Pro Gly Leu Lys Leu Phe Phe Asn Arg Gly Phe Glu Val Leu Pro  
145 150 155 160

Glu Leu Pro Gly Val Ala Ser Ala Val Ala Val Glu Ser Ile His Ala  
165 170 175

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				180					185					190		
5	Arg	Asp	Trp	Leu	Lys	Gly	His	Leu	Asp	Ala	Leu	Arg	Ala	Gln	Gly	Met
			195					200					205			
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		210					215					220				
15	Arg	Ala	Leu	Ala	Ala	Arg	Leu	Arg	Ser	Glu	Gly	Tyr	Val	Pro	Phe	Val
	225					230					235					240
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	1				5					10					15	
35	Asn	Asp	Gly	Gln	His	Trp	Arg	Arg	Leu	Gln	Asn	Asp	Ser	Ala	Tyr	Leu
				20					25					30		
40	Ala	Glu	His	Gly	Ile	Thr	Ala	Val	Trp	Ile	Pro	Pro	Ala	Tyr	Lys	Gly
			35					40					45			
45	Thr	Ser	Gln	Ala	Asp	Val	Gly	Tyr	Gly	Ala	Tyr	Asp	Leu	Tyr	Asp	Leu
		50					55					60				
50	Gly	Glu	Phe	His	Gln	Lys	Gly	Thr	Val	Arg	Thr	Lys	Tyr	Gly	Thr	Lys
	65					70					75					80
55	Gly	Glu	Leu	Gln	Ser	Ala	Ile	Lys	Ser	Leu	His	Ser	Arg	Asp	Ile	Asn
					85					90					95	
60	Val	Tyr	Gly	Asp	Trp	Ile	Asn	His	Lys	Gly	Gly	Ala	Asp	Ala	Thr	Glu
				100					105					110		
65	Asp	Val	Thr	Ala	Val	Glu	Val	Asp	Pro	Ala	Asp	Arg	Asn	Arg	Val	Ile
			115					120					125			

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	Ser	Gly	Glu	His	Leu	Ile	Lys	Ala	Trp	Thr	His	Phe	His	Phe	Pro	Gly	
	130						135					140					
5	Arg	Gly	Ser	Thr	Tyr	Ser	Asp	Phe	Lys	Trp	His	Trp	Tyr	His	Phe	Asp	
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	Gly	Thr	Asp	Trp	Asp	Glu	Ser	Arg	Lys	Leu	Asn	Arg	Ile	Tyr	Lys	Phe	
10					165					170					175		
	Gln	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Ser	Asn	Glu	Asn	Gly	Asn	Tyr	
				180					185					190			
15	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Ile	Asp	Tyr	Asp	His	Pro	Asp	Val	Ala	
			195					200					205				
	Ala	Glu	Ile	Lys	Arg	Trp	Gly	Thr	Trp	Tyr	Ala	Asn	Glu	Leu	Gln	Leu	
20		210					215					220					
	Asp	Gly	Phe	Arg	Leu	Asp	Ala	Val	Lys	His	Ile	Lys	Phe	Ser	Phe	Leu	
	225					230					235					240	
25	Arg	Asp	Trp	Val	Asn	His	Val	Arg	Glu	Lys	Thr	Gly	Lys	Glu	Met	Phe	
					245					250					255		
	Thr	Val	Ala	Glu	Tyr	Trp	Gln	Asn	Asp	Leu	Gly	Ala	Leu	Glu	Asn	Tyr	
30				260					265					270			
	Leu	Asn	Lys	Thr	Asn	Phe	Asn	His	Ser	Val	Phe	Asp	Val	Pro	Leu	His	
35			275					280					285				
	Tyr	Gln	Phe	His	Ala	Ala	Ser	Thr	Gln	Gly	Gly	Gly	Tyr	Asp	Met	Arg	
	290						295					300					
40	Lys	Leu	Leu	Asn	Gly	Thr	Trp	Ser	Lys	His	Pro	Leu	Lys	Ser	Val	Thr	
	305					310					315					320	
	Phe	Val	Asp	Asn	His	Asp	Thr	Gln	Pro	Gly	Gln	Ser	Leu	Glu	Ser	Thr	
45					325					330					335		
	Val	Gln	Thr	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	Phe	Ile	Leu	Thr	Arg	
50					340				345					350			
	Glu	Ser	Gly	Tyr	Pro	Gln	Val	Phe	Tyr	Gly	Asp	Met	Tyr	Gly	Thr	Lys	
			355					360					365				
55	Gly	Asp	Ser	Gln	Arg	Glu	Ile	Pro	Ala	Leu	Lys	His	Lys	Ile	Glu	Pro	
	370						375					380					

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	Ile	Leu	Lys	Ala	Arg	Lys	Gln	Tyr	Ala	Tyr	Gly	Ala	Gln	His	Asp	Tyr	385	390	395	400
5	Phe	Asp	His	His	Asp	Ile	Val	Gly	Trp	Thr	Arg	Glu	Gly	Asp	Ser	Ser		405	410	415
10	Val	Ala	Asn	Ser	Gly	Leu	Ala	Ala	Leu	Ile	Thr	Asp	Gly	Pro	Gly	Gly		420	425	430
15	Ala	Lys	Arg	Met	Tyr	Val	Gly	Arg	Gln	Asn	Ala	Gly	Glu	Thr	Trp	His		435	440	445
20	Asp	Ile	Thr	Gly	Asn	Arg	Ser	Glu	Pro	Trp	Ile	Asn	Ser	Glu	Gly	Trp		450	455	460
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30	His	His	Asn	Gly	Thr	Asn	Gly	Thr	Met	Met	Gln	Tyr	Phe	Glu	Trp	Tyr	1	5	10	15
35	Leu	Pro	Asn	Asp	Gly	Asn	His	Trp	Asn	Arg	Leu	Arg	Ser	Asp	Ala	Ser		20	25	30
40	Asn	Leu	Lys	Asp	Lys	Gly	Ile	Ser	Ala	Val	Trp	Ile	Pro	Pro	Ala	Trp		35	40	45
45	Lys	Gly	Ala	Ser	Gln	Asn	Asp	Val	Gly	Tyr	Gly	Ala	Tyr	Asp	Leu	Tyr		50	55	60
50	Asp	Leu	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Ile	Arg	Thr	Lys	Tyr	Gly	65	70	75	80
	Thr	Arg	Asn	Gln	Leu	Gln	Ala	Ala	Val	Asn	Ala	Leu	Lys	Ser	Asn	Gly		85	90	95
55	Ile	Gln	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	Gly	Gly	Ala	Asp		100	105	110
	Ala	Thr	Glu	Met	Val	Arg	Ala	Val	Glu	Val	Asn	Pro	Asn	Asn	Arg	Asn		115	120	125

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	Gln	Glu	Val	Ser	Gly	Glu	Tyr	Thr	Ile	Glu	Ala	Trp	Thr	Lys	Phe	Asp	
	130						135					140					
5	Phe	Pro	Gly	Arg	Gly	Asn	Thr	His	Ser	Asn	Phe	Lys	Trp	Arg	Trp	Tyr	
	145					150					155					160	
10	His	Phe	Asp	Gly	Val	Asp	Trp	Asp	Gln	Ser	Arg	Lys	Leu	Asn	Asn	Arg	
					165					170					175		
15	Ile	Tyr	Lys	Phe	Arg	Gly	Asp	Gly	Lys	Gly	Trp	Asp	Trp	Glu	Val	Asp	
				180					185					190			
20	Thr	Glu	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Ile	Asp	Met	
			195					200					205				
25	Asp	His	Pro	Glu	Val	Val	Asn	Glu	Leu	Arg	Asn	Trp	Gly	Val	Trp	Tyr	
	210						215					220					
30	Thr	Asn	Thr	Leu	Gly	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His	
	225					230					235					240	
35	Ile	Lys	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Ile	Asn	His	Val	Arg	Ser	Ala	
					245					250					255		
40	Thr	Gly	Lys	Asn	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	
				260					265					270			
45	Gly	Ala	Ile	Glu	Asn	Tyr	Leu	Asn	Lys	Thr	Asn	Trp	Asn	His	Ser	Val	
			275					280					285				
50	Phe	Asp	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Lys	Ser	Gly	
	290						295					300					
55	Gly	Asn	Tyr	Asp	Met	Arg	Gln	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg	
	305					310					315				320		
60	His	Pro	Met	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	
					325					330					335		
65	Glu	Glu	Ala	Leu	Glu	Ser	Phe	Val	Glu	Glu	Trp	Phe	Lys	Pro	Leu	Ala	
				340					345					350			
70	Tyr	Ala	Leu	Thr	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	
			355					360					365				
75	Gly	Asp	Tyr	Tyr	Gly	Ile	Pro	Thr	His	Gly	Val	Pro	Ala	Met	Lys	Ser	
	370						375					380					

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	Lys	Ile	Asp	Pro	Ile	Leu	Glu	Ala	Arg	Gln	Lys	Tyr	Ala	Tyr	Gly	Arg	385	390	395	400
5	Gln	Asn	Asp	Tyr	Leu	Asp	His	His	Asn	Ile	Ile	Gly	Trp	Thr	Arg	Glu	405	410	415	
10	Gly	Asn	Thr	Ala	His	Pro	Asn	Ser	Gly	Leu	Ala	Thr	Ile	Met	Ser	Asp	420	425	430	
15	Gly	Ala	Gly	Gly	Asn	Lys	Trp	Met	Phe	Val	Gly	Arg	Asn	Lys	Ala	Gly	435	440	445	
20	Gln	Val	Trp	Thr	Asp	Ile	Thr	Gly	Asn	Arg	Ala	Gly	Thr	Val	Thr	Ile	450	455	460	
25	Asn	Ala	Asp	Gly	Trp	Gly	Asn	Phe	Ser	Val	Asn	Gly	Gly	Ser	Val	Ser	465	470	475	480
30	Ile	Trp	Val	Asn	Lys												485			
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40	Leu	Pro	Asn	Asp	Gly	Asn	His	Trp	Asn	Arg	Leu	Arg	Asp	Asp	Ala	Ser	20	25	30	
45	Asn	Leu	Arg	Asn	Arg	Gly	Ile	Thr	Ala	Ile	Trp	Ile	Pro	Pro	Ala	Trp	35	40	45	
50	Lys	Gly	Thr	Ser	Gln	Asn	Asp	Val	Gly	Tyr	Gly	Ala	Tyr	Asp	Leu	Tyr	50	55	60	
55	Asp	Leu	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Val	Arg	Thr	Lys	Tyr	Gly	65	70	75	80
	Thr	Arg	Ser	Gln	Leu	Glu	Ser	Ala	Ile	His	Ala	Leu	Lys	Asn	Asn	Gly	85	90	95	
	Val	Gln	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	Gly	Gly	Ala	Asp	100	105	110	

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	Ala	Thr	Glu	Asn	Val	Leu	Ala	Val	Glu	Val	Asn	Pro	Asn	Asn	Arg	Asn	
			115					120					125				
5	Gln	Glu	Ile	Ser	Gly	Asp	Tyr	Thr	Ile	Glu	Ala	Trp	Thr	Lys	Phe	Asp	
		130					135					140					
10	Phe	Pro	Gly	Arg	Gly	Asn	Thr	Tyr	Ser	Asp	Phe	Lys	Trp	Arg	Trp	Tyr	
	145					150					155					160	
15	His	Phe	Asp	Gly	Val	Asp	Trp	Asp	Gln	Ser	Arg	Gln	Phe	Gln	Asn	Arg	
					165					170					175		
20	Ile	Tyr	Lys	Phe	Arg	Gly	Asp	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Asp	
				180					185					190			
25	Ser	Glu	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Val	Asp	Met	
			195					200					205				
30	Asp	His	Pro	Glu	Val	Val	Asn	Glu	Leu	Arg	Arg	Trp	Gly	Glu	Trp	Tyr	
	210						215					220					
35	Thr	Asn	Thr	Leu	Asn	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His	
	225					230					235					240	
40	Ile	Lys	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Leu	Thr	His	Val	Arg	Asn	Ala	
					245					250					255		
45	Thr	Gly	Lys	Glu	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	
				260					265					270			
50	Gly	Ala	Leu	Glu	Asn	Tyr	Leu	Asn	Lys	Thr	Asn	Trp	Asn	His	Ser	Val	
			275					280					285				
55	Phe	Asp	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Asn	Ser	Gly	
	290						295					300					
60	Gly	Asn	Tyr	Asp	Met	Ala	Lys	Leu	Leu	Asn	Gly	Thr	Val	Val	Gln	Lys	
	305					310					315					320	
65	His	Pro	Met	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	
					325					330					335		
70	Gly	Glu	Ser	Leu	Glu	Ser	Phe	Val	Gln	Glu	Trp	Phe	Lys	Pro	Leu	Ala	
				340					345					350			
75	Tyr	Ala	Leu	Ile	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	

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	355		360		365	
5	Gly Asp Tyr Tyr Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala	370	375	380		
10	Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr	385	390	395	400	
	Gln His Asp Tyr Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu	405	410	415		
15	Gly Asn Thr Thr His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp	420	425	430		
20	Gly Pro Gly Gly Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly	435	440	445		
	Gln Val Trp His Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile	450	455	460		
25	Asn Ala Asp Gly Trp Ala Asn Phe Ser Val Asn Gly Gly Ser Val Ser	465	470	475	480	
30	Ile Trp Val Lys Arg	485				
35	<210> 5 <211> 484 <212> PRT <213> Bacillus sp.					
40	His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr	1	5	10	15	
45	Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Asn Ser Asp Ala Ser	20	25	30		
50	Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp	35	40	45		
55	Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr	50	55	60		
	Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly	65	70	75	80	
	Thr Arg Ser Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly					



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	85							90							95						
5	Ile	Gln	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	Gly	Gly	Ala	Asp					
				100					105					110							
	Ala	Thr	Glu	Met	Val	Arg	Ala	Val	Glu	Val	Asn	Pro	Asn	Asn	Arg	Asn					
10			115					120					125								
	Gln	Glu	Val	Thr	Gly	Glu	Tyr	Thr	Ile	Glu	Ala	Trp	Thr	Arg	Phe	Asp					
			130				135					140									
15	Phe	Pro	Gly	Arg	Gly	Asn	Thr	His	Ser	Ser	Phe	Lys	Trp	Arg	Trp	Tyr					
	145					150					155					160					
	His	Phe	Asp	Gly	Val	Asp	Trp	Asp	Gln	Ser	Arg	Arg	Leu	Asn	Asn	Arg					
20					165					170					175						
	Ile	Tyr	Lys	Phe	Arg	Gly	His	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Asp					
				180					185					190							
25	Thr	Glu	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Ile	Asp	Met					
			195					200					205								
30	Asp	His	Pro	Glu	Val	Val	Asn	Glu	Leu	Arg	Asn	Trp	Gly	Val	Trp	Tyr					
		210					215					220									
	Thr	Asn	Thr	Leu	Gly	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His					
35						230					235					240					
	Ile	Lys	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Ile	Asn	His	Val	Arg	Ser	Ala					
					245					250					255						
40	Thr	Gly	Lys	Asn	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu					
				260					265					270							
45	Gly	Ala	Ile	Glu	Asn	Tyr	Leu	Gln	Lys	Thr	Asn	Trp	Asn	His	Ser	Val					
			275					280					285								
	Phe	Asp	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Lys	Ser	Gly					
50		290					295					300									
	Gly	Asn	Tyr	Asp	Met	Arg	Asn	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg					
	305					310					315				320						
55	His	Pro	Ser	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro					
					325					330					335						

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Glu Glu Ala Leu Glu Ser Phe Val Glu Glu Trp Phe Lys Pro Leu Ala  
340 345 350

5 Tyr Ala Leu Thr Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr  
355 360 365

10 Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Arg Ser  
370 375 380

Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Lys  
385 390 395 400

15 Gln Asn Asp Tyr Leu Asp His His Asn Ile Gly Trp Thr Arg Glu Gly  
405 410 415

20 Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp Gly  
420 425 430

25 Ala Gly Gly Ser Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly Gln  
435 440 445

Val Trp Ser Asp Ile Thr Gly Asn Arg Thr Gly Thr Val Thr Ile Asn  
450 455 460

30 Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser Ile  
465 470 475 480

35 Trp Val Asn Lys

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Pro Asp Asp Gly Thr Leu Trp Thr Lys Val Ala Asn Glu Ala Asn Asn  
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50 Leu Ser Ser Leu Gly Ile Thr Ala Leu Trp Leu Pro Pro Ala Tyr Lys  
35 40 45

55 Gly Thr Ser Arg Ser Asp Val Gly Tyr Gly Val Tyr Asp Leu Tyr Asp  
50 55 60

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	Leu	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Val	Arg	Thr	Lys	Tyr	Gly	Thr	65	70	75	80
5	Lys	Ala	Gln	Tyr	Leu	Gln	Ala	Ile	Gln	Ala	Ala	His	Ala	Ala	Gly	Met	85	90	95	
10	Gln	Val	Tyr	Ala	Asp	Val	Val	Phe	Asp	His	Lys	Gly	Gly	Ala	Asp	Gly	100	105	110	
15	Thr	Glu	Trp	Val	Asp	Ala	Val	Glu	Val	Asn	Pro	Ser	Asp	Arg	Asn	Gln	115	120	125	
20	Glu	Ile	Ser	Gly	Thr	Tyr	Gln	Ile	Gln	Ala	Trp	Thr	Lys	Phe	Asp	Phe	130	135	140	
25	Pro	Gly	Arg	Gly	Asn	Thr	Tyr	Ser	Ser	Phe	Lys	Trp	Arg	Trp	Tyr	His	145	150	155	160
30	Phe	Asp	Gly	Val	Asp	Trp	Asp	Glu	Ser	Arg	Lys	Leu	Ser	Arg	Ile	Tyr	165	170	175	
35	Lys	Phe	Arg	Gly	Ile	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Asp	Thr	Glu	180	185	190	
40	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Leu	Asp	Met	Asp	His	195	200	205	
45	Pro	Glu	Val	Val	Thr	Glu	Leu	Lys	Asn	Trp	Gly	Lys	Trp	Tyr	Val	Asn	210	215	220	
50	Thr	Thr	Asn	Ile	Asp	Gly	Phe	Arg	Leu	Asp	Ala	Val	Lys	His	Ile	Lys	225	230	235	240
55	Phe	Ser	Phe	Phe	Pro	Asp	Trp	Leu	Ser	Tyr	Val	Arg	Ser	Gln	Thr	Gly	245	250	255	
	Lys	Pro	Leu	Phe	Thr	Val	Gly	Glu	Tyr	Trp	Ser	Tyr	Asp	Ile	Asn	Lys	260	265	270	
	Leu	His	Asn	Tyr	Ile	Thr	Lys	Thr	Asn	Gly	Thr	Met	Ser	Leu	Phe	Asp	275	280	285	
	Ala	Pro	Leu	His	Asn	Lys	Phe	Tyr	Thr	Ala	Ser	Lys	Ser	Gly	Gly	Ala	290	295	300	
	Phe	Asp	Met	Arg	Thr	Leu	Met	Thr	Asn	Thr	Leu	Met	Lys	Asp	Gln	Pro	305	310	315	320

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	Thr	Leu	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Thr	Glu	Pro	Gly	Gln	
					325					330						335	
5	Ala	Leu	Gln	Ser	Trp	Val	Asp	Pro	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	
				340					345					350			
10	Phe	Ile	Leu	Thr	Arg	Gln	Glu	Gly	Tyr	Pro	Cys	Val	Phe	Tyr	Gly	Asp	
			355					360					365				
15	Tyr	Tyr	Gly	Ile	Pro	Gln	Tyr	Asn	Ile	Pro	Ser	Leu	Lys	Ser	Lys	Ile	
		370					375					380					
20	Asp	Pro	Leu	Leu	Ile	Ala	Arg	Arg	Asp	Tyr	Ala	Tyr	Gly	Thr	Gln	His	
	385				390					395						400	
25	Asp	Tyr	Leu	Asp	His	Ser	Asp	Ile	Ile	Gly	Trp	Thr	Arg	Glu	Gly	Val	
					405					410						415	
30	Thr	Glu	Lys	Pro	Gly	Ser	Gly	Leu	Ala	Ala	Leu	Ile	Thr	Asp	Gly	Pro	
				420				425					430				
35	Gly	Gly	Ser	Lys	Trp	Met	Tyr	Val	Gly	Lys	Gln	His	Ala	Gly	Lys	Val	
			435				440					445					
40	Phe	Tyr	Asp	Leu	Thr	Gly	Asn	Arg	Ser	Asp	Thr	Val	Thr	Ile	Asn	Ser	
		450					455					460					
45	Asp	Gly	Trp	Gly	Glu	Phe	Lys	Val	Asn	Gly	Gly	Ser	Val	Ser	Val	Trp	
	465				470				475							480	
50	Val	Pro	Arg	Lys	Thr	Thr	Val	Ser	Thr	Ile	Ala	Arg	Pro	Ile	Thr	Thr	
					485				490							495	
55	Arg	Pro	Trp	Thr	Gly	Glu	Phe	Val	Arg	Trp	Thr	Glu	Pro	Arg	Leu	Val	
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	Pro	Asp	Asp	Gly	Thr	Leu	Trp	Thr	Lys	Val	Ala	Asn	Glu	Ala	Asn	Asn	
				20					25					30			
5	Leu	Ser	Ser	Leu	Gly	Ile	Thr	Ala	Leu	Trp	Leu	Pro	Pro	Ala	Tyr	Lys	
			35					40					45				
	Gly	Thr	Ser	Arg	Ser	Asp	Val	Gly	Tyr	Gly	Val	Tyr	Asp	Leu	Tyr	Asp	
10		50					55					60					
	Leu	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Val	Arg	Thr	Lys	Tyr	Gly	Thr	
	65					70					75					80	
15	Lys	Ala	Gln	Tyr	Leu	Gln	Ala	Ile	Gln	Ala	Ala	His	Ala	Ala	Gly	Met	
					85					90					95		
	Gln	Val	Tyr	Ala	Asp	Val	Val	Phe	Asp	His	Lys	Gly	Gly	Ala	Asp	Gly	
20				100					105					110			
	Thr	Glu	Trp	Val	Asp	Ala	Val	Glu	Val	Asn	Pro	Ser	Asp	Arg	Asn	Gln	
25			115					120					125				
	Glu	Ile	Ser	Gly	Thr	Tyr	Gln	Ile	Gln	Ala	Trp	Thr	Lys	Phe	Asp	Phe	
		130					135					140					
30	Pro	Gly	Arg	Gly	Asn	Thr	Tyr	Ser	Ser	Phe	Lys	Trp	Arg	Trp	Tyr	His	
	145					150					155					160	
	Phe	Asp	Gly	Val	Asp	Trp	Asp	Glu	Ser	Arg	Lys	Leu	Ser	Arg	Ile	Tyr	
35					165					170					175		
	Lys	Phe	Arg	Gly	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Asp	Thr	Glu	Asn	
				180					185					190			
40	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Leu	Asp	Met	Asp	His	Pro	
			195					200					205				
	Glu	Val	Val	Thr	Glu	Leu	Lys	Asn	Trp	Gly	Lys	Trp	Tyr	Val	Asn	Thr	
45		210					215					220					
	Thr	Asn	Ile	Asp	Gly	Phe	Arg	Leu	Asp	Ala	Val	Lys	His	Ile	Lys	Phe	
50		225				230					235					240	
	Ser	Phe	Phe	Pro	Asp	Trp	Leu	Ser	Tyr	Val	Arg	Ser	Gln	Thr	Gly	Lys	
					245					250					255		
55	Pro	Leu	Phe	Thr	Val	Gly	Glu	Tyr	Trp	Ser	Tyr	Asp	Ile	Asn	Lys	Leu	
				260					265					270			

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	His	Asn	Tyr	Ile	Thr	Lys	Thr	Asn	Gly	Thr	Met	Ser	Leu	Phe	Asp	Ala	
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5	Pro	Leu	His	Asn	Lys	Phe	Tyr	Thr	Ala	Ser	Lys	Ser	Gly	Gly	Ala	Phe	
		290					295					300					
10	Asp	Met	Arg	Thr	Leu	Met	Thr	Asn	Thr	Leu	Met	Lys	Asp	Gln	Pro	Thr	
	305					310					315					320	
15	Leu	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Thr	Glu	Pro	Gly	Gln	Ala	
					325					330					335		
20	Leu	Gln	Ser	Trp	Val	Asp	Pro	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	Phe	
				340					345					350			
25	Ile	Leu	Thr	Arg	Gln	Glu	Gly	Tyr	Pro	Cys	Val	Phe	Tyr	Gly	Asp	Tyr	
			355					360					365				
30	Tyr	Gly	Ile	Pro	Gln	Tyr	Asn	Ile	Pro	Ser	Leu	Lys	Ser	Lys	Ile	Asp	
		370					375					380					
35	Pro	Leu	Leu	Ile	Ala	Arg	Arg	Asp	Tyr	Ala	Tyr	Gly	Thr	Gln	His	Asp	
	385					390					395					400	
40	Tyr	Leu	Asp	His	Ser	Asp	Ile	Ile	Gly	Trp	Thr	Arg	Glu	Gly	Val	Thr	
					405					410					415		
45	Glu	Lys	Pro	Gly	Ser	Gly	Leu	Ala	Ala	Leu	Ile	Thr	Asp	Gly	Pro	Gly	
				420					425					430			
50	Gly	Ser	Lys	Trp	Met	Tyr	Val	Gly	Lys	Gln	His	Ala	Gly	Lys	Val	Phe	
			435					440					445				
55	Tyr	Asp	Leu	Thr	Gly	Asn	Arg	Ser	Asp	Thr	Val	Thr	Ile	Asn	Ser	Asp	
		450					455					460					
60	Gly	Trp	Gly	Glu	Phe	Lys	Val	Asn	Gly	Gly	Ser	Val	Ser	Val	Trp	Val	
	465					470					475					480	
65	Pro	Arg	Lys	Thr	Thr												
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5	His 1	His	Asn	Gly	Thr 5	Asn	Gly	Thr	Met	Met 10	Gln	Tyr	Phe	Glu	Trp 15	Tyr
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15	Asn	Leu	Lys 35	Ser	Lys	Gly	Ile	Thr 40	Ala	Val	Trp	Ile	Pro 45	Pro	Ala	Trp
20	Lys	Gly 50	Ala	Ser	Gln	Asn	Asp 55	Val	Gly	Tyr	Gly	Ala 60	Tyr	Asp	Leu	Tyr
25	Asp 65	Leu	Gly	Glu	Phe	Asn 70	Gln	Lys	Gly	Thr	Val 75	Arg	Thr	Lys	Tyr	Gly 80
30	Thr	Arg	Ser	Gln	Leu 85	Gln	Ala	Ala	Val	Thr 90	Ser	Leu	Lys	Asn	Asn 95	Gly
35	Ile	Gln	Val	Tyr 100	Gly	Asp	Val	Val	Met 105	Asn	His	Lys	Gly	Gly 110	Ala	Asp
40	Ala	Thr	Glu 115	Met	Val	Arg	Ala	Val 120	Glu	Val	Asn	Pro	Asn 125	Asn	Arg	Asn
45	Gln	Glu	Val	Thr	Gly	Glu	Tyr 135	Thr	Ile	Glu	Ala	Trp 140	Thr	Arg	Phe	Asp
50	Phe 145	Pro	Gly	Arg	Gly	Asn 150	Thr	His	Ser	Ser	Phe 155	Lys	Trp	Arg	Trp	Tyr 160
55	His	Phe	Asp	Gly	Val 165	Asp	Trp	Asp	Gln	Ser 170	Arg	Arg	Leu	Asn	Asn 175	Arg
60	Ile	Tyr	Lys	Phe 180	Arg	Gly	Lys	Ala	Trp 185	Asp	Trp	Glu	Val	Asp 190	Thr	Glu
65	Asn	Gly	Asn 195	Tyr	Asp	Tyr	Leu	Met 200	Tyr	Ala	Asp	Ile	Asp 205	Met	Asp	His
70	Pro	Glu 210	Val	Val	Asn	Glu	Leu 215	Arg	Asn	Trp	Gly	Val 220	Trp	Tyr	Thr	Asn
75	Thr 225	Leu	Gly	Leu	Asp	Gly 230	Phe	Arg	Ile	Asp	Ala 235	Val	Lys	His	Ile	Lys 240

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	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Ile	Asn	His	Val	Arg	Ser	Ala	Thr	Gly	
					245					250					255		
5	Lys	Asn	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	Gly	Ala	
				260					265					270			
10	Ile	Glu	Asn	Tyr	Leu	Gln	Lys	Thr	Asn	Trp	Asn	His	Ser	Val	Phe	Asp	
			275					280					285				
15	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Lys	Ser	Gly	Gly	Asn	
		290					295					300					
20	Tyr	Asp	Met	Arg	Asn	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg	His	Pro	
	305					310					315					320	
25	Ser	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	Glu	Glu	
					325					330					335		
30	Ala	Leu	Glu	Ser	Phe	Val	Glu	Glu	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	
				340					345					350			
35	Leu	Thr	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	Gly	Asp	
			355					360					365				
40	Tyr	Tyr	Gly	Ile	Pro	Thr	His	Gly	Val	Pro	Ala	Met	Arg	Ser	Lys	Ile	
		370					375					380					
45	Asp	Pro	Ile	Leu	Glu	Ala	Arg	Gln	Lys	Tyr	Ala	Tyr	Gly	Pro	Gln	His	
	385					390					395					400	
50	Asp	Tyr	Leu	Asp	His	Pro	Asp	Val	Ile	Gly	Trp	Thr	Arg	Glu	Gly	Asp	
					405					410					415		
55	Ser	Ser	His	Pro	Lys	Ser	Gly	Leu	Ala	Thr	Leu	Ile	Thr	Asp	Gly	Pro	
				420					425					430			
60	Gly	Gly	Ser	Lys	Arg	Met	Tyr	Ala	Gly	Leu	Lys	Asn	Ala	Gly	Glu	Thr	
			435					440					445				
65	Trp	Tyr	Asp	Ile	Thr	Gly	Asn	Arg	Ser	Asp	Thr	Val	Lys	Ile	Gly	Ser	
	450						455					460					
70	Asp	Gly	Trp	Gly	Glu	Phe	His	Val	Asn	Asp	Gly	Ser	Val	Ser	Ile	Tyr	
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 <213> Paenibacillus curdlanolyticus

<400> 9

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Asp Gly Ala His Trp Asn Arg Leu Asn Asn Asp Ala Gln Asn Leu Lys  
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Asn Val Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly Gly  
 35 40 45

Ser Ser Ala Asp Val Gly Tyr Gly Val Tyr Asp Thr Tyr Asp Leu Gly  
 50 55 60

Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys Ser  
 65 70 75 80

Glu Leu Ile Ser Ala Val Asn Asn Leu His Ala Lys Gly Ile Ala Val  
 85 90 95

Tyr Gly Asp Val Val Leu Asn His Arg Met Asn Ala Asp Ala Thr Glu  
 100 105 110

Leu Val Asp Ala Val Glu Val Asp Pro Asn Asn Arg Asn Val Glu Thr  
 115 120 125

Thr Ser Thr Tyr Gln Ile Gln Ala Trp Thr Gln Tyr Asp Phe Pro Gly  
 130 135 140

Arg Gly Asn Thr Tyr Ser Ser Phe Lys Trp Arg Trp Tyr His Phe Asp  
 145 150 155 160

Gly Val Asp Trp Asp Gln Ser Arg Gly Leu Asn Arg Ile Tyr Lys Leu  
 165 170 175

Arg Gly Asp Gly Lys Asp Trp Asp Trp Glu Val Asp Ser Glu Tyr Gly  
 180 185 190

Asn Tyr Asp Tyr Leu Met Gly Ala Asp Leu Asp Phe Asn His Pro Asp  
 195 200 205

Val Val Asn Glu Thr Lys Thr Trp Gly Lys Trp Phe Val Asn Thr Val

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	210					215						220					
5	Asn	Leu	Asp	Gly	Val	Arg	Leu	Asp	Ala	Val	Lys	His	Ile	Lys	Phe	Asp	
	225					230					235					240	
	Phe	Met	Arg	Asp	Trp	Val	Asn	Asn	Val	Arg	Ser	Thr	Thr	Gly	Lys	Asn	
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	Leu	Phe	Ala	Val	Gly	Glu	Tyr	Trp	His	Tyr	Asp	Val	Asn	Lys	Leu	Asn	
				260					265					270			
15	Ser	Tyr	Ile	Thr	Lys	Thr	Asn	Gly	Thr	Met	Ser	Leu	Phe	Asp	Val	Pro	
			275					280					285				
	Leu	His	Phe	Arg	Phe	Tyr	Asp	Ala	Ser	Asn	Gly	Gly	Gly	Gly	Tyr	Asp	
20		290					295					300					
	Met	Arg	Asn	Leu	Leu	Asn	Asn	Thr	Leu	Met	Ser	Ser	Asn	Pro	Met	Lys	
	305					310					315					320	
25																	
	Ala	Val	Thr	Phe	Val	Glu	Asn	His	Asp	Thr	Gln	Pro	Thr	Gln	Ala	Leu	
					325					330					335		
30	Gln	Ser	Thr	Val	Gln	Ser	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	Thr	Ile	
				340					345					350			
	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Cys	Val	Phe	Tyr	Gly	Asp	Tyr	Tyr	
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	Gly	Thr	Ser	Asp	Gly	Lys	Ile	Ser	Ser	Tyr	Lys	Pro	Ile	Met	Asp	Lys	
		370					375					380					
40																	
	Leu	Leu	Asn	Ala	Arg	Lys	Val	Tyr	Ala	Tyr	Gly	Thr	Gln	Arg	Asp	Tyr	
	385					390					395					400	
45	Phe	Asp	His	Pro	Asp	Ile	Val	Gly	Trp	Thr	Arg	Glu	Gly	Asp	Ala	Ala	
					405					410					415		
	His	Ala	Gly	Ser	Gly	Leu	Ala	Thr	Leu	Ile	Thr	Asp	Gly	Pro	Gly	Gly	
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	Ser	Lys	Trp	Met	Tyr	Val	Gly	Thr	Ser	Lys	Ala	Gly	Gln	Val	Trp	Thr	
			435					440					445				
55																	
	Asp	Lys	Thr	Gly	Asn	Arg	Ser	Gly	Thr	Val	Thr	Ile	Asp	Ala	Asn	Gly	
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Trp Gly Asn Phe Trp Val Asn Gly Gly Ser Val Ser Val Trp Ala Lys  
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10 <400> 10

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20 Ser Ser Val Gly Ile Thr Ala Val Trp Thr Pro Pro Ala Tyr Lys Gly  
35 40 45

Thr Ser Gln Ala Asp Val Gly Tyr Gly Pro Tyr Asp Leu Tyr Asp Leu  
50 55 60

25 Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys  
65 70 75 80

30 Gly Glu Leu Lys Ser Ala Val Asn Thr Leu His Ser Asn Gly Ile Gln  
85 90 95

35 Val Tyr Gly Asp Val Val Met Asn His Lys Ala Gly Ala Asp Tyr Thr  
100 105 110

Glu Asn Val Thr Ala Val Glu Val Asn Pro Ser Asn Arg Asn Gln Glu  
115 120 125

40 Thr Ser Gly Glu Tyr Asn Ile Gln Ala Trp Thr Gly Phe Asn Phe Pro  
130 135 140

45 Gly Arg Gly Thr Thr Tyr Ser Asn Phe Lys Trp Gln Trp Phe His Phe  
145 150 155 160

50 Asp Gly Thr Asp Trp Asp Gln Ser Arg Ser Leu Ser Arg Ile Phe Lys  
165 170 175

Phe Thr Gly Lys Ala Trp Asp Trp Pro Val Ser Ser Glu Asn Gly Asn  
180 185 190

55 Tyr Asp Tyr Leu Met Tyr Ala Asp Tyr Asp Tyr Asp His Pro Asp Val  
195 200 205

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	225					230					235					240	
	Leu	Lys	Asp	Trp	Val	Asp	Asn	Ala	Arg	Ala	Ala	Thr	Gly	Lys	Glu	Met	
10					245					250					255		
	Phe	Thr	Val	Gly	Glu	Tyr	Trp	Gln	Asn	Asp	Leu	Gly	Ala	Leu	Asn	Asn	
				260					265					270			
15	Tyr	Leu	Ala	Lys	Val	Asn	Tyr	Asn	Gln	Ser	Leu	Phe	Asp	Ala	Pro	Leu	
			275					280					285				
	His	Tyr	Asn	Phe	Tyr	Ala	Ala	Ser	Thr	Gly	Gly	Gly	Tyr	Tyr	Asp	Met	
20			290				295					300					
	Arg	Asn	Ile	Leu	Asn	Asn	Thr	Leu	Val	Ala	Ser	Asn	Pro	Thr	Lys	Ala	
25	305					310					315					320	
	Val	Thr	Leu	Val	Glu	Asn	His	Asp	Thr	Gln	Pro	Gly	Gln	Ser	Leu	Glu	
					325					330					335		
30	Ser	Thr	Val	Gln	Pro	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	Phe	Ile	Leu	
				340					345					350			
	Thr	Arg	Ser	Gly	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	Gly	Asp	Met	Tyr	Gly	
35			355					360					365				
	Thr	Lys	Gly	Thr	Thr	Thr	Arg	Glu	Ile	Pro	Ala	Leu	Lys	Ser	Lys	Ile	
		370					375					380					
40	Glu	Pro	Leu	Leu	Lys	Ala	Arg	Lys	Asp	Tyr	Ala	Tyr	Gly	Thr	Gln	Arg	
	385					390					395					400	
	Asp	Tyr	Ile	Asp	Asn	Pro	Asp	Val	Ile	Gly	Trp	Thr	Arg	Glu	Gly	Asp	
45					405					410					415		
	Ser	Thr	Lys	Ala	Lys	Ser	Gly	Leu	Ala	Thr	Val	Ile	Thr	Asp	Gly	Pro	
50				420					425					430			
	Gly	Gly	Ser	Lys	Arg	Met	Tyr	Val	Gly	Thr	Ser	Asn	Ala	Gly	Glu	Ile	
			435					440					445				
55	Trp	Tyr	Asp	Leu	Thr	Gly	Asn	Asn	Ser	Thr	Lys	Ile	Thr	Ile	Gly	Ser	
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Asp Gly Tyr Ala Thr Phe Pro Val Asn Lys Gly Ser Val Ser Val Trp  
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5 Val Gln Gln

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20 Trp Ser Phe Asn Thr Leu Lys His Asn Met Lys Asp Ile His Asp Ala  
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Gly Tyr Thr Ala Ile Gln Thr Ser Pro Ile Asn Gln Val Lys Glu Gly  
35 40 45

25 Asn Gln Gly Asp Lys Ser Met Ser Asn Trp Tyr Trp Leu Tyr Gln Pro  
50 55 60

30 Thr Ser Tyr Gln Ile Gly Asn Arg Tyr Leu Gly Thr Glu Gln Glu Phe  
65 70 75 80

35 Lys Glu Met Cys Ala Ala Ala Glu Glu Tyr Gly Ile Lys Val Ile Val  
85 90 95

Asp Ala Val Ile Asn His Thr Thr Ser Asp Tyr Ala Ala Ile Ser Asn  
100 105 110

40 Glu Val Lys Ser Ile Pro Asn Trp Thr His Gly Asn Thr Gln Ile Lys  
115 120 125

45 Asn Trp Ser Asp Arg Trp Asp Val Thr Gln Asn Ser Leu Leu Gly Leu  
130 135 140

50 Tyr Asp Trp Asn Thr Gln Asn Thr Gln Val Gln Ser Tyr Leu Lys Arg  
145 150 155 160

Phe Leu Asp Arg Ala Leu Asn Asp Gly Ala Asp Gly Phe Arg Phe Asp  
165 170 175

55 Ala Ala Lys His Ile Glu Leu Pro Asp Asp Gly Ser Tyr Gly Ser Gln  
180 185 190

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	Phe	Trp	Pro	Asn	Ile	Thr	Asn	Thr	Ser	Ala	Glu	Phe	Gln	Tyr	Gly	Glu	
			195					200					205				
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		210					215					220					
	Asp	Val	Thr	Ala	Ser	Asn	Tyr	Gly	His	Ser	Ile	Arg	Ser	Ala	Leu	Lys	
10	225					230					235					240	
	Asn	Arg	Asn	Leu	Gly	Val	Ser	Asn	Ile	Ser	His	Tyr	Ala	Ser	Asp	Val	
				245						250					255		
15	Ser	Ala	Asp	Lys	Leu	Val	Thr	Trp	Val	Glu	Ser	His	Asp	Thr	Tyr	Ala	
			260						265					270			
	Asn	Asp	Asp	Glu	Glu	Ser	Thr	Trp	Met	Ser	Asp	Asp	Asp	Ile	Arg	Leu	
20			275					280					285				
	Gly	Trp	Ala	Val	Ile	Ala	Ser	Arg	Ser	Gly	Ser	Thr	Pro	Leu	Phe	Phe	
25		290					295					300					
	Ser	Arg	Pro	Glu	Gly	Gly	Gly	Asn	Gly	Val	Arg	Phe	Pro	Gly	Lys	Ser	
	305					310					315					320	
	Gln	Ile	Gly	Asp	Arg	Gly	Ser	Ala	Leu	Phe	Glu	Asp	Gln	Ala	Ile	Thr	
30				325						330					335		
	Ala	Val	Asn	Arg	Phe	His	Asn	Val	Met	Ala	Gly	Gln	Pro	Glu	Glu	Leu	
35				340					345					350			
	Ser	Asn	Pro	Asn	Gly	Asn	Asn	Gln	Ile	Phe	Met	Asn	Gln	Arg	Gly	Ser	
			355					360					365				
40	His	Gly	Val	Val	Leu	Ala	Asn	Ala	Gly	Ser	Ser	Ser	Val	Ser	Ile	Asn	
	370						375					380					
	Thr	Ala	Thr	Lys	Leu	Pro	Asp	Gly	Arg	Tyr	Asp	Asn	Lys	Ala	Gly	Ala	
45	385				390						395					400	
	Gly	Ser	Phe	Gln	Val	Asn	Asp	Gly	Lys	Leu	Thr	Gly	Thr	Ile	Asn	Ala	
50				405						410					415		
	Arg	Ser	Val	Ala	Val	Leu	Tyr	Pro	Asp	Asp	Ile	Ala	Lys	Ala	Pro	His	
				420					425					430			
55	Val	Phe	Leu	Glu	Asn	Tyr	Lys	Thr	Gly	Val	Thr	His	Ser	Phe	Asn	Asp	
			435					440					445				

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	Gln	Leu	Thr	Ile	Thr	Leu	Arg	Ala	Asp	Ala	Asn	Thr	Thr	Lys	Ala	Val		
	450						455					460						
5	Tyr	Gln	Ile	Asn	Asn	Gly	Pro	Glu	Thr	Ala	Phe	Lys	Asp	Gly	Asp	Gln		
	465					470					475					480		
10	Phe	Thr	Ile	Gly	Lys	Gly	Asp	Pro	Phe	Gly	Lys	Thr	Tyr	Thr	Ile	Met		
				485						490						495		
15	Leu	Lys	Gly	Thr	Asn	Ser	Asp	Gly	Val	Thr	Arg	Thr	Glu	Lys	Tyr	Ser		
				500					505						510			
20	Phe	Val	Lys	Arg	Asp	Pro	Ala	Ser	Ala	Lys	Thr	Ile	Gly	Tyr	Gln	Asn		
			515					520					525					
25	Pro	Asn	His	Trp	Ser	Gln	Val	Asn	Ala	Tyr	Ile	Tyr	Lys	His	Asp	Gly		
		530					535					540						
30	Ser	Arg	Val	Ile	Glu	Leu	Thr	Gly	Ser	Trp	Pro	Gly	Lys	Pro	Met	Thr		
	545					550				555						560		
35	Lys	Asn	Ala	Asp	Gly	Ile	Tyr	Thr	Leu	Thr	Leu	Pro	Ala	Asp	Thr	Asp		
				565					570						575			
40	Thr	Thr	Asn	Ala	Lys	Val	Ile	Phe	Asn	Asn	Gly	Ser	Ala	Gln	Val	Pro		
				580				585						590				
45	Gly	Gln	Asn	Gln	Pro	Gly	Phe	Asp	Tyr	Val	Leu	Asn	Gly	Leu	Tyr	Asn		
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	1				5					10					15			
65	Asn	Asp	Gly	Gln	His	Trp	Asn	Arg	Leu	His	Asp	Asp	Ala	Ala	Ala	Leu		
				20					25					30				
70	Ser	Asp	Ala	Gly	Ile	Thr	Ala	Ile	Trp	Ile	Pro	Pro	Ala	Tyr	Lys	Gly		
			35					40					45					

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	Asn	Ser	Gln	Ala	Asp	Val	Gly	Tyr	Gly	Ala	Tyr	Asp	Leu	Tyr	Asp	Leu
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5	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Val	Arg	Thr	Lys	Tyr	Gly	Thr	Lys
	65					70					75					80
	Ala	Gln	Leu	Glu	Arg	Ala	Ile	Gly	Ser	Leu	Lys	Ser	Asn	Asp	Ile	Asn
10					85					90					95	
	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	Met	Gly	Ala	Asp	Phe	Thr
				100					105					110		
15	Glu	Ala	Val	Gln	Ala	Val	Gln	Val	Asn	Pro	Thr	Asn	Arg	Trp	Gln	Asp
			115					120					125			
	Ile	Ser	Gly	Ala	Tyr	Thr	Ile	Asp	Ala	Trp	Thr	Gly	Phe	Asp	Phe	Ser
20		130					135					140				
	Gly	Arg	Asn	Asn	Ala	Tyr	Ser	Asp	Phe	Lys	Trp	Arg	Trp	Phe	His	Phe
25	145					150					155					160
	Asn	Gly	Val	Asp	Trp	Asp	Gln	Arg	Tyr	Gln	Glu	Asn	His	Ile	Phe	Arg
				165						170					175	
30	Phe	Ala	Asn	Thr	Asn	Trp	Asn	Trp	Arg	Val	Asp	Glu	Glu	Asn	Gly	Asn
				180					185					190		
	Tyr	Asp	Tyr	Leu	Leu	Gly	Ser	Asn	Ile	Asp	Phe	Ser	His	Pro	Glu	Val
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	Gln	Asp	Glu	Leu	Lys	Asp	Trp	Gly	Ser	Trp	Phe	Thr	Asp	Glu	Leu	Asp
40		210					215					220				
	Leu	Asp	Gly	Tyr	Arg	Leu	Asp	Ala	Ile	Lys	His	Ile	Pro	Phe	Trp	Tyr
	225					230					235					240
45	Thr	Ser	Asp	Trp	Val	Arg	His	Gln	Arg	Asn	Glu	Ala	Asp	Gln	Asp	Leu
					245					250					255	
	Phe	Val	Val	Gly	Glu	Tyr	Trp	Lys	Asp	Asp	Val	Gly	Ala	Leu	Glu	Phe
50				260					265					270		
	Tyr	Leu	Asp	Glu	Met	Asn	Trp	Glu	Met	Ser	Leu	Phe	Asp	Val	Pro	Leu
			275					280					285			
55	Asn	Tyr	Asn	Phe	Tyr	Arg	Ala	Ser	Gln	Gln	Gly	Gly	Ser	Tyr	Asp	Met



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	290		295		300												
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	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Thr	Gln	Pro	Gly	Glu	Ser	Leu	Glu	
					325					330					335		
10	Ser	Trp	Val	Ala	Asp	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	Thr	Ile	Leu	
				340					345					350			
15	Thr	Arg	Glu	Gly	Gly	Tyr	Pro	Asn	Val	Phe	Tyr	Gly	Asp	Tyr	Tyr	Gly	
			355					360					365				
	Ile	Pro	Asn	Asp	Asn	Ile	Ser	Ala	Lys	Lys	Asp	Met	Ile	Asp	Glu	Leu	
20		370					375					380					
	Leu	Asp	Ala	Arg	Gln	Asn	Tyr	Ala	Tyr	Gly	Thr	Gln	His	Asp	Tyr	Phe	
	385					390					395					400	
25	Asp	His	Trp	Asp	Val	Val	Gly	Trp	Thr	Arg	Glu	Gly	Ser	Ser	Ser	Arg	
					405					410						415	
	Pro	Asn	Ser	Gly	Leu	Ala	Thr	Ile	Met	Ser	Asn	Gly	Pro	Gly	Gly	Ser	
30				420					425					430			
	Lys	Trp	Met	Tyr	Val	Gly	Arg	Gln	Asn	Ala	Gly	Gln	Thr	Trp	Thr	Asp	
			435					440					445				
35	Leu	Thr	Gly	Asn	Asn	Gly	Ala	Ser	Val	Thr	Ile	Asn	Gly	Asp	Gly	Trp	
		450					455					460					
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	1				5					10					15		
	Leu	Gly	Val	Asn	Ala	Gln	Phe	Leu	Trp	Phe	Ser	Pro	Glu	Arg	Tyr	Asn	
55				20					25					30			
	Lys	Gln	Ile	Asp	Arg	Leu	Gln	Asp	Leu	Gly	Leu	Glu	Trp	Val	Arg	Leu	

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	35	40	45
5	Asp Leu His Trp Asp Arg Leu Glu Thr Ala Glu Asp Gln Tyr Gln Leu 50 55 60		
10	Ala Ser Leu Asp Gln Leu Val Lys Asp Leu Glu Ala Arg Gln Leu Lys 65 70 75 80		
15	Ser Val Phe Tyr Leu Val Gly Ser Ala Arg Phe Ile Thr Thr Ala Pro 85 90 95		
20	Phe Tyr Ser Pro Phe Gln Asp Gln Tyr Pro Pro Arg Asp Pro Glu Val 100 105 110		
25	Phe Ala Arg Arg Met Ala Met Leu Ser Gln Arg Tyr Pro Ser Val Ala 115 120 125		
30	Ala Trp Gln Val Trp Asn Glu Pro Asn Leu Ile Gly Phe Trp Arg Pro 130 135 140		
35	Lys Ala Asp Pro Glu Gly Tyr Ala Lys Leu Leu Gln Ala Ser Thr Ile 145 150 155 160		
40	Ala Leu Arg Met Val Asp Pro Glu Lys Pro Val Val Ser Ala Gly Met 165 170 175		
45	Ala Phe Phe Ser Glu Met Pro Asp Gly Arg Thr Met Phe Asp Ala Leu 180 185 190		
50	Gly His Leu Gly Val Glu Ser Leu Gly Thr Ile Ala Thr Tyr His Pro 195 200 205		
55	Tyr Thr Gln Leu Pro Glu Gly Asn Tyr Pro Trp Asn Leu Asp Phe Val 210 215 220		
	Ser His Ala Asn Gln Ile Asn Arg Ala Leu Arg Asn Ala Gly Val Pro 225 230 235 240		
	Ala Ile Trp Ser Thr Glu Trp Gly Trp Ser Ala Tyr Lys Gly Pro Lys 245 250 255		
	Glu Leu Gln Asp Ile Ile Gly Val Glu Gly Gln Ala Asp Tyr Val Leu 260 265 270		
	Arg Arg Leu Ala Leu Met Ser Ala Leu Asp Tyr Asp Arg Ile Phe Leu 275 280 285		

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Phe Thr Leu Ser Asp Leu Asp Gln Arg Ala Ser Val Arg Asp Arg Asp  
290 295 300

5 Tyr Gly Leu Leu Asp Leu Asp Ala Asn Pro Lys Pro Val Tyr Leu Ala  
305 310 315 320

10 Leu Gln Arg Phe Leu Lys Val Thr Gly Pro Lys Leu Arg Pro Ala Asp  
325 330 335

15 Pro Pro Val Thr Glu Asp Leu Pro Asp Gly Ser Phe Ser Ile Gly Trp  
340 345 350

20 Thr Arg Glu Asp Gly Arg Asn Val Trp Leu Phe Trp Ser Ala Arg Gly  
355 360 365

25 Gly Asn Val Arg Leu Pro Lys Leu Lys Glu Ala Thr Leu His Asp Pro  
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30 Leu Ser Gly Lys Val Thr Pro Leu Ser Gly Ser Asp Gly Leu Glu Val  
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35 Pro Val Lys Ser Ser Leu Gln Met Leu Val Trp Glu  
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55 Thr Gly Ala His Ile Ser Asp Ala Ile Lys Ala Gly His Ala Asp Val  
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60 Cys Thr Ile Glu Arg Ser Gly Ala Asp Lys Arg Arg Gln Glu Ser Leu  
35 40 45

65 Lys Gly Ile Pro Thr Lys Pro Gly Phe Asp Arg Asp Glu Trp Pro Met  
50 55 60

70 Ala Met Cys Glu Glu Gly Gly Lys Gly Ala Ser Val Arg Tyr Val Ser  
65 70 75 80

75 Ser Ser Asp Asn Arg Gly Ala Gly Ser Trp Val Gly Asn Arg Leu Asn  
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Gly Tyr Ala Asp Gly Thr Arg Ile Leu Phe Ile Val Gln  
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Glu Thr Gly Ser His Ile Arg Asp Ala Ile Ala Glu Gly His Pro Asp  
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Ile Cys Thr Ile Asp Asp Gly Ala Asp Lys Arg Arg Glu Glu Ser Leu  
35 40 45

20  
Lys Gly Ile Pro Thr Lys Pro Gly Tyr Asp Arg Asp Glu Trp Pro Met  
50 55 60

25  
Ala Val Cys Glu Glu Gly Gly Ala Gly Ala Asp Val Arg Tyr Val Thr  
65 70 75 80

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Pro Ser Asp Asn Arg Gly Ala Gly Ser Trp Val Gly Asn Gln Met Ser  
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Ser Tyr Pro Asp Gly Thr Arg Val Leu Phe Ile Val Gln  
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Thr Gly Ala His Ile Ser Asp Ala Ile Lys Ala Gly His Ser Asp Val  
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Cys Thr Ile Glu Arg Ser Gly Ala Asp Lys Arg Arg Gln Glu Ser Leu  
35 40 45

Lys Gly Ile Pro Thr Lys Pro Gly Phe Asp Arg Asp Glu Trp Pro Met  
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55  
Ala Met Cys Glu Glu Gly Gly Lys Gly Ala Ser Val Arg Tyr Val Ser

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	65				70					75				80		
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25	Glu	Val	Lys	Gly	Gln	Ser	Ala	Leu	Pro	Phe	Asp	Val	Asp	Cys	Trp	Ala
				20					25					30		
30	Ile	Leu	Cys	Lys	Gly	Ala	Pro	Asn	Val	Leu	Gln	Arg	Val	Asn	Glu	Lys
			35					40					45			
35	Thr	Lys	Asn	Ser	Asn	Arg	Asp	Arg	Ser	Gly	Ala	Asn	Lys	Gly	Pro	Phe
	50						55					60				
40	Lys	Asp	Pro	Gln	Lys	Trp	Gly	Ile	Lys	Ala	Leu	Pro	Pro	Lys	Asn	Pro
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45	Ser	Trp	Ser	Ala	Gln	Asp	Phe	Lys	Ser	Pro	Glu	Glu	Tyr	Ala	Phe	Ala
					85					90					95	
50	Ser	Ser	Leu	Gln	Gly	Gly	Thr	Asn	Ala	Ile	Leu	Ala	Pro	Val	Asn	Leu
			100						105					110		
55	Ala	Ser	Gln	Asn	Ser	Gln	Gly	Gly	Val	Leu	Asn	Gly	Phe	Tyr	Ser	Ala
			115					120					125			
60	Asn	Lys	Val	Ala	Gln	Phe	Asp	Pro	Ser	Lys	Pro	Gln	Gln	Thr	Lys	Gly
		130					135					140				
65	Thr	Trp	Phe	Gln	Ile	Thr	Lys	Phe	Thr	Gly	Ala	Ala	Gly	Pro	Tyr	Cys
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70	Lys	Ala	Leu	Gly	Ser	Asn	Asp	Lys	Ser	Val	Cys	Asp	Lys	Asn	Lys	Asn
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75	Ile	Ala	Gly	Asp	Trp	Gly	Phe	Asp	Pro	Ala	Lys	Trp	Ala	Tyr	Gln	Tyr

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	Ala	Arg	Thr	Gln	Leu	Ala	Gly	Leu	Thr	Val	Ala	Val	Ala	Gly	Ser	Gly
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20	Thr	Gly	Tyr	Ser	Arg	Asp	Leu	Phe	Pro	Thr	Trp	Asp	Ala	Ile	Ser	Gly
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25	Asn	Cys	Asn	Ala	Arg	Glu	Tyr	Val	Leu	Lys	Arg	Asp	Gly	Glu	Gly	Val
		50					55					60				
	Gln	Val	Asn	Asn	Ala	Cys	Glu	Ser	Gln	Ser	Gly	Thr	Trp	Ile	Ser	Pro
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	Tyr	Asp	Asn	Ala	Ser	Phe	Thr	Asn	Ala	Ser	Ser	Leu	Asp	Ile	Asp	His
					85					90					95	
35	Met	Val	Pro	Leu	Lys	Asn	Ala	Trp	Ile	Ser	Gly	Ala	Ser	Ser	Trp	Thr
				100					105						110	
	Thr	Ala	Gln	Arg	Glu	Ala	Leu	Ala	Asn	Asp	Val	Ser	Arg	Pro	Gln	Leu
40			115					120					125			
	Trp	Ala	Val	Ser	Ala	Ser	Ala	Asn	Arg	Ser	Lys	Gly	Asp	Arg	Ser	Pro
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45	Asp	Gln	Trp	Lys	Pro	Pro	Leu	Thr	Ser	Phe	Tyr	Cys	Thr	Tyr	Ala	Lys
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	Ser	Trp	Ile	Asp	Val	Lys	Ser	Phe	Tyr	Lys	Leu	Thr	Ile	Thr	Ser	Ala
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10 Gly Trp Gly Val Ser Leu Ala Trp Trp Ala Lys Ala Phe Gly Asn Arg  
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15 Asp Asp Leu Ala Asn Val Phe Phe Thr Arg Asn Asn Gln Val Ile Asn  
35 40 45

Gly Gln Asn Leu Pro Gly Leu Gly Phe Asn Ile Ala Arg Tyr Asn Ala  
50 55 60

20 Gly Ala Cys Ser Thr Asn Thr Tyr Asn Gly Ser Ser Met Val Val Ser  
65 70 75 80

25 Ser Ser Ile Lys Pro Ser Arg Gln Val Asp Gly Tyr Trp Leu Asp Trp  
85 90 95

Ala Ser Thr Asp Pro Ala Ser Ser Ser Trp Asn Trp Asn Val Asp Ala  
100 105 110

30 Asn Gln Arg Ala Met Leu Gln Lys Ala Lys Ala Asn Gly Ala Asn Ile  
115 120 125

35 Phe Glu Leu Phe Ser Asn Ser Pro Met Trp Trp Met Cys Leu Asn His  
130 135 140

40 Asn Pro Ser Gly Ser Gly Ser Ser Asp Asn Leu Gln Ser Trp Asn Tyr  
145 150 155 160

Gln Asn His Ala Val Tyr Leu Ala Asn Ile Ala Gln His Ala Gln Gln  
165 170 175

45 Asn Trp Gly Ile Gln Phe Gln Ser Val Glu Ala Phe Asn Glu Pro Ser  
180 185 190

50 Ser Gly Trp Gly Pro Thr Gly Thr Gln Glu Gly Cys His Phe Ala Val  
195 200 205

Ser Thr Met Ala Thr Val Ile Gly Tyr Leu Asn Thr Glu Leu Ala Gln  
210 215 220

55 Arg Gly Leu Ser Ser Phe Ile Ser Ala Ser Asp Glu Thr Ser Tyr Asp

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	225		230		235		240									
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				245					250						255	
10	Val	Lys	Arg	Val	Asn	Val	His	Gly	Tyr	Gln	Gly	Gly	Gly	Gly	Arg	Arg
			260						265					270		
15	Asp	Thr	Leu	Tyr	Ser	Leu	Val	Ser	Gln	Ala	Gly	Lys	Arg	Leu	Trp	Asn
			275					280					285			
20	Ser	Glu	Tyr	Gly	Asp	Ala	Asp	Ala	Ser	Gly	Lys	Ser	Met	Tyr	Thr	Asn
		290					295					300				
25	Leu	Leu	Leu	Asp	Phe	Thr	Trp	Leu	His	Pro	Thr	Ala	Trp	Val	Tyr	Trp
	305					310					315					320
30	Gln	Ala	Ile	Asp	Gly	Ser	Gly	Trp	Gly	Leu	Ile	Val	Gly	Asp	Asn	Asp
				325						330					335	
35	Gln	Leu	Thr	Leu	Ser	Ser	Ala	Ser	Thr	Lys	Tyr	Phe	Val	Leu	Ala	Gln
			340						345					350		
40	Leu	Thr	Arg	His	Ile	Arg	Pro	Gly	Met	Gln	Ile	Leu	Thr	Thr	Pro	Asp
			355					360					365			
45	Gly	Asn	Thr	Val	Ala	Ala	Tyr	Asp	Ser	Gly	Ser	Gln	Lys	Leu	Val	Ile
		370					375					380				
50	Val	Ala	Ala	Asn	Trp	Gly	Ser	Ala	Gln	Thr	Ile	Thr	Phe	Asp	Leu	Thr
	385					390					395				400	
55	Arg	Ala	Lys	Thr	Ala	Gly	Ser	Asn	Gly	Ala	Thr	Val	Pro	Arg	Trp	Ser
				405						410					415	
60	Thr	Gln	Thr	Ser	Gly	Gly	Asp	Gln	Tyr	Lys	Ser	Tyr	Ser	Asp	Thr	Lys
			420						425					430		
65	Ile	Asn	Asn	Gly	Lys	Phe	Ser	Val	Ser	Phe	Ser	Thr	Gly	Gln	Val	Gln
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Gly Trp Gly Thr Ser Leu Ala Trp Trp Gly Asn Val Phe Gly Thr Arg  
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Asp Asp Phe Ala Asp Leu Phe Phe Thr Thr Lys Ser Val Thr Tyr Asn  
35 40 45

Gly Thr Ser Leu Pro Gly Leu Gly Leu Asn Ile Ala Arg Tyr Asn Leu  
50 55 60

Gly Ala Cys Ser Trp Asn Ala Val Asn Gly Glu Thr Met Val Lys Ser  
65 70 75 80

Pro Asn Ile Pro Ala Phe Lys Gln Ile Glu Gly Phe Trp Gln Asp Trp  
85 90 95

Asn Asn Glu Asp Pro Thr Ser Ser Ala Trp Asp Trp Thr Ala Asp Ala  
100 105 110

Thr Gln Arg Ala Met Leu Val Lys Ala Thr Gln Arg Gly Ala Val Thr  
115 120 125

Glu Leu Phe Ala Asn Ser Pro Met Trp Trp Met Cys Tyr Asn His Asn  
130 135 140

Pro Ser Gly Ala Ala Asp Gly Gly Asn Asn Leu Gln Thr Trp Asn Tyr  
145 150 155 160

Arg Gln His Ala Ser His Leu Ala Ala Val Ala Leu Tyr Ala Arg Thr  
165 170 175

Asn Trp Gly Val Asn Phe Ala Thr Val Asp Pro Phe Asn Glu Pro Ala  
180 185 190

Ser Ser Trp Trp Thr Ala Ser Gly Thr Gln Glu Gly Cys His Leu Asp  
195 200 205

Pro Ala Val Gln Ala Ala Val Leu Pro Tyr Met Arg Ser Glu Leu Asp  
210 215 220

Lys Arg Gly Leu Thr Gly Val Arg Ile Ser Ala Ser Asp Glu Thr Asn  
225 230 235 240

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	Tyr	Asp	Thr	Ala	Arg	Ser	Thr	Trp	Ser	Ser	Phe	Gly	Ser	Ala	Thr	Lys	
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5	Ala	Leu	Val	Ser	Gln	Val	Asn	Val	His	Gly	Tyr	Gln	Gly	Thr	Gly	Gly	
				260					265					270			
10	Arg	Arg	Asp	Leu	Leu	Tyr	Thr	Asp	Val	Val	Thr	Thr	Ser	Gly	Lys	Lys	
			275					280					285				
15	Leu	Trp	Asn	Ser	Glu	Thr	Gly	Asp	Ser	Asp	Gly	Thr	Gly	Leu	Ser	Met	
	290						295					300					
20	Ala	Arg	Asn	Leu	Cys	Tyr	Asp	Phe	Arg	Trp	Leu	His	Pro	Thr	Ala	Trp	
	305					310					315					320	
25	Cys	Tyr	Trp	Gln	Val	Met	Asp	Pro	Ser	Thr	Gly	Trp	Ala	Met	Ile	Ala	
				325						330					335		
30	Tyr	Asp	Ala	Asn	Thr	Leu	Gln	Pro	Thr	Thr	Val	Gln	Pro	Lys	Tyr	Tyr	
				340					345					350			
35	Val	Met	Ala	Gln	Phe	Ser	Arg	His	Ile	Arg	Pro	Gly	Met	Thr	Ile	Leu	
			355					360					365				
40	Asp	Thr	Gly	Val	Ser	Phe	Ala	Ala	Ala	Ala	Tyr	Asp	Ala	Ser	Ala	Arg	
	370						375					380					
45	Arg	Leu	Val	Leu	Val	Ala	Val	Asn	Thr	Ser	Thr	Ser	Pro	Gln	Thr	Phe	
	385					390					395					400	
50	Thr	Phe	Asp	Leu	Ser	Arg	Phe	Thr	Thr	Val	Thr	Gly	Gly	Ser	Gly	Gly	
				405						410					415		
55	Leu	Val	Pro	Arg	Trp	Asn	Thr	Val	Thr	Gly	Gly	Gly	Asp	Met	Tyr	Arg	
				420					425					430			
60	Ala	Tyr	Thr	Asn	Thr	Tyr	Val	Thr	Gly	Lys	Ser	Val	Ser	Ala	Thr	Phe	
			435					440					445				
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75	<211>	464															
80	<212>	PRT															
85	<213>	Streptomyces avermitilis															
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5	Gly	Trp	Gly	Thr	Ser	Leu	Ala	Trp	Trp	Ala	Asn	Val	Phe	Gly	Ala	Arg	
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	Asp	Asp	Phe	Ala	Asp	Leu	Phe	Phe	Thr	Thr	Lys	Ser	Val	Thr	Tyr	Asn	
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	Gly	Arg	Thr	Leu	Pro	Gly	Leu	Gly	Leu	Asn	Ile	Ala	Arg	Tyr	Asn	Leu	
		50					55					60					
15	Gly	Ala	Cys	Ser	Trp	Asn	Ser	Val	Ser	Gly	Glu	Ser	Met	Val	Ala	Ser	
	65					70					75					80	
	Ala	Asn	Ile	Pro	Ala	Phe	Lys	Gln	Ile	Glu	Gly	Tyr	Trp	Gln	Asp	Trp	
20					85					90					95		
	Asn	Asn	Glu	Asp	Pro	Thr	Ser	Ser	Ala	Trp	Lys	Trp	Thr	Ala	Asp	Ala	
25				100					105					110			
	Ala	Gln	Arg	Thr	Met	Leu	Val	Lys	Ala	Thr	Ala	Arg	Gly	Ala	Thr	Thr	
			115					120					125				
30	Glu	Leu	Phe	Ala	Asn	Ser	Pro	Met	Trp	Trp	Met	Cys	Leu	Asn	His	Asn	
		130					135					140					
	Pro	Ser	Gly	Ala	Ser	Gly	Gly	Gly	Asn	Asn	Leu	Gln	Ser	Trp	Asn	Tyr	
35	145					150					155					160	
	Arg	Gln	His	Ala	Ser	His	Leu	Ala	Ala	Val	Ala	Leu	Tyr	Ala	Lys	Ser	
				165						170					175		
40	Asn	Trp	Gly	Val	Asn	Phe	Ala	Thr	Val	Asp	Pro	Phe	Asn	Glu	Pro	Ser	
				180					185					190			
	Ser	Ser	Trp	Trp	Thr	Ala	Thr	Gly	Thr	Gln	Glu	Gly	Cys	His	Met	Asp	
45			195					200					205				
	Ala	Ser	Val	Gln	Ala	Ala	Val	Leu	Pro	Tyr	Leu	Arg	Ser	Glu	Leu	Asp	
50		210					215					220					
	Arg	Arg	Gly	Leu	Thr	Gly	Thr	Lys	Ile	Ser	Ala	Ser	Asp	Glu	Thr	Ser	
	225					230					235					240	
55	Tyr	Asp	Leu	Ala	Arg	Thr	Thr	Trp	Gly	Ser	Phe	Gly	Ser	Ser	Thr	Lys	
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5	Arg	Arg	Asp	Leu	Leu	Tyr	Thr	Asp	Val	Val	Thr	Thr	Ala	Gly	Lys	Ala	
			275					280					285				
10	Leu	Trp	Asn	Ser	Glu	Thr	Gly	Asp	Ser	Asp	Gly	Thr	Gly	Leu	Thr	Leu	
		290					295					300					
15	Ala	Ser	Asn	Leu	Cys	Leu	Asp	Phe	Arg	Trp	Leu	His	Pro	Thr	Ala	Trp	
	305					310					315					320	
20	Val	Tyr	Trp	Gln	Val	Met	Asp	Pro	Ser	Ser	Gly	Trp	Ala	Met	Ile	Ala	
					325					330					335		
25	Tyr	Asp	Ala	Ser	Thr	Leu	Gln	Pro	Gly	Ala	Val	Gln	Thr	Lys	Tyr	Tyr	
				340					345					350			
30	Val	Met	Ala	Gln	Phe	Ser	Arg	His	Ile	Arg	Ala	Gly	Met	Thr	Ile	Val	
			355					360					365				
35	Asp	Thr	Gly	Val	Gly	Tyr	Ala	Ala	Ala	Ala	Tyr	Asp	Ala	Thr	Ala	Arg	
		370					375					380					
40	Arg	Leu	Val	Ile	Val	Ala	Val	Asn	Thr	Ser	Thr	Ser	Ala	Gln	Thr	Leu	
	385					390					395					400	
45	Thr	Phe	Asp	Leu	Ser	Arg	Phe	Ser	Thr	Val	Thr	Gly	Gly	Thr	Gly	Gly	
				405						410					415		
50	Leu	Val	Arg	Arg	Trp	Asn	Thr	Val	Thr	Gly	Gly	Gly	Gly	Asp	Leu	Tyr	
				420					425					430			
55	Ala	Ala	His	Ser	Asp	Thr	Tyr	Leu	Ser	Gly	Lys	Ser	Leu	Ser	Val	Pro	
			435					440					445				
60	Phe	Ala	Ala	Gly	Ala	Val	Gln	Thr	Leu	Glu	Val	Asp	Gly	Val	Thr	Val	
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70	<211>	458															
75	<212>	PRT															
80	<213>	Trichoderma harzianum															
85	<400>	22															
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	1				5					10					15		

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	Gly	Trp	Gly	Val	Ser	Leu	Ala	Trp	Trp	Ala	Lys	Ala	Phe	Gly	Asn	Arg	
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5	Asp	Asp	Leu	Ala	Asn	Val	Phe	Phe	Thr	Arg	Asn	Asn	Gln	Val	Ile	Asn	
			35					40					45				
	Gly	Gln	Asn	Leu	Pro	Gly	Leu	Gly	Phe	Asn	Ile	Ala	Arg	Tyr	Asn	Ala	
10		50					55					60					
	Gly	Ala	Cys	Ser	Thr	Asn	Thr	Tyr	Asn	Gly	Ser	Ser	Met	Val	Val	Ser	
	65					70					75					80	
15	Ser	Ser	Ile	Lys	Pro	Ser	Arg	Gln	Val	Asp	Gly	Tyr	Trp	Leu	Asp	Trp	
					85					90					95		
	Ala	Ser	Thr	Asp	Pro	Ala	Ser	Ser	Ser	Trp	Asn	Trp	Asn	Val	Asp	Ala	
20					100					105				110			
	Asn	Gln	Arg	Ala	Met	Leu	Gln	Lys	Ala	Lys	Ala	Asn	Gly	Ala	Asn	Ile	
25				115				120					125				
	Phe	Glu	Leu	Phe	Ser	Asn	Ser	Pro	Met	Trp	Trp	Met	Cys	Leu	Asn	His	
		130					135					140					
30	Asn	Pro	Ser	Gly	Ser	Gly	Ser	Ser	Asp	Asn	Leu	Gln	Ser	Trp	Asn	Tyr	
	145					150					155					160	
	Gln	Asn	His	Ala	Val	Tyr	Leu	Ala	Asn	Ile	Ala	Gln	His	Ala	Gln	Gln	
35					165					170					175		
	Asn	Trp	Gly	Ile	Gln	Phe	Gln	Ser	Val	Glu	Ala	Phe	Asn	Glu	Pro	Ser	
				180					185					190			
40	Ser	Gly	Trp	Gly	Pro	Thr	Gly	Thr	Gln	Glu	Gly	Cys	His	Phe	Ala	Val	
			195					200					205				
	Ser	Thr	Met	Ala	Thr	Val	Ile	Gly	Tyr	Leu	Asn	Thr	Glu	Leu	Ala	Gln	
45			210				215					220					
	Arg	Gly	Leu	Ser	Ser	Phe	Ile	Ser	Ala	Ser	Asp	Glu	Thr	Ser	Tyr	Asp	
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	Leu	Ala	Ile	Ser	Thr	Trp	Gln	Gly	Leu	Gly	Ser	Ser	Ala	Gln	Asn	Ala	
					245					250					255		
55	Val	Lys	Arg	Val	Asn	Val	His	Gly	Tyr	Gln	Gly	Gly	Gly	Gly	Arg	Arg	
				260					265					270			

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	Asp	Thr	Leu	Tyr	Ser	Leu	Val	Ser	Gln	Ala	Gly	Lys	Arg	Leu	Trp	Asn	
			275						280				285				
5	Ser	Glu	Tyr	Gly	Asp	Ala	Asp	Ala	Ser	Gly	Lys	Ser	Met	Tyr	Thr	Asn	
		290					295					300					
10	Leu	Leu	Leu	Asp	Phe	Thr	Trp	Leu	His	Pro	Thr	Ala	Trp	Val	Tyr	Trp	
	305					310					315					320	
15	Gln	Ala	Ile	Asp	Gly	Ser	Gly	Trp	Gly	Leu	Ile	Val	Gly	Asp	Asn	Asp	
					325					330					335		
20	Gln	Leu	Thr	Leu	Ser	Ser	Ala	Ser	Thr	Lys	Tyr	Phe	Val	Leu	Ala	Gln	
				340					345					350			
25	Leu	Thr	Arg	His	Ile	Arg	Pro	Gly	Met	Gln	Ile	Leu	Thr	Thr	Pro	Asp	
			355					360					365				
30	Gly	Asn	Thr	Val	Ala	Ala	Tyr	Asp	Ser	Gly	Ser	Gln	Lys	Leu	Val	Ile	
	370						375					380					
35	Val	Ala	Ala	Asn	Trp	Gly	Ser	Ala	Gln	Thr	Ile	Thr	Phe	Asp	Leu	Thr	
	385					390					395					400	
40	Arg	Ala	Lys	Thr	Ala	Gly	Ser	Asn	Gly	Ala	Thr	Val	Pro	Arg	Trp	Ser	
					405					410					415		
45	Thr	Gln	Thr	Ser	Gly	Gly	Asp	Gln	Tyr	Lys	Ser	Tyr	Ser	Asp	Thr	Lys	
				420					425					430			
50	Ile	Asn	Asn	Gly	Lys	Phe	Ser	Val	Ser	Phe	Ser	Thr	Gly	Gln	Val	Gln	
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	1				5					10					15		
	Gly	Trp	Gly	Thr	Ser	Leu	Ala	Trp	Trp	Gly	Asn	Val	Phe	Gly	Thr	Arg	
				20					25					30			

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	Asp	Asp	Phe	Ala	Asp	Leu	Phe	Phe	Thr	Thr	Lys	Ser	Val	Thr	Tyr	Asn
			35					40					45			
5	Gly	Thr	Ser	Leu	Pro	Gly	Leu	Gly	Leu	Asn	Ile	Ala	Arg	Tyr	Asn	Leu
		50					55					60				
10	Gly	Ala	Cys	Ser	Trp	Asn	Ala	Val	Asn	Gly	Glu	Thr	Met	Val	Lys	Ser
	65					70					75					80
15	Pro	Asn	Ile	Pro	Ala	Phe	Lys	Gln	Ile	Glu	Gly	Phe	Trp	Gln	Asp	Trp
					85					90					95	
20	Asn	Asn	Glu	Asp	Pro	Thr	Ser	Ser	Ala	Trp	Asp	Trp	Thr	Ala	Asp	Ala
				100					105					110		
25	Thr	Gln	Arg	Ala	Met	Leu	Val	Lys	Ala	Thr	Gln	Arg	Gly	Ala	Val	Thr
			115					120					125			
30	Glu	Leu	Phe	Ala	Asn	Ser	Pro	Met	Trp	Trp	Met	Cys	Tyr	Asn	His	Asn
		130					135					140				
35	Pro	Ser	Gly	Ala	Ala	Asp	Gly	Gly	Asn	Asn	Leu	Gln	Thr	Trp	Asn	Tyr
	145					150					155					160
40	Arg	Gln	His	Ala	Ser	His	Leu	Ala	Ala	Val	Ala	Leu	Tyr	Ala	Arg	Thr
					165					170					175	
45	Asn	Trp	Gly	Val	Asn	Phe	Ala	Thr	Val	Asp	Pro	Phe	Asn	Glu	Pro	Ala
				180					185					190		
50	Ser	Ser	Trp	Trp	Thr	Ala	Ser	Gly	Thr	Gln	Glu	Gly	Cys	His	Leu	Asp
			195					200					205			
55	Pro	Ala	Val	Gln	Ala	Ala	Val	Leu	Pro	Tyr	Met	Arg	Ser	Glu	Leu	Asp
		210					215					220				
60	Lys	Arg	Gly	Leu	Thr	Gly	Val	Arg	Ile	Ser	Ala	Ser	Asp	Glu	Thr	Asn
	225					230					235					240
65	Tyr	Asp	Thr	Ala	Arg	Ser	Thr	Trp	Ser	Ser	Phe	Gly	Ser	Ala	Thr	Lys
					245					250					255	
70	Ala	Leu	Val	Ser	Gln	Val	Asn	Val	His	Gly	Tyr	Gln	Gly	Thr	Gly	Gly
				260					265					270		
75	Arg	Arg	Asp	Leu	Leu	Tyr	Thr	Asp	Val	Val	Thr	Thr	Ser	Gly	Lys	Lys

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	275		280		285
5	Leu Trp Asn Ser Glu Thr Gly Asp Ser Asp Gly Thr Gly Leu Ser Met				
	290		295		300
10	Ala Arg Asn Leu Cys Tyr Asp Phe Arg Trp Leu His Pro Thr Ala Trp				
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					320
15	Cys Tyr Trp Gln Val Met Asp Pro Ser Thr Gly Trp Ala Met Ile Ala				
			325		330
					335
20	Tyr Asp Ala Asn Thr Leu Gln Pro Thr Thr Val Gln Pro Lys Tyr Tyr				
			340		345
					350
25	Val Met Ala Gln Phe Ser Arg His Ile Arg Pro Gly Met Thr Ile Leu				
			355		360
					365
30	Asp Thr Gly Val Ser Phe Ala Ala Ala Ala Tyr Asp Ala Ser Ala Arg				
			370		375
					380
35	Arg Leu Val Leu Val Ala Val Asn Thr Ser Thr Ser Pro Gln Thr Phe				
			385		390
					395
40	Thr Phe Asp Leu Ser Arg Phe Thr Thr Val Thr Gly Gly Ser Gly Gly				
			405		410
					415
45	Leu Val Pro Arg Trp Asn Thr Val Thr Gly Gly Gly Asp Met Tyr Arg				
			420		425
					430
50	Ala Tyr Thr Asn Thr Tyr Val Thr Gly Lys Ser Val Ser Ala Thr Phe				
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55	Ala Ala Gly Ser Val Gln Thr Leu Gln Val Asp Gly Val Thr Thr				
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		20		25	30
	Asp Asp Phe Ala Asp Leu Phe Phe Thr Thr Lys Ser Val Thr Tyr Asn				



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	35	40	45
5	Gly Arg Thr Leu Pro Gly Leu Gly Leu Asn Ile Ala Arg Tyr Asn Leu 50 55 60		
10	Gly Ala Cys Ser Trp Asn Ser Val Ser Gly Glu Ser Met Val Ala Ser 65 70 75 80		
15	Ala Asn Ile Pro Ala Phe Lys Gln Ile Glu Gly Tyr Trp Gln Asp Trp 85 90 95		
20	Asn Asn Glu Asp Pro Thr Ser Ser Ala Trp Lys Trp Thr Ala Asp Ala 100 105 110		
25	Ala Gln Arg Thr Met Leu Val Lys Ala Thr Ala Arg Gly Ala Thr Thr 115 120 125		
30	Glu Leu Phe Ala Asn Ser Pro Met Trp Trp Met Cys Leu Asn His Asn 130 135 140		
35	Pro Ser Gly Ala Ser Gly Gly Gly Asn Asn Leu Gln Ser Trp Asn Tyr 145 150 155 160		
40	Arg Gln His Ala Ser His Leu Ala Ala Val Ala Leu Tyr Ala Lys Ser 165 170 175		
45	Asn Trp Gly Val Asn Phe Ala Thr Val Asp Pro Phe Asn Glu Pro Ser 180 185 190		
50	Ser Ser Trp Trp Thr Ala Thr Gly Thr Gln Glu Gly Cys His Met Asp 195 200 205		
55	Ala Ser Val Gln Ala Ala Val Leu Pro Tyr Leu Arg Ser Glu Leu Asp 210 215 220		
	Arg Arg Gly Leu Thr Gly Thr Lys Ile Ser Ala Ser Asp Glu Thr Ser 225 230 235 240		
	Tyr Asp Leu Ala Arg Thr Thr Trp Gly Ser Phe Gly Ser Ser Thr Lys 245 250 255		
	Ala Leu Val Asn Arg Val Asn Val His Gly Tyr Gln Gly Ser Gly Gly 260 265 270		
	Arg Arg Asp Leu Leu Tyr Thr Asp Val Val Thr Thr Ala Gly Lys Ala 275 280 285		

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	Leu	Trp	Asn	Ser	Glu	Thr	Gly	Asp	Ser	Asp	Gly	Thr	Gly	Leu	Thr	Leu	
	290						295					300					
5	Ala	Ser	Asn	Leu	Cys	Leu	Asp	Phe	Arg	Trp	Leu	His	Pro	Thr	Ala	Trp	
	305					310					315					320	
10	Val	Tyr	Trp	Gln	Val	Met	Asp	Pro	Ser	Ser	Gly	Trp	Ala	Met	Ile	Ala	
					325					330					335		
15	Tyr	Asp	Ala	Ser	Thr	Leu	Gln	Pro	Gly	Ala	Val	Gln	Thr	Lys	Tyr	Tyr	
				340					345					350			
20	Val	Met	Ala	Gln	Phe	Ser	Arg	His	Ile	Arg	Ala	Gly	Met	Thr	Ile	Val	
			355					360					365				
25	Asp	Thr	Gly	Val	Gly	Tyr	Ala	Ala	Ala	Ala	Tyr	Asp	Ala	Thr	Ala	Arg	
			370				375					380					
30	Arg	Leu	Val	Ile	Val	Ala	Val	Asn	Thr	Ser	Thr	Ser	Ala	Gln	Thr	Leu	
	385					390					395					400	
35	Thr	Phe	Asp	Leu	Ser	Arg	Phe	Ser	Thr	Val	Thr	Gly	Gly	Thr	Gly	Gly	
				405						410					415		
40	Leu	Val	Arg	Arg	Trp	Asn	Thr	Val	Thr	Gly	Gly	Gly	Gly	Asp	Leu	Tyr	
				420				425						430			
45	Ala	Ala	His	Ser	Asp	Thr	Tyr	Leu	Ser	Gly	Lys	Ser	Leu	Ser	Val	Pro	
			435					440					445				
50	Phe	Ala	Ala	Gly	Ala	Val	Gln	Thr	Leu	Glu	Val	Asp	Gly	Val	Thr	Val	
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70	Gly	Trp	Gly	Val	Ser	Leu	Ala	Trp	Trp	Ala	Lys	Ala	Phe	Gly	Asn	Arg	
				20					25					30			
75	Asp	Asp	Leu	Ala	Asn	Val	Phe	Phe	Thr	Arg	Asn	Asn	Gln	Val	Ile	Asn	
			35					40					45				

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	Gly	Gln	Asn	Leu	Pro	Gly	Leu	Gly	Phe	Asn	Ile	Ala	Arg	Tyr	Asn	Ala	
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5	Gly	Ala	Cys	Ser	Thr	Asn	Thr	Tyr	Asn	Gly	Ser	Ser	Met	Val	Val	Ser	
	65					70					75					80	
	Ser	Ser	Ile	Lys	Pro	Ser	Arg	Gln	Val	Asp	Gly	Tyr	Trp	Leu	Asp	Trp	
10					85					90					95		
	Ala	Ser	Thr	Asp	Pro	Ala	Ser	Ser	Ser	Trp	Asn	Trp	Asn	Val	Asp	Ala	
				100					105					110			
15	Asn	Gln	Arg	Ala	Met	Leu	Gln	Lys	Ala	Lys	Ala	Asn	Gly	Ala	Asn	Ile	
			115					120					125				
	Phe	Glu	Leu	Phe	Ser	Asn	Ser	Pro	Met	Trp	Trp	Met	Cys	Leu	Asn	His	
20		130					135					140					
	Asn	Pro	Ser	Gly	Ser	Gly	Ser	Ser	Asp	Asn	Leu	Gln	Ser	Trp	Asn	Tyr	
	145					150					155					160	
25	Gln	Asn	His	Ala	Val	Tyr	Leu	Ala	Asn	Ile	Ala	Gln	His	Ala	Gln	Gln	
				165						170					175		
	Asn	Trp	Gly	Ile	Gln	Phe	Gln	Ser	Val	Glu	Ala	Phe	Asn	Glu	Pro	Ser	
30				180					185					190			
	Ser	Gly	Trp	Gly	Pro	Thr	Gly	Thr	Gln	Glu	Gly	Cys	His	Phe	Ala	Val	
35			195					200					205				
	Ser	Thr	Met	Ala	Thr	Val	Ile	Gly	Tyr	Leu	Asn	Thr	Glu	Leu	Ala	Gln	
		210					215					220					
40	Arg	Gly	Leu	Ser	Ser	Phe	Ile	Ser	Ala	Ser	Asp	Glu	Thr	Ser	Tyr	Asp	
	225					230					235					240	
	Leu	Ala	Ile	Ser	Thr	Trp	Gln	Gly	Leu	Gly	Ser	Ser	Ala	Gln	Asn	Ala	
45					245					250					255		
	Val	Lys	Arg	Val	Asn	Val	His	Gly	Tyr	Gln	Gly	Gly	Gly	Gly	Arg	Arg	
50				260					265					270			
	Asp	Thr	Leu	Tyr	Ser	Leu	Val	Ser	Gln	Ala	Gly	Lys	Arg	Leu	Trp	Asn	
			275					280					285				
55	Ser	Glu	Tyr	Gly	Asp	Ala	Asp	Ala	Ser	Gly	Lys	Ser	Met	Tyr	Thr	Asn	
		290					295					300					

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	Leu	Leu	Leu	Asp	Phe	Thr	Trp	Leu	His	Pro	Thr	Ala	Trp	Val	Tyr	Trp	
	305					310					315					320	
5	Gln	Ala	Ile	Asp	Gly	Ser	Gly	Trp	Gly	Leu	Ile	Val	Gly	Asp	Asn	Asp	
					325					330					335		
10	Gln	Leu	Thr	Leu	Ser	Ser	Ala	Ser	Thr	Lys	Tyr	Phe	Val	Leu	Ala	Gln	
				340					345					350			
15	Leu	Thr	Arg	His	Ile	Arg	Pro	Gly	Met	Gln	Ile	Leu	Thr	Thr	Pro	Asp	
			355					360					365				
20	Gly	Asn	Thr	Val	Ala	Ala	Tyr	Asp	Ser	Gly	Ser	Gln	Lys	Leu	Val	Ile	
	370						375					380					
25	Val	Ala	Ala	Asn	Trp	Gly	Ser	Ala	Gln	Thr	Ile	Thr	Phe	Asp	Leu	Thr	
	385					390					395					400	
30	Arg	Ala	Lys	Thr	Ala	Gly	Ser	Asn	Gly	Ala	Thr	Val	Pro	Arg	Trp	Ser	
					405					410					415		
35	Thr	Gln	Thr	Ser	Gly	Gly	Asp	Gln	Tyr	Lys	Ser	Tyr	Ser	Asp	Thr	Lys	
				420					425					430			
40	Ile	Asn	Asn	Gly	Lys	Phe	Ser	Val	Ser	Phe	Ser	Thr	Gly	Gln	Val	Gln	
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	450						455										
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	1				5					10					15		
60	Val	Met	Asn	Glu	Ile	Ala	Gly	Phe	Ser	Gly	Thr	Gly	Tyr	Val	Gly	Gly	
				20					25					30			
65	Trp	Asp	Glu	Asp	Ala	Asp	Thr	Val	Ser	Leu	Thr	Phe	Thr	Ser	Asp	Ala	
			35					40					45				
70	Thr	Lys	Leu	Tyr	Asp	Val	Lys	Ile	Arg	Tyr	Ser	Gly	Pro	Tyr	Gly	Ser	
	50						55					60					

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	Lys	Tyr	Thr	Arg	Ile	Ser	Tyr	Asn	Gly	Ala	Thr	Gly	Gly	Asp	Ile	Ser	65	70	75	80
5	Leu	Pro	Glu	Thr	Thr	Glu	Trp	Ala	Thr	Val	Asn	Ala	Gly	Gln	Ala	Leu		85	90	95
10	Leu	Asn	Ala	Gly	Ser	Asn	Thr	Ile	Lys	Leu	His	Asn	Asn	Trp	Gly	Trp	100	105	110	
15	Tyr	Leu	Ile	Asp	Ala	Val	Ile	Leu	Thr	Pro	Ser	Val	Pro	Arg	Pro	Pro	115	120	125	
20	His	Gln	Val	Thr	Asp	Ala	Leu	Val	Asn	Thr	Asn	Ser	Asn	Ala	Val	Thr	130	135	140	
25	Lys	Gln	Leu	Met	Lys	Phe	Leu	Val	Ser	Lys	Tyr	His	Lys	Ala	Tyr	Ile	145	150	155	160
30	Thr	Gly	Gln	Gln	Glu	Leu	His	Ala	His	Gln	Trp	Val	Glu	Lys	Asn	Val	165	170	175	
35	Gly	Lys	Ser	Pro	Ala	Ile	Leu	Gly	Leu	Asp	Phe	Met	Asp	Tyr	Ser	Pro	180	185	190	
40	Ser	Arg	Val	Glu	Phe	Gly	Thr	Thr	Ser	Gln	Ala	Val	Glu	Gln	Ala	Ile	195	200	205	
45	Asp	Phe	Asp	Lys	Arg	Gly	Gly	Ile	Val	Thr	Phe	Ala	Trp	His	Trp	Asn	210	215	220	
50	Ala	Pro	Ser	Gly	Leu	Ile	Asn	Thr	Pro	Gly	Ser	Glu	Trp	Trp	Arg	Gly	225	230	235	240
55	Phe	Tyr	Thr	Glu	His	Thr	Thr	Phe	Asp	Val	Ala	Ala	Ala	Leu	Gln	Asn	245	250	255	
60	Thr	Thr	Asn	Ala	Asn	Tyr	Asn	Leu	Leu	Ile	Arg	Asp	Ile	Asp	Ala	Ile	260	265	270	
65	Ala	Val	Gln	Leu	Lys	Arg	Leu	Gln	Thr	Ala	Gly	Val	Pro	Val	Leu	Trp	275	280	285	
70	Arg	Pro	Leu	His	Glu	Ala	Glu	Gly	Gly	Trp	Phe	Trp	Trp	Gly	Ala	Lys	290	295	300	
75	Gly	Pro	Glu	Pro	Ala	Lys	Lys	Leu	Tyr	Lys	Ile	Leu	Tyr	Asp	Arg	Leu	305	310	315	320

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	Thr	Asn	Tyr	His	Lys	Leu	Asn	Asn	Leu	Ile	Trp	Val	Trp	Asn	Ser	Val
					325					330					335	
5	Ala	Lys	Asp	Trp	Tyr	Pro	Gly	Asp	Glu	Ile	Val	Asp	Val	Leu	Ser	Phe
				340					345					350		
10	Asp	Ser	Tyr	Pro	Ala	Gln	Pro	Gly	Asp	His	Gly	Pro	Val	Ser	Ala	Gln
			355					360					365			
15	Tyr	Asn	Ala	Leu	Val	Glu	Leu	Gly	Lys	Asp	Lys	Lys	Leu	Ile	Ala	Ala
	370						375					380				
20	Thr	Glu	Val	Gly	Thr	Ile	Pro	Asp	Pro	Asp	Leu	Met	Gln	Leu	Tyr	Glu
	385					390					395					400
25	Ser	Tyr	Trp	Ser	Phe	Phe	Val	Thr	Trp	Glu	Gly	Glu	Phe	Ile	Glu	Asn
					405					410					415	
30	Gly	Val	His	Asn	Ser	Leu	Glu	Phe	Leu	Lys	Lys	Leu	Tyr	Asn	Asn	Ser
				420					425					430		
35	Phe	Val	Leu	Asn	Leu	Asp	Thr	Ile	Gln	Gly	Trp	Lys	Asn	Gly	Ala	Gly
		435					440						445			
40	Ser	Ser	Thr	Thr	Thr	Val	Lys	Ser	Thr	Thr	Thr	Thr	Pro	Thr	Thr	Thr
	450						455						460			
45	Ile	Lys	Ser	Thr	Thr	Thr	Thr	Pro	Val	Thr	Thr	Pro	Thr	Thr	Val	Lys
	465					470					475				480	
50	Thr	Thr	Thr	Thr	Pro	Thr	Thr	Thr	Ala	Thr	Thr	Val	Lys	Ser	Thr	Thr
					485				490						495	
55	Thr	Thr	Ala	Gly	Pro	Thr	Pro	Thr	Ala	Val	Ala	Gly	Arg	Trp	Gln	Gln
				500					505					510		
60	Cys	Gly	Gly	Ile	Gly	Phe	Thr	Gly	Pro	Thr	Thr	Cys	Glu	Ala	Gly	Thr
		515						520					525			
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15	Trp	Asp	Glu	Asp	Ala	Asp	Thr	Val	Ser	Leu	Thr	Phe	Thr	Ser	Asp	Ala	35	40	45	
20	Thr	Lys	Leu	Tyr	Asp	Val	Lys	Ile	Arg	Tyr	Ser	Gly	Pro	Tyr	Gly	Ser	50	55	60	
25	Lys	Tyr	Thr	Arg	Ile	Ser	Tyr	Asn	Gly	Ala	Thr	Gly	Gly	Asp	Ile	Ser	65	70	75	80
30	Leu	Pro	Glu	Thr	Thr	Glu	Trp	Ala	Thr	Val	Asn	Ala	Gly	Gln	Ala	Leu	85	90	95	
35	Leu	Asn	Ala	Gly	Ser	Asn	Thr	Ile	Lys	Leu	His	Asn	Asn	Trp	Gly	Trp	100	105	110	
40	Tyr	Leu	Ile	Asp	Ala	Val	Ile	Leu	Thr	Pro	Ser	Val	Pro	Arg	Pro	Pro	115	120	125	
45	His	Gln	Val	Thr	Asp	Ala	Leu	Val	Asn	Thr	Asn	Ser	Asn	Ala	Val	Thr	130	135	140	
50	Lys	Gln	Leu	Met	Lys	Phe	Leu	Val	Ser	Lys	Tyr	His	Lys	Ala	Tyr	Ile	145	150	155	160
55	Thr	Gly	Gln	Gln	Glu	Leu	His	Ala	His	Gln	Trp	Val	Glu	Lys	Asn	Val	165	170	175	
	Gly	Lys	Ser	Pro	Ala	Ile	Leu	Gly	Leu	Asp	Phe	Met	Asp	Tyr	Ser	Pro	180	185	190	
	Ser	Arg	Val	Glu	Phe	Gly	Thr	Thr	Ser	Gln	Ala	Val	Glu	Gln	Ala	Ile	195	200	205	
	Asp	Phe	Asp	Lys	Arg	Gly	Gly	Ile	Val	Thr	Phe	Ala	Trp	His	Trp	Asn	210	215	220	
	Ala	Pro	Ser	Gly	Leu	Ile	Asn	Thr	Pro	Gly	Ser	Glu	Trp	Trp	Arg	Gly	225	230	235	240
	Phe	Tyr	Thr	Glu	His	Thr	Thr	Phe	Asp	Val	Ala	Ala	Ala	Leu	Gln	Asn				

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	245	250	255
5	Thr Thr Asn Ala Asn Tyr Asn Leu Leu Ile Arg Asp Ile Asp Ala Ile 260 265 270		
10	Ala Val Gln Leu Lys Arg Leu Gln Thr Ala Gly Val Pro Val Leu Trp 275 280 285		
15	Arg Pro Leu His Glu Ala Glu Gly Gly Trp Phe Trp Trp Gly Ala Lys 290 295 300		
20	Gly Pro Glu Pro Ala Lys Lys Leu Tyr Lys Ile Leu Tyr Asp Arg Leu 305 310 315 320		
25	Thr Asn Tyr His Lys Leu Asn Asn Leu Ile Trp Val Trp Asn Ser Val 325 330 335		
30	Ala Lys Asp Trp Tyr Pro Gly Asp Glu Ile Val Asp Val Leu Ser Phe 340 345 350		
35	Asp Ser Tyr Pro Ala Gln Pro Gly Asp His Gly Pro Val Ser Ala Gln 355 360 365		
40	Tyr Asn Ala Leu Val Glu Leu Gly Lys Asp Lys Lys Leu Ile Ala Ala 370 375 380		
45	Thr Glu Val Gly Thr Ile Pro Asp Pro Asp Leu Met Gln Leu Tyr Glu 385 390 395 400		
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55	Gly Val His Asn Ser Leu Glu Phe Leu Lys Lys Leu Tyr Asn Asn Ser 420 425 430		
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185

190

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55 Val Ile His Lys Thr Asn Gly Asp Glu  
355 360

## Claims

1. A cleaning composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity and selected from the endo-alpha-1,4-polygalactosminidase class (EC 3.2.1.109) of enzymes.
2. A cleaning composition according to claim 1 wherein the glycoside hydrolase enzyme having glycoside hydrolase activity is a variant having at least 60%, preferably at least 70%, more preferably at least 80% identity to SEQ ID NO:1.
3. A cleaning composition according to claim 1 or claim 2 wherein the glycoside hydrolase is from GH family 114.
4. A cleaning composition according to any preceding claim wherein the glycoside hydrolase comprises PeIAh.
5. A cleaning composition according to any preceding claim wherein the glycoside hydrolase enzyme is obtainable

from *Pseudomonas*, preferably from *Pseudomonas aeruginosa*.

6. A cleaning composition according to any preceding claim wherein the glycoside hydrolase enzyme is an isolated glycoside hydrolase.
7. A cleaning composition according to any preceding claim wherein the composition further comprises additional enzyme selected from galactanases, mannanases, nucleases, and mixtures thereof.
8. A cleaning composition according to claim 7 wherein the composition additionally comprises a nuclease enzyme, preferably a deoxyribonuclease enzyme.
9. A cleaning composition according to any preceding claim wherein the composition further comprises one, preferably two or three or more additional enzymes selected from lipases, proteases, pectate lyases, cellulases, cutinases, and mixtures thereof.
10. A cleaning composition according to any preceding claim wherein the composition further comprises a  $\beta$ -N-acetylglucosaminidase enzyme from E.C. 3.2.1.52, preferably an enzyme having at least 70% identity to SEQ ID NO:12.
11. A cleaning composition according to any preceding claim wherein the cleaning composition further comprises from 1% to 80 wt% , preferably from 5 to 80 wt% of the cleaning composition, of a surfactant system, preferably comprising an anionic surfactant.
12. A cleaning composition according to claim 11 wherein the surfactant system additionally comprises a nonionic surfactant, and preferably the weight ratio of the anionic to nonionic surfactant is from 25:1 to 1:2.
13. A cleaning composition according to claim 11 or claim 12 wherein the anionic surfactant is selected from alkyl benzene sulphonates and (optionally alkoxylated) alkyl sulfates and mixtures thereof, preferably the anionic surfactant comprising at least 50 wt% alkyl benzene sulphonate surfactant.
14. A method of cleaning a surface, preferably a textile, comprising mixing the cleaning composition according to any preceding claim with water to form an aqueous liquor and contacting a surface, preferably a textile, with the aqueous liquor in a laundering step, preferably wherein the glycoside hydrolase enzyme is present in the aqueous liquor in an amount of from 0.01ppm to 1000 ppm enzyme, based on active protein or from 0.05 or from 0.1ppm to 750 or 500ppm.
15. Use of a composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity and belonging to the endo-alpha-1,4-polygalactosminidase class (EC 3.2.1.109) of enzymes, to enhance stain removal from a surface, preferably a fabric surface, particularly greasy-stain removal, body soil removal and/or for reduction of malodour from the surface.



## EUROPEAN SEARCH REPORT

Application Number  
EP 17 20 4716

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 2010/125047 A1 (LANT NEIL JOSEPH [GB]) 20 May 2010 (2010-05-20) * paragraph [0079] - paragraph [0104]; claims; examples *	1-15	INV. C11D3/386
X	US 2009/176680 A1 (PATTERSON STEVEN GEORGE [GB] ET AL) 9 July 2009 (2009-07-09) * paragraph [0006] - paragraph [0015]; claims; examples *	1-15	
X	EP 2 363 455 A1 (PROCTER & GAMBLE [US]) 7 September 2011 (2011-09-07) * paragraph [0087]; claim 9; examples *	1-15	
X	US 2014/066353 A1 (WANG XIAOLI [CN] ET AL) 6 March 2014 (2014-03-06) * paragraph [0305]; claim 17; examples *	1-15	
X	WO 2015/184526 A1 (HOSPITAL FOR SICK CHILDREN [CA]; UNIV MCGILL [CA]) 10 December 2015 (2015-12-10) * claim 37; example 21 *	1-5, 14, 15	TECHNICAL FIELDS SEARCHED (IPC) C11D
The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 11 January 2018	Examiner Vernier, Frédéric
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on  
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