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(54) **CLEANING COMPOSITIONS INCLUDING ENZYMES**

(57) Cleaning compositions that include an amylase and a glycosyl hydrolase enzyme, particularly from GH family 39. Methods of making and using such cleaning compositions. Use of a composition having an amylase enzyme and a glycosyl hydrolase enzyme, particularly from GH family 39 for enhancing stain removal from a surface such as a fabric surface and/or malodour reduction.

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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 that include a 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 tending 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 glycosyl hydrolases can provide particularly improved cleaning.

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.

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SUMMARY OF THE INVENTION

[0006] The present invention provides a cleaning and/or treatment composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity, wherein the enzyme is a member of a glycoside hydrolase family GH 39.

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 to SEQ ID NO: 1, and less than, or up to 100% identity with SEQ ID NO: 1.

[0008] 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.

40 **[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 glycoside hydrolases from family GH 39 to enhance soil and/or stain removal from a surface, preferably a fabric, and/or for malodour reduction from a surface, preferably the glycoside hydrolase 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 less than or up to 100% identity with SEQ ID NO: 1, to enhance soil and/or stain removal from a surface, preferably a fabric, and/or for malodour reduction from a surface.

45 **[0010]** A preferred composition comprises a second glycosyl hydrolase from the endo-alpha-1,4-polygalactosaminidase class (EC 3.2.1.109) of enzymes.

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DETAILED DESCRIPTION OF THE INVENTION

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

55 **[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 composition comprises a glycoside hydrolase enzyme having glycoside hydrolase activity and selected from GH family 39 glycoside hydrolases. The enzyme essential to the present invention preferably 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:1.

[0035] Preferably the glycoside hydrolase is from GH family 39.

[0036] Preferably, the glycoside hydrolase enzyme comprises 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 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 is PslGH, optionally in addition to further glycoside hydrolases.

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

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

[0040] Preferably the glycoside hydrolase enzyme is present in a 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 composition comprising the glycoside hydrolase described herein may also give rise to/be useful for 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 herein (SEQ ID No. 2 in WO 96/23874): 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: 3 herein (SEQ ID No. 12 in WO 06/002643):

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 as SEQ ID NO: 4 herein (SEQ ID No. 4 in WO06/002643), 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 as SEQ ID NO:5 herein, the wild-type enzyme from *Bacillus* sp.707 (SEQ ID NO:7 in US 6,093, 562), 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: 6 or SEQ ID NO:7 herein (SEQ ID NOS: 1 and 2 from WO 09/149130), 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 as SEQ ID NO: 8 herein (SEQ ID NO:1 in WO2016091688), 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 α -amylase" from *Paenibacillus curdlanolyticus* YK9 as SEQ ID NO: 9 herein (SEQ ID NO:3 in WO2014099523).

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

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

(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®, ENZYSIZE®, 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 Additional Glycosyl Hydrolase

[0044] The composition of the invention preferably comprises an additional glycosyl hydrolase. A preferred additional glycosyl hydrolase comprises a glycosyl hydrolase from the endo- α -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:12. Preferably the additional glycoside hydrolase is from GH family 114.

[0045] Preferably, the additional glycoside hydrolase enzyme is a microbial enzyme, it may be fungal or bacterial in origin, though bacterial glycoside hydrolases are most preferred. Fungal glycoside hydrolases may be most preferred. Such additional 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: 12 of the present invention from *Pseudomonas aeruginosa*. Preferably such additional glycoside hydrolase in the cleaning composition of the invention is PelAh.

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 fluorescers; 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 composition preferably comprises 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 lipases, proteases, pectate lyases, cellulases, cutinases, and mixtures thereof. Preferably in addition, the cleaning composition comprises 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, lipoxxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, β -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: 13 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: 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.

[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: 16 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: 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.

[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*, *Phaeoemoniella chlamydospora*, *Fusarium verticillioides* 7600, *Fusarium oxysporum* f. sp. cubense race 4, *Colletotrichum graminicola* M1.001, *Fusarium oxysporum* FOSC 3-a, *Fusarium avenaceum*, *Fusarium langsethiae*, *Grossmannia clavigera* kw1407, *Claviceps purpurea* 20.1, *Verticillium longisporum*, *Fusarium oxysporum* f. sp. cubense race 1, *Maguaporthes oryzae* 70-15, *Beauveria bassiana* D1-5, *Fusarium pseudograminearum* CS3096, *Neonectria ditissima*, *Magnaporthe oryzae* ATCC 64411, *Cordyceps militaries* 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 attenuata*, *Ustilago violacea*, *Diplodia seriata*, *Ophiostoma piceae* UAMH 11346, *Pseudogymnoascus pannorum* 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 *Pyrenopeziza 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-galactanase 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: 18 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: 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.

[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: 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.

[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: 18, SEQ ID NO: 19 or SEQ ID NO: 20.

[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. Particularly preferred are mannanases 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- β -1,4-mannase; β -mannanase B; 3-1,4-mannan 4-mannanohydrolase; endo-3-mannanase; and β -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: 21 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: 22 from *Chaetomium virens*.

[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: 23 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: 24 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: 25 from *Myrothecium roridum*.

[0073] Preferably the mannanase is an isolated mannanase.

[0074] Preferably the mannanase 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 mannanase enzyme is present in the laundering aqueous solution in an amount of from 0.01 ppm to 1000 ppm of the mannanase enzyme, or from 0.05 or from 0.1 ppm to 750 or 500 ppm.

Xanthan-degrading enzyme

[0075] 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

[0076] In a preferred composition, the composition may additionally comprise an acetylglucosaminidase enzyme, preferably a β -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:26.

Proteases

[0077] 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 W92/17577 (Novozymes A/S), including the variants of this *Bacillus* sp TY145 subtilase described in WO2015024739, and WO2016066757.

[0078] Preferred proteases include those derived from *Bacillus gibsonii* or *Bacillus Lentus*.

[0079] 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, WO2011/07211 US2011/0237487, WO2011/140316 WO2012/151480, EP2510092, EP2566960 OR EP2705145.

Lipases

[0080] 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 15Å 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

[0081] 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

[0082] 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

[0083] 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.

[0084] Surfactants suitable for use in the surfactant system may be derived from natural and/or renewable sources.

[0085] 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.

[0086] 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.

[0087] Preferably the composition further comprises a nonionic surfactant, especially an alcohol alkoxyate in particular an alcohol ethoxyate nonionic surfactant. 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

[0088] 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

[0089] 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 which 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

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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 preferably sodium.

[0095] 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.

[0096] 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.

[0097] 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

[0098] 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.

[0099] Other suitable non-ionic surfactants for use herein include fatty alcohol polyglycol ethers, alkylpolyglucosides and fatty acid glucamides.

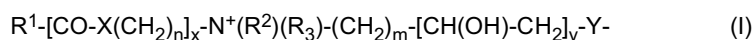
Amphoteric surfactant

[0100] 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 α 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.

[0101] 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

[0102] 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

R¹ 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, NR⁴ with C1-4 Alkyl residue R⁴, O or S,

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

x 0 or 1, preferably 1,

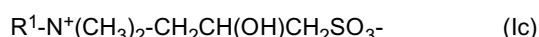
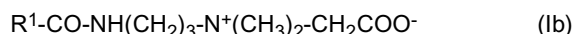
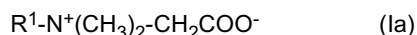
R², R³ are independently a C1-4 alkyl residue, potentially hydroxy substituted such as a hydroxyethyl, preferably a methyl.

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

y 0 or 1 and

Y is COO, SO₃, OPO(OR⁵)O or P(O)(OR⁵)O, whereby R⁵ is a hydrogen atom H or a C1-4 alkyl residue.

[0103] 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);



R¹-CO-NH-(CH₂)₃-N⁺(CH₃)₂-CH₂CH(OH)CH₂SO₃⁻ (Id) in which R¹ as the same meaning as in formula I. Particularly preferred betaines are the Carbobetaine [wherein Y=COO⁻], in particular the Carbobetaine of the formula (Ia) and (Ib), more preferred are the Alkylamidobetaine of the formula (Ib).

[0104] Examples of suitable betaines and sulfobetaine are the following [designated in accordance with INCI]: Almondamidopropyl of betaines, Apricotamidopropyl betaines, Avocamidopropyl of betaines, Babassuamidopropyl of betaines, Behenamidopropyl betaines, Behenyl of betaines, betaines, Canolamidopropyl betaines, Capryl/Capramidopropyl betaines, Carnitine, Cetyl 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 Acetoxypromyl 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

[0105] 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

[0106] 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.

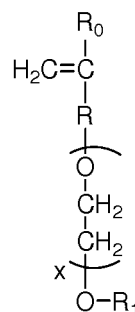
Polymers

[0107] 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.

[0108] 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.

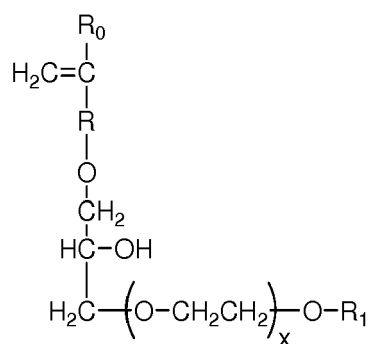
[0109] Other suitable carboxylate polymers are co-polymers comprising: (i) from 50 to less than 98 wt% structural units derived from one or more monomers comprising carboxyl groups; (ii) from 1 to less than 49 wt% structural units derived from one or more monomers comprising sulfonate moieties; and (iii) 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.

[0110] The composition may comprise one or more amphiphilic cleaning polymers such as the compound having the following general structure: $bis((C_2H_5O)(C_2H_4O)_n)(CH_3)-N^+-C_xH_{2x}-N^+-(CH_3)-bis((C_2H_5O)(C_2H_4O)_n)$, 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.

[0111] 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%.

[0112] 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.

[0113] 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.

[0114] 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%.

[0115] 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%.

[0116] Preferably the composition comprises one or more soil release polymers.

[0117] 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.

[0118] 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.

[0119] 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.

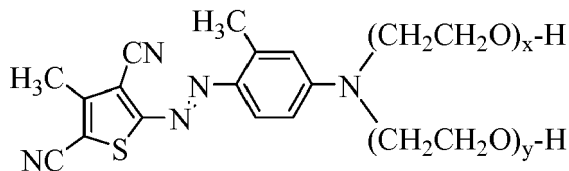
Amines

[0120] 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

[0121] 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

[0122] 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

[0123] 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

[0124] 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.

[0125] 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.

[0126] 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.

[0127] 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

[0128] The present disclosure relates to methods of using the cleaning compositions 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.

[0129] The present invention also provides use of a composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity, wherein the enzyme is a member of a glycoside hydrolase family GH 39 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 has at least 60% identity or 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 and up to less than or 100% identity to SEQ ID NO: 1. Typically contact of the glycoside hydrolase enzyme with the surface will be in a laundering process in which the glycoside hydrolase enzyme or composition comprising the glycoside hydrolase enzyme is mixed with water to provide an aqueous (wash) liquor which is contacted with the surface.

[0130] 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 laundering solutions. 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 laundering solutions can comprise amounts sufficient to form from about 500 to 25,000 ppm, or from 500 to 15,000 ppm of composition in aqueous liquor, or from about 1,000 to 5,000 ppm or 3000ppm of the cleaning compositions herein will be provided in aqueous liquor.

[0131] Typically, the aqueous liquor is formed by contacting the detergent with wash water in such an amount so that the concentration of the detergent in the aqueous 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 laundry detergent composition in the aqueous 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.

[0132] 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 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 wash 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 a fabric, the water to fabric ratio is typically from about 1:1 to about 30:1. Typically the wash 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.

[0133] 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.

[0134] 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

[0135] 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.

[0136] 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	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>
	% 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
Optical Brightener 1	0.20	0.25	0.01	0.01	0.50

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(continued)

<u>Ingredients</u>	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>
	% weight of compartment				
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
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				

<u>Ingredient</u>	<u>F</u>	<u>G</u>	<u>H</u>	<u>I</u>	<u>J</u>	<u>K</u>
	% 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					

[0137] Based on total cleaning and/or treatment composition/compartment weight. Enzyme levels are reported as raw material.

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Examples 19 to 24

[0138] Granular laundry detergent compositions for hand washing or washing machines, typically top-loading washing machines.

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Ingredient	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>
	% 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	0.007	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
Sulfate/Moisture	Balance					

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Examples 25-30

[0139] Granular laundry detergent compositions typically for front-loading automatic washing machines.

Ingredient	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>
	% 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
Chelant 2	0.2	-	0.2	-	0.2	0.2
MgSO ₄	-	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	-	-

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(continued)

Ingredient	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>
	% weight					
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.

[0140]

Ingredients	<u>31</u>	<u>32</u>	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>37</u>
	% weight						
AE _{1.8} S	6.77	5.16	1.36	1.30	-	-	-
AE ₃ 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 ₁₂₋₁₄ dimethyl Amine Oxide	0.30	0.73	0.23	0.37	-	-	-
C ₁₂₋₁₈ 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
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
Polymer 2	0.30	0.33	0.23	0.17	-	-	-
Polymer 3	-	-	-	-	-	-	0.80

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(continued)

	Ingredients	<u>31</u>	<u>32</u>	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>37</u>
		% weight						
5	Polymer 4	0.80	0.81	0.60	0.40	1.00	1.00	-
	1,2-Propanediol	-	6.60	-	3.30	0.50	2.00	8.00
	Structurant	0.10	-	-	-	-	-	0.10
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
	Protease	0.80	0.60	0.70	0.90	0.70	0.60	1.50
15	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
	Amylase 2	-	0.20	0.10	0.15	0.07	-	0.10
	Xyloglucanase	0.20	0.10	-	-	0.05	0.05	0.20
20	Lipase	0.40	0.20	0.30	0.10	0.20	-	-
	Polishing enzyme	-	0.04	-	-	-	0.004	-
	Nuclease	0.05	0.03	0.01	0.03	0.03	0.003	0.003
25	Dispersin B	-	-	-	0.05	0.03	0.001	0.001
	Acid Violet 50	0.05	-	-	-	-	-	0.005
	Direct Violet 9	-	-	-	-	-	0.05	-
	Violet DD	-	0.035	0.02	0.037	0.04	-	-
30	Water insoluble plant fiber	0.2	-	-	-	1.2	-	-
	Dye control agent	-	0.3	-	0.5	-	0.3	-
	Alkoxylated polyaryl/ polyalkyl phenol	-	-	1.2	-	-	-	3.1
35	Water, dyes & minors	Balance						
	pH	8.2						

[0141] Based on total cleaning and/or treatment composition weight. Unless indicated otherwise, enzyme levels are reported as raw material.

40

AE1.8S is C₁₂₋₁₅ alkyl methoxy sulfate with an average degree of ethoxylation of 1.8
 AE3S is C₁₂₋₁₅ alkyl ethoxy sulfate with an av degree of ethoxylation of 3
 AE7 is C₁₂₋₁₃ alcohol ethoxylate, with an average degree of ethoxylation of 7
 AE8 is C₁₂₋₁₃ alcohol ethoxylate, with an average degree of ethoxylation of 8
 45 AE9 is C₁₂₋₁₃ alcohol ethoxylate, with an average degree of ethoxylation of 9
 Alkoxylated polyaryl is alkoxylated polyaryl/polyalkyl phenol for example Emulsogen® TS160, Hostapal®
 / polyalkyl phenol 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
 50 Amylase 3 is Stainzyme® Plus, 20 mg active/g,
 AS is C₁₂₋₁₄ alkylsulfate
 Cellulase 2 is Celluclean™, 15.6 mg active/g
 Xyloglucanase is Whitezyme®, 20mg active/g
 Chelant 1 is diethylene triamine pentaacetic acid
 55 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

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	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®).
	Dye Control Agent	is for example Suparex® O.IN (M1), Nylofixan® P (M2), Nylofixan® PM (M3), or Nylofixan® HF (M4)
5	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 ₉ -C ₁₅ (HLAS is acid form).
	Lipase	is Lipex®, 18 mg active/g
	Mannanase	is Mannaway®, 25 mg active/g
10	Nuclease	is a Phosphodiesterase according to any of SEQ ID NOs: 2 to 6, preferably SEQ ID NO: 2, 3 and/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
	Optical Brightener 2	is disodium 4,4'-bis-(2-sulfostyryl)biphenyl (sodium salt)
	Optical Brightener 3	is Optiblanc SPL10® from 3V Sigma
15	Perfume encapsulate	is a core-shell melamine formaldehyde perfume microcapsules
	Photobleach	is a sulfonated zinc phthalocyanine
	Polishing enzyme	is Para-nitrobenzyl esterase, reported as 1000mg active/g
	Polyetheramine	as described in present disclosure.
20	Polymer 1	is bis((C ₂ H ₅ O)(C ₂ H ₄ O)n)(CH ₃)-N ⁺ -C _x H _{2x} -N ⁺ -(CH ₃)-bis((C ₂ H ₅ O)(C ₂ H ₄ O)n), wherein n = 20-30, x = 3 to 8 or sulphated or sulfonated variants thereof
	Polymer 2	is ethoxylated (EO ₁₅) tetraethylene pentamine
	Polymer 3	is ethoxylated polyethylenimine
	Polymer 4	is ethoxylated hexamethylene diamine
	Polymer 5	is Acusol 305, provided by Rohm&Haas
25	Polymer 6	is a polyethylene glycol polymer grafted with vinyl acetate side chains, provided by BASF.
	Protease	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 C12-14 Dimethylhydroxyethyl ammonium chloride
30	S-ACMC	is Reactive Blue 19 Azo-CM-Cellulose provided by Megazyme
	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

35 **[0142]** 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

<110> P&G

<120> CLEANING COMPOSITIONS INCLUDING ENZYMES

<130> CM04646FM

<160> 26

<170> PatentIn version 3.5

<210> 1

<211> 412

<212> PRT

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Lys Gln Ile Asp Arg Leu Gln Asp Leu Gly Leu Glu Trp Val Arg Leu
 35 40 45

Asp Leu His Trp Asp Arg Leu Glu Thr Ala Glu Asp Gln Tyr Gln Leu
 50 55 60

Ala Ser Leu Asp Gln Leu Val Lys Asp Leu Glu Ala Arg Gln Leu Lys
 65 70 75 80

Ser Val Phe Tyr Leu Val Gly Ser Ala Arg Phe Ile Thr Thr Ala Pro
 85 90 95

Phe Tyr Ser Pro Phe Gln Asp Gln Tyr Pro Pro Arg Asp Pro Glu Val
 100 105 110

Phe Ala Arg Arg Met Ala Met Leu Ser Gln Arg Tyr Pro Ser Val Ala
 115 120 125

Ala Trp Gln Val Trp Asn Glu Pro Asn Leu Ile Gly Phe Trp Arg Pro
 130 135 140

Lys Ala Asp Pro Glu Gly Tyr Ala Lys Leu Leu Gln Ala Ser Thr Ile
 145 150 155 160

Ala Leu Arg Met Val Asp Pro Glu Lys Pro Val Val Ser Ala Gly Met
 165 170 175

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	Glu	Leu	Gln	Asp	Ile	Ile	Gly	Val	Glu	Gly	Gln	Ala	Asp	Tyr	Val	Leu	
20				260					265					270			
	Arg	Arg	Leu	Ala	Leu	Met	Ser	Ala	Leu	Asp	Tyr	Asp	Arg	Ile	Phe	Leu	
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		290					295					300					
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<213> B. licheniformis

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20	Thr	Ser	Gln	Ala	Asp	Val	Gly	Tyr	Gly	Ala	Tyr	Asp	Leu	Tyr	Asp	Leu	50	55	60	
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5	Thr	Val	Ala	Glu	Tyr	Trp	Gln	Asn	Asp	Leu	Gly	Ala	Leu	Glu	Asn	Tyr	
				260					265					270			
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	Tyr	Gln	Phe	His	Ala	Ala	Ser	Thr	Gln	Gly	Gly	Gly	Tyr	Asp	Met	Arg	
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	Phe	Asp	His	His	Asp	Ile	Val	Gly	Trp	Thr	Arg	Glu	Gly	Asp	Ser	Ser	
					405					410					415		
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	Ala	Lys	Arg	Met	Tyr	Val	Gly	Arg	Gln	Asn	Ala	Gly	Glu	Thr	Trp	His	
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<212> PRT

<213> Bacillus sp.

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

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	Ile	Lys	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Ile	Asn	His	Val	Arg	Ser	Ala	
					245					250					255		
5	Thr	Gly	Lys	Asn	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	
				260					265					270			
10	Gly	Ala	Ile	Glu	Asn	Tyr	Leu	Asn	Lys	Thr	Asn	Trp	Asn	His	Ser	Val	
			275					280					285				
15	Phe	Asp	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Lys	Ser	Gly	
		290					295					300					
20	Gly	Asn	Tyr	Asp	Met	Arg	Gln	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg	
	305					310					315				320		
25	His	Pro	Met	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	
					325					330					335		
30	Glu	Glu	Ala	Leu	Glu	Ser	Phe	Val	Glu	Glu	Trp	Phe	Lys	Pro	Leu	Ala	
				340					345					350			
35	Tyr	Ala	Leu	Thr	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	
			355					360					365				
40	Gly	Asp	Tyr	Tyr	Gly	Ile	Pro	Thr	His	Gly	Val	Pro	Ala	Met	Lys	Ser	
		370					375					380					
45	Lys	Ile	Asp	Pro	Ile	Leu	Glu	Ala	Arg	Gln	Lys	Tyr	Ala	Tyr	Gly	Arg	
	385					390					395					400	
50	Gln	Asn	Asp	Tyr	Leu	Asp	His	His	Asn	Ile	Ile	Gly	Trp	Thr	Arg	Glu	
					405					410					415		
55	Gly	Asn	Thr	Ala	His	Pro	Asn	Ser	Gly	Leu	Ala	Thr	Ile	Met	Ser	Asp	
				420					425					430			
60	Gly	Ala	Gly	Gly	Asn	Lys	Trp	Met	Phe	Val	Gly	Arg	Asn	Lys	Ala	Gly	
			435					440					445				
65	Gln	Val	Trp	Thr	Asp	Ile	Thr	Gly	Asn	Arg	Ala	Gly	Thr	Val	Thr	Ile	
		450				455						460					
70	Asn	Ala	Asp	Gly	Trp	Gly	Asn	Phe	Ser	Val	Asn	Gly	Gly	Ser	Val	Ser	
	465					470					475					480	
75	Ile	Trp	Val	Asn	Lys												

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485

5 <210> 4
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 <212> PRT
 <213> Bacillus sp.
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10 His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His
 1 5 10 15

15 Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser
 20 25 30

20 Asn Leu Arg Asn Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp
 35 40 45

25 Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
 50 55 60

30 Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
 65 70 75 80

35 Thr Arg Ser Gln Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly
 85 90 95

40 Val Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
 100 105 110

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

50 Gln Glu Ile Ser Gly Asp Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
 130 135 140

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

His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Gln Phe Gln Asn Arg
 165 170 175

Ile Tyr Lys Phe Arg Gly Asp Gly Lys Ala Trp Asp Trp Glu Val Asp
 180 185 190

Ser Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met
 195 200 205

Asp His Pro Glu Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr

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	210					215						220					
5	Thr	Asn	Thr	Leu	Asn	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His	
	225					230					235					240	
	Ile	Lys	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Leu	Thr	His	Val	Arg	Asn	Ala	
10					245					250					255		
	Thr	Gly	Lys	Glu	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	
				260					265					270			
15	Gly	Ala	Leu	Glu	Asn	Tyr	Leu	Asn	Lys	Thr	Asn	Trp	Asn	His	Ser	Val	
			275					280					285				
	Phe	Asp	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Asn	Ser	Gly	
20		290					295					300					
	Gly	Asn	Tyr	Asp	Met	Ala	Lys	Leu	Leu	Asn	Gly	Thr	Val	Val	Gln	Lys	
25	305					310					315				320		
	His	Pro	Met	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	
					325					330					335		
30	Gly	Glu	Ser	Leu	Glu	Ser	Phe	Val	Gln	Glu	Trp	Phe	Lys	Pro	Leu	Ala	
				340					345					350			
	Tyr	Ala	Leu	Ile	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	
35			355					360					365				
	Gly	Asp	Tyr	Tyr	Gly	Ile	Pro	Thr	His	Ser	Val	Pro	Ala	Met	Lys	Ala	
		370					375					380					
40	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	
45					405					410					415		
	Gly	Asn	Thr	Thr	His	Pro	Asn	Ser	Gly	Leu	Ala	Thr	Ile	Met	Ser	Asp	
50				420					425					430			
	Gly	Pro	Gly	Gly	Glu	Lys	Trp	Met	Tyr	Val	Gly	Gln	Asn	Lys	Ala	Gly	
			435					440					445				
55	Gln	Val	Trp	His	Asp	Ile	Thr	Gly	Asn	Lys	Pro	Gly	Thr	Val	Thr	Ile	
		450					455					460					

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

5 Ile Trp Val Lys Arg
485

10 <210> 5
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<213> Bacillus sp.

<400> 5

15 His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
1 5 10 15

20 Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Asn Ser Asp Ala Ser
20 25 30

25 Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp
35 40 45

Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
50 55 60

30 Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
65 70 75 80

35 Thr Arg Ser Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly
85 90 95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
100 105 110

40 Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn
115 120 125

45 Gln Glu Val Thr Gly Glu Tyr Thr Ile Glu Ala Trp Thr Arg Phe Asp
130 135 140

50 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
165 170 175

55 Ile Tyr Lys Phe Arg Gly His Gly Lys Ala Trp Asp Trp Glu Val Asp
180 185 190

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

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

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

Trp Val Asn Lys

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<211> 515

<212> PRT

15 <213> Geobacillus Stearothermophilus

<400> 6

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Ala Ala Pro Phe Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr Leu
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Pro Asp Asp Gly Thr Leu Trp Thr Lys Val Ala Asn Glu Ala Asn Asn
20 25 30

25

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

30

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

35

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

Lys Ala Gln Tyr Leu Gln Ala Ile Gln Ala Ala His Ala Ala Gly Met
85 90 95

40

Gln Val Tyr Ala Asp Val Val Phe Asp His Lys Gly Gly Ala Asp Gly
100 105 110

45

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

50

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

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

55

Phe Asp Gly Val Asp Trp Asp Glu Ser Arg Lys Leu Ser Arg Ile Tyr
165 170 175

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	Lys	Phe	Arg	Gly	Ile	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Asp	Thr	Glu	180	185	190
5	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Leu	Asp	Met	Asp	His	195	200	205
10	Pro	Glu	Val	Val	Thr	Glu	Leu	Lys	Asn	Trp	Gly	Lys	Trp	Tyr	Val	Asn	210	215	220
	Thr	Thr	Asn	Ile	Asp	Gly	Phe	Arg	Leu	Asp	Ala	Val	Lys	His	Ile	Lys	225	230	235
15	Phe	Ser	Phe	Phe	Pro	Asp	Trp	Leu	Ser	Tyr	Val	Arg	Ser	Gln	Thr	Gly	245	250	255
20	Lys	Pro	Leu	Phe	Thr	Val	Gly	Glu	Tyr	Trp	Ser	Tyr	Asp	Ile	Asn	Lys	260	265	270
25	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
30	Phe	Asp	Met	Arg	Thr	Leu	Met	Thr	Asn	Thr	Leu	Met	Lys	Asp	Gln	Pro	305	310	315
35	Thr	Leu	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Thr	Glu	Pro	Gly	Gln	325	330	335
40	Ala	Leu	Gln	Ser	Trp	Val	Asp	Pro	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	340	345	350
	Phe	Ile	Leu	Thr	Arg	Gln	Glu	Gly	Tyr	Pro	Cys	Val	Phe	Tyr	Gly	Asp	355	360	365
45	Tyr	Tyr	Gly	Ile	Pro	Gln	Tyr	Asn	Ile	Pro	Ser	Leu	Lys	Ser	Lys	Ile	370	375	380
50	Asp	Pro	Leu	Leu	Ile	Ala	Arg	Arg	Asp	Tyr	Ala	Tyr	Gly	Thr	Gln	His	385	390	395
	Asp	Tyr	Leu	Asp	His	Ser	Asp	Ile	Ile	Gly	Trp	Thr	Arg	Glu	Gly	Val	405	410	415
55	Thr	Glu	Lys	Pro	Gly	Ser	Gly	Leu	Ala	Ala	Leu	Ile	Thr	Asp	Gly	Pro	420	425	430

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Gly Gly Ser Lys Trp Met Tyr Val Gly Lys Gln His Ala Gly Lys Val
435 440 445

5 Phe Tyr Asp Leu Thr Gly Asn Arg Ser Asp Thr Val Thr Ile Asn Ser
450 455 460

10 Asp Gly Trp Gly Glu Phe Lys Val Asn Gly Gly Ser Val Ser Val Trp
465 470 475 480

15 Val Pro Arg Lys Thr Thr Val Ser Thr Ile Ala Arg Pro Ile Thr Thr
485 490 495

20 Arg Pro Trp Thr Gly Glu Phe Val Arg Trp Thr Glu Pro Arg Leu Val
500 505 510

25 Ala Trp Pro
515

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<400> 7

30 Ala Ala Pro Phe Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr Leu
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35 Pro Asp Asp Gly Thr Leu Trp Thr Lys Val Ala Asn Glu Ala Asn Asn
20 25 30

40 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
50 55 60

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

Lys Ala Gln Tyr Leu Gln Ala Ile Gln Ala Ala His Ala Ala Gly Met
85 90 95

50 Gln Val Tyr Ala Asp Val Val Phe Asp His Lys Gly Gly Ala Asp Gly
100 105 110

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

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

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	370		375		380
5	Pro 385	Leu Leu Ile Ala	Arg Arg Asp 390	Tyr Ala Tyr 395	Gly Thr Gln His Asp 400
10		Tyr Leu Asp His 405	Ser Asp Ile Ile	Gly Trp Thr 410	Arg Glu Gly Val Thr 415
15	Glu Lys Pro 420	Gly Ser Gly Leu Ala 425	Ala Leu Ile Thr	Asp Gly Pro Gly 430	
20	Gly Ser Lys 435	Trp Met Tyr Val 440	Gly Lys Gln His Ala 445	Gly Lys Val Phe	
25	Tyr Asp Leu Thr 450	Gly Asn Arg Ser Asp 455	Thr Val Thr Ile Asn Ser Asp 460		
30	Gly Trp Gly Glu Phe 465	Lys Val Asn Gly Gly 470	Ser Val Ser Val Trp Val 475		
35	Pro Arg Lys Thr Thr 485				
40	<210> 8	<211> 483	<212> PRT	<213> Artificial	
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60	Leu Pro Asn Asp Gly 20	Asn His Trp Asn Arg Leu Asn Ser Asp Ala Ser 30			
65	Asn Leu Lys 35	Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp 45			
70	Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr 50				
75	Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly 65				
80	Thr Arg Ser Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly 85				

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	Ile	Gln	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	Gly	Gly	Ala	Asp	
				100					105						110		
5	Ala	Thr	Glu	Met	Val	Arg	Ala	Val	Glu	Val	Asn	Pro	Asn	Asn	Arg	Asn	
			115					120					125				
10	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	
20	His	Phe	Asp	Gly	Val	Asp	Trp	Asp	Gln	Ser	Arg	Arg	Leu	Asn	Asn	Arg	
					165					170					175		
25	Ile	Tyr	Lys	Phe	Arg	Gly	Lys	Ala	Trp	Asp	Trp	Glu	Val	Asp	Thr	Glu	
				180					185					190			
30	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Ile	Asp	Met	Asp	His	
			195					200					205				
35	Pro	Glu	Val	Val	Asn	Glu	Leu	Arg	Asn	Trp	Gly	Val	Trp	Tyr	Thr	Asn	
		210					215					220					
40	Thr	Leu	Gly	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His	Ile	Lys	
	225					230					235					240	
45	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Ile	Asn	His	Val	Arg	Ser	Ala	Thr	Gly	
					245					250					255		
50	Lys	Asn	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	Gly	Ala	
				260					265					270			
55	Ile	Glu	Asn	Tyr	Leu	Gln	Lys	Thr	Asn	Trp	Asn	His	Ser	Val	Phe	Asp	
		275					280						285				
60	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Lys	Ser	Gly	Gly	Asn	
		290					295					300					
65	Tyr	Asp	Met	Arg	Asn	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg	His	Pro	
	305					310					315					320	
70	Ser	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	Glu	Glu	
					325					330					335		
75	Ala	Leu	Glu	Ser	Phe	Val	Glu	Glu	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	

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	340		345		350
5	Leu Thr Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr Gly Asp	355	360	365	
10	Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Arg Ser Lys Ile	370	375	380	
15	Asp Pro Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Pro Gln His	385	390	395	400
20	Asp Tyr Leu Asp His Pro Asp Val Ile Gly Trp Thr Arg Glu Gly Asp	405	410	415	
25	Ser Ser His Pro Lys Ser Gly Leu Ala Thr Leu Ile Thr Asp Gly Pro	420	425	430	
30	Gly Gly Ser Lys Arg Met Tyr Ala Gly Leu Lys Asn Ala Gly Glu Thr	435	440	445	
35	Trp Tyr Asp Ile Thr Gly Asn Arg Ser Asp Thr Val Lys Ile Gly Ser	450	455	460	
40	Asp Gly Trp Gly Glu Phe His Val Asn Asp Gly Ser Val Ser Ile Tyr	465	470	475	480
45	Val Gln Lys				
50	<210> 9				
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	Asp Gly Ala His Trp Asn Arg Leu Asn Asn Asp Ala Gln Asn Leu Lys	20	25	30	
	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				

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

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	Ala	Val	Thr	Phe	Val	Glu	Asn	His	Asp	Thr	Gln	Pro	Thr	Gln	Ala	Leu
					325					330					335	
5	Gln	Ser	Thr	Val	Gln	Ser	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	Thr	Ile
				340					345					350		
10	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Cys	Val	Phe	Tyr	Gly	Asp	Tyr	Tyr
			355					360					365			
15	Gly	Thr	Ser	Asp	Gly	Lys	Ile	Ser	Ser	Tyr	Lys	Pro	Ile	Met	Asp	Lys
		370					375					380				
20	Leu	Leu	Asn	Ala	Arg	Lys	Val	Tyr	Ala	Tyr	Gly	Thr	Gln	Arg	Asp	Tyr
	385					390					395					400
25	Phe	Asp	His	Pro	Asp	Ile	Val	Gly	Trp	Thr	Arg	Glu	Gly	Asp	Ala	Ala
					405					410					415	
30	His	Ala	Gly	Ser	Gly	Leu	Ala	Thr	Leu	Ile	Thr	Asp	Gly	Pro	Gly	Gly
				420					425					430		
35	Ser	Lys	Trp	Met	Tyr	Val	Gly	Thr	Ser	Lys	Ala	Gly	Gln	Val	Trp	Thr
			435					440					445			
40	Asp	Lys	Thr	Gly	Asn	Arg	Ser	Gly	Thr	Val	Thr	Ile	Asp	Ala	Asn	Gly
		450					455					460				
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				20					25					30		
65	Ser	Ser	Val	Gly	Ile	Thr	Ala	Val	Trp	Thr	Pro	Pro	Ala	Tyr	Lys	Gly
			35					40					45			
70	Thr	Ser	Gln	Ala	Asp	Val	Gly	Tyr	Gly	Pro	Tyr	Asp	Leu	Tyr	Asp	Leu
		50					55					60				

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	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Val	Arg	Thr	Lys	Tyr	Gly	Thr	Lys	65	70	75	80
5	Gly	Glu	Leu	Lys	Ser	Ala	Val	Asn	Thr	Leu	His	Ser	Asn	Gly	Ile	Gln		85	90	95
10	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	Ala	Gly	Ala	Asp	Tyr	Thr		100	105	110
15	Glu	Asn	Val	Thr	Ala	Val	Glu	Val	Asn	Pro	Ser	Asn	Arg	Asn	Gln	Glu		115	120	125
20	Thr	Ser	Gly	Glu	Tyr	Asn	Ile	Gln	Ala	Trp	Thr	Gly	Phe	Asn	Phe	Pro		130	135	140
25	Gly	Arg	Gly	Thr	Thr	Tyr	Ser	Asn	Phe	Lys	Trp	Gln	Trp	Phe	His	Phe		145	150	155
30	Asp	Gly	Thr	Asp	Trp	Asp	Gln	Ser	Arg	Ser	Leu	Ser	Arg	Ile	Phe	Lys		165	170	175
35	Phe	Thr	Gly	Lys	Ala	Trp	Asp	Trp	Pro	Val	Ser	Ser	Glu	Asn	Gly	Asn		180	185	190
40	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Tyr	Asp	Tyr	Asp	His	Pro	Asp	Val		195	200	205
45	Val	Asn	Glu	Met	Lys	Lys	Trp	Gly	Val	Trp	Tyr	Ala	Asn	Glu	Val	Gly		210	215	220
50	Leu	Asp	Gly	Tyr	Arg	Leu	Asp	Ala	Val	Lys	His	Ile	Lys	Phe	Ser	Phe		225	230	235
55	Leu	Lys	Asp	Trp	Val	Asp	Asn	Ala	Arg	Ala	Ala	Thr	Gly	Lys	Glu	Met		245	250	255
	Phe	Thr	Val	Gly	Glu	Tyr	Trp	Gln	Asn	Asp	Leu	Gly	Ala	Leu	Asn	Asn		260	265	270
	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		290	295	300
	Arg	Asn	Ile	Leu	Asn	Asn	Thr	Leu	Val	Ala	Ser	Asn	Pro	Thr	Lys	Ala		305	310	315
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Val Thr Leu Val Glu Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu
325 330 335

5 Ser Thr Val Gln Pro Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu
340 345 350

10 Thr Arg Ser Gly Gly Tyr Pro Ser Val Phe Tyr Gly Asp Met Tyr Gly
355 360 365

15 Thr Lys Gly Thr Thr Thr Arg Glu Ile Pro Ala Leu Lys Ser Lys Ile
370 375 380

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

20 Asp Tyr Ile Asp Asn Pro Asp Val Ile Gly Trp Thr Arg Glu Gly Asp
405 410 415

25 Ser Thr Lys Ala Lys Ser Gly Leu Ala Thr Val Ile Thr Asp Gly Pro
420 425 430

Gly Gly Ser Lys Arg Met Tyr Val Gly Thr Ser Asn Ala Gly Glu Ile
435 440 445

30 Trp Tyr Asp Leu Thr Gly Asn Asn Ser Thr Lys Ile Thr Ile Gly Ser
450 455 460

35 Asp Gly Tyr Ala Thr Phe Pro Val Asn Lys Gly Ser Val Ser Val Trp
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Val Gln Gln

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

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

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	Ser	Arg	Pro	Glu	Gly	Gly	Gly	Asn	Gly	Val	Arg	Phe	Pro	Gly	Lys	Ser	305	310	315	320
5	Gln	Ile	Gly	Asp	Arg	Gly	Ser	Ala	Leu	Phe	Glu	Asp	Gln	Ala	Ile	Thr		325	330	335
10	Ala	Val	Asn	Arg	Phe	His	Asn	Val	Met	Ala	Gly	Gln	Pro	Glu	Glu	Leu		340	345	350
15	Ser	Asn	Pro	Asn	Gly	Asn	Asn	Gln	Ile	Phe	Met	Asn	Gln	Arg	Gly	Ser		355	360	365
20	His	Gly	Val	Val	Leu	Ala	Asn	Ala	Gly	Ser	Ser	Ser	Val	Ser	Ile	Asn		370	375	380
25	Thr	Ala	Thr	Lys	Leu	Pro	Asp	Gly	Arg	Tyr	Asp	Asn	Lys	Ala	Gly	Ala		385	390	400
	Gly	Ser	Phe	Gln	Val	Asn	Asp	Gly	Lys	Leu	Thr	Gly	Thr	Ile	Asn	Ala		405	410	415
30	Arg	Ser	Val	Ala	Val	Leu	Tyr	Pro	Asp	Asp	Ile	Ala	Lys	Ala	Pro	His		420	425	430
35	Val	Phe	Leu	Glu	Asn	Tyr	Lys	Thr	Gly	Val	Thr	His	Ser	Phe	Asn	Asp		435	440	445
40	Gln	Leu	Thr	Ile	Thr	Leu	Arg	Ala	Asp	Ala	Asn	Thr	Thr	Lys	Ala	Val		450	455	460
45	Tyr	Gln	Ile	Asn	Asn	Gly	Pro	Glu	Thr	Ala	Phe	Lys	Asp	Gly	Asp	Gln		465	470	475
50	Phe	Thr	Ile	Gly	Lys	Gly	Asp	Pro	Phe	Gly	Lys	Thr	Tyr	Thr	Ile	Met		485	490	495
55	Leu	Lys	Gly	Thr	Asn	Ser	Asp	Gly	Val	Thr	Arg	Thr	Glu	Lys	Tyr	Ser		500	505	510
	Phe	Val	Lys	Arg	Asp	Pro	Ala	Ser	Ala	Lys	Thr	Ile	Gly	Tyr	Gln	Asn		515	520	525
	Pro	Asn	His	Trp	Ser	Gln	Val	Asn	Ala	Tyr	Ile	Tyr	Lys	His	Asp	Gly		530	535	540
	Ser	Arg	Val	Ile	Glu	Leu	Thr	Gly	Ser	Trp	Pro	Gly	Lys	Pro	Met	Thr				

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	545		550		555		560									
5	Lys	Asn	Ala	Asp	Gly	Ile	Tyr	Thr	Leu	Thr	Leu	Pro	Ala	Asp	Thr	Asp
					565					570					575	
10	Thr	Thr	Asn	Ala	Lys	Val	Ile	Phe	Asn	Asn	Gly	Ser	Ala	Gln	Val	Pro
				580					585					590		
15	Gly	Gln	Asn	Gln	Pro	Gly	Phe	Asp	Tyr	Val	Leu	Asn	Gly	Leu	Tyr	Asn
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30	Ala	Glu	Leu	Ser	Gln	Phe	Asp	Trp	Val	Val	Leu	Glu	Ala	Ala	His	Leu
				20					25					30		
35	Lys	Pro	Ala	Asp	Val	Gly	Tyr	Leu	Lys	Glu	Gln	Gly	Ser	Thr	Pro	Phe
			35					40					45			
40	Ala	Tyr	Leu	Ser	Val	Gly	Glu	Phe	Asp	Gly	Asp	Ala	Ala	Ala	Ile	Ala
		50					55					60				
45	Asp	Ser	Gly	Leu	Ala	Arg	Gly	Lys	Ser	Ala	Val	Arg	Asn	Gln	Ala	Trp
	65					70					75					80
50	Asn	Ser	Gln	Val	Met	Asp	Leu	Ala	Ala	Pro	Ser	Trp	Arg	Ala	His	Leu
				85						90					95	
55	Leu	Lys	Arg	Ala	Ala	Glu	Leu	Arg	Lys	Gln	Gly	Tyr	Ala	Gly	Leu	Phe
				100					105					110		
60	Leu	Asp	Thr	Leu	Asp	Ser	Phe	Gln	Leu	Gln	Ala	Glu	Glu	Arg	Arg	Glu
			115					120					125			
65	Gly	Gln	Arg	Arg	Ala	Leu	Ala	Ser	Phe	Leu	Ala	Gln	Leu	His	Arg	Gln
		130					135					140				
70	Glu	Pro	Gly	Leu	Lys	Leu	Phe	Phe	Asn	Arg	Gly	Phe	Glu	Val	Leu	Pro

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	145		150		155		160									
5	Glu	Leu	Pro	Gly	Val	Ala	Ser	Ala	Val	Ala	Val	Glu	Ser	Ile	His	Ala
				165						170					175	
10	Gly	Trp	Asp	Ala	Ala	Ala	Gly	Gln	Tyr	Arg	Glu	Val	Pro	Gln	Asp	Asp
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15	Arg	Asp	Trp	Leu	Lys	Gly	His	Leu	Asp	Ala	Leu	Arg	Ala	Gln	Gly	Met
			195					200					205			
20	Pro	Ile	Val	Ala	Ile	Asp	Tyr	Leu	Pro	Pro	Glu	Arg	Arg	Asp	Glu	Ala
			210				215					220				
25	Arg	Ala	Leu	Ala	Ala	Arg	Leu	Arg	Ser	Glu	Gly	Tyr	Val	Pro	Phe	Val
	225					230					235					240
30	Ser	Thr	Pro	Ala	Leu	Asp	Tyr	Leu	Gly	Val	Ser	Asp	Val	Glu	Val	Gln
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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
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70	Ala	Met	Cys	Glu	Glu	Gly	Gly	Lys	Gly	Ala	Ser	Val	Arg	Tyr	Val	Ser
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75	Ser	Ser	Asp	Asn	Arg	Gly	Ala	Gly	Ser	Trp	Val	Gly	Asn	Arg	Leu	Asn
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Ile Cys Thr Ile Asp Asp Gly Ala Asp Lys Arg Arg Glu Glu Ser Leu
35 40 45

25

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

Ala Val Cys Glu Glu Gly Gly Ala Gly Ala Asp Val Arg Tyr Val Thr
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30

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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35

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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
50 55 60

55

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

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20 25 30

Ile Leu Cys Lys Gly Ala Pro Asn Val Leu Gln Arg Val Asn Glu Lys
35 40 45

25 Thr Lys Asn Ser Asn Arg Asp Arg Ser Gly Ala Asn Lys Gly Pro Phe
50 55 60

30 Lys Asp Pro Gln Lys Trp Gly Ile Lys Ala Leu Pro Pro Lys Asn Pro
65 70 75 80

35 Ser Trp Ser Ala Gln Asp Phe Lys Ser Pro Glu Glu Tyr Ala Phe Ala
85 90 95

Ser Ser Leu Gln Gly Gly Thr Asn Ala Ile Leu Ala Pro Val Asn Leu
100 105 110

40 Ala Ser Gln Asn Ser Gln Gly Gly Val Leu Asn Gly Phe Tyr Ser Ala
115 120 125

45 Asn Lys Val Ala Gln Phe Asp Pro Ser Lys Pro Gln Gln Thr Lys Gly
130 135 140

50 Thr Trp Phe Gln Ile Thr Lys Phe Thr Gly Ala Ala Gly Pro Tyr Cys
145 150 155 160

Lys Ala Leu Gly Ser Asn Asp Lys Ser Val Cys Asp Lys Asn Lys Asn
165 170 175

55 Ile Ala Gly Asp Trp Gly Phe Asp Pro Ala Lys Trp Ala Tyr Gln Tyr
180 185 190

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Asp Glu Lys Asn Asn Lys Phe Asn Tyr Val Gly Lys
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10 <400> 17

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20 25 30

20 Thr Gly Tyr Ser Arg Asp Leu Phe Pro Thr Trp Asp Ala Ile Ser Gly
35 40 45

Asn Cys Asn Ala Arg Glu Tyr Val Leu Lys Arg Asp Gly Glu Gly Val
50 55 60

25 Gln Val Asn Asn Ala Cys Glu Ser Gln Ser Gly Thr Trp Ile Ser Pro
65 70 75 80

30 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
115 120 125

40 Trp Ala Val Ser Ala Ser Ala Asn Arg Ser Lys Gly Asp Arg Ser Pro
130 135 140

45 Asp Gln Trp Lys Pro Pro Leu Thr Ser Phe Tyr Cys Thr Tyr Ala Lys
145 150 155 160

Ser Trp Ile Asp Val Lys Ser Phe Tyr Lys Leu Thr Ile Thr Ser Ala
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<212> PRT

<213> Streptomyces davawensis

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

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Gly Arg Thr Leu Pro Gly Leu Gly Leu Asn Ile Ala Arg Tyr Asn Leu
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Gly Ala Cys Ser Trp Asn Ser Val Ser Gly Glu Ser Met Val Ala Ser
65 70 75 80

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

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Asn Asn Glu Asp Pro Thr Ser Ser Ala Trp Lys Trp Thr Ala Asp Ala
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Ala Gln Arg Thr Met Leu Val Lys Ala Thr Ala Arg Gly Ala Thr Thr
115 120 125

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

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Pro Ser Gly Ala Ser Gly Gly Gly Asn Asn Leu Gln Ser Trp Asn Tyr
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

45

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

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Ala Ser Val Gln Ala Ala Val Leu Pro Tyr Leu Arg Ser Glu Leu Asp
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Arg Arg Gly Leu Thr Gly Thr Lys Ile Ser Ala Ser Asp Glu Thr Ser
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55

Tyr Asp Leu Ala Arg Thr Thr Trp Gly Ser Phe Gly Ser Ser Thr Lys

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5	Ala Leu Val Asn Arg Val Asn Val His Gly Tyr Gln Gly Ser Gly Gly 260 265 270		
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20	Ala Ser Asn Leu Cys Leu Asp Phe Arg Trp Leu His Pro Thr Ala Trp 305 310 315 320		
25	Val Tyr Trp Gln Val Met Asp Pro Ser Ser Gly Trp Ala Met Ile Ala 325 330 335		
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35	Val Met Ala Gln Phe Ser Arg His Ile Arg Ala Gly Met Thr Ile Val 355 360 365		
40	Asp Thr Gly Val Gly Tyr Ala Ala Ala Ala Tyr Asp Ala Thr Ala Arg 370 375 380		
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25	Ser	Ser	Ile	Lys	Pro	Ser	Arg	Gln	Val	Asp	Gly	Tyr	Trp	Leu	Asp	Trp
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50	Gln	Asn	His	Ala	Val	Tyr	Leu	Ala	Asn	Ile	Ala	Gln	His	Ala	Gln	Gln
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55	Asn	Trp	Gly	Ile	Gln	Phe	Gln	Ser	Val	Glu	Ala	Phe	Asn	Glu	Pro	Ser
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75	Leu	Ala	Ile	Ser	Thr	Trp	Gln	Gly	Leu	Gly	Ser	Ser	Ala	Gln	Asn	Ala
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			275					280					285			
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15	Leu	Leu	Leu	Asp	Phe	Thr	Trp	Leu	His	Pro	Thr	Ala	Trp	Val	Tyr	Trp
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20	Gln	Ala	Ile	Asp	Gly	Ser	Gly	Trp	Gly	Leu	Ile	Val	Gly	Asp	Asn	Asp
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25	Gln	Leu	Thr	Leu	Ser	Ser	Ala	Ser	Thr	Lys	Tyr	Phe	Val	Leu	Ala	Gln
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40	Val	Ala	Ala	Asn	Trp	Gly	Ser	Ala	Gln	Thr	Ile	Thr	Phe	Asp	Leu	Thr
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45	Arg	Ala	Lys	Thr	Ala	Gly	Ser	Asn	Gly	Ala	Thr	Val	Pro	Arg	Trp	Ser
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50	Thr	Gln	Thr	Ser	Gly	Gly	Asp	Gln	Tyr	Lys	Ser	Tyr	Ser	Asp	Thr	Lys
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20	Leu	Pro	Glu	Thr	Thr	Glu	Trp	Ala	Thr	Val	Asn	Ala	Gly	Gln	Ala	Leu	
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25	Leu	Asn	Ala	Gly	Ser	Asn	Thr	Ile	Lys	Leu	His	Asn	Asn	Trp	Gly	Trp	
				100					105					110			
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			115					120					125				
35	His	Gln	Val	Thr	Asp	Ala	Leu	Val	Asn	Thr	Asn	Ser	Asn	Ala	Val	Thr	
		130					135					140					
40	Lys	Gln	Leu	Met	Lys	Phe	Leu	Val	Ser	Lys	Tyr	His	Lys	Ala	Tyr	Ile	
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45	Thr	Gly	Gln	Gln	Glu	Leu	His	Ala	His	Gln	Trp	Val	Glu	Lys	Asn	Val	
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50	Gly	Lys	Ser	Pro	Ala	Ile	Leu	Gly	Leu	Asp	Phe	Met	Asp	Tyr	Ser	Pro	
				180					185					190			
55	Ser	Arg	Val	Glu	Phe	Gly	Thr	Thr	Ser	Gln	Ala	Val	Glu	Gln	Ala	Ile	
			195					200					205				
60	Asp	Phe	Asp	Lys	Arg	Gly	Gly	Ile	Val	Thr	Phe	Ala	Trp	His	Trp	Asn	
	210						215					220					
65	Ala	Pro	Ser	Gly	Leu	Ile	Asn	Thr	Pro	Gly	Ser	Glu	Trp	Trp	Arg	Gly	
	225					230					235					240	
70	Phe	Tyr	Thr	Glu	His	Thr	Thr	Phe	Asp	Val	Ala	Ala	Ala	Leu	Gln	Asn	
					245					250					255		
75	Thr	Thr	Asn	Ala	Asn	Tyr	Asn	Leu	Leu	Ile	Arg	Asp	Ile	Asp	Ala	Ile	
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		290					295					300					
10	Gly	Pro	Glu	Pro	Ala	Lys	Lys	Leu	Tyr	Lys	Ile	Leu	Tyr	Asp	Arg	Leu	
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20	Asp	Ser	Tyr	Pro	Ala	Gln	Pro	Gly	Asp	His	Gly	Pro	Val	Ser	Ala	Gln	
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30	Ser	Tyr	Trp	Ser	Phe	Phe	Val	Thr	Trp	Glu	Gly	Glu	Phe	Ile	Glu	Asn	
					405					410					415		
35	Gly	Val	His	Asn	Ser	Leu	Glu	Phe	Leu	Lys	Lys	Leu	Tyr	Asn	Asn	Ser	
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25 Ala Ala Ile Tyr Gly Asp Lys Arg Thr Ser Val Val Leu Asn Gly Gly
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30 Ala Ser Ser Glu Val Tyr Phe Pro Ala Gly Glu Thr Trp Thr Asn Val
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35 Ala Ala Gly Gln Leu Leu Leu Asn Gln Gly Ser Asn Thr Ile Asp Ile
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Val Ser Asn Trp Gly Trp Tyr Leu Ile Asp Ser Ile Thr Leu Thr Pro
115 120 125

40 Ser Thr Pro Arg Pro Ala His Gln Ile Asn Glu Ala Pro Val Asn Ala
130 135 140

45 Ala Ala Asp Lys Asn Ala Lys Ala Leu Tyr Ser Tyr Leu Arg Ser Ile
145 150 155 160

50 Tyr Gly Lys Lys Ile Leu Ser Gly Gln Gln Glu Leu Ser Leu Ser Asn
165 170 175

Trp Ile Ala Gln Gln Thr Gly Lys Thr Pro Ala Leu Val Ser Val Asp
180 185 190

55 Leu Met Asp Tyr Ser Pro Ser Arg Val Glu Arg Gly Thr Val Gly Thr
195 200 205

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	His	Arg	Trp	Trp	Ser	Gly	Phe	Tyr	Thr	Ser	Ala	Thr	Asp	Phe	Asp	Val	
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	Ala	Ala	Ala	Leu	Ser	Ser	Thr	Thr	Asn	Ala	Asn	Tyr	Thr	Leu	Leu	Ile	
				260					265					270			
15	Arg	Asp	Ile	Asp	Ala	Ile	Ala	Val	Gln	Leu	Lys	Arg	Leu	Gln	Ser	Ala	
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	Gly	Val	Pro	Val	Leu	Phe	Arg	Pro	Leu	His	Glu	Ala	Glu	Gly	Gly	Trp	
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	Phe	Trp	Trp	Gly	Ala	Lys	Gly	Pro	Glu	Pro	Ala	Lys	Lys	Leu	Trp	Gly	
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					325					330					335		
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				340					345					350			
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	Ser	Thr	Gln	Tyr	Asn	Gln	Leu	Ile	Glu	Leu	Gly	Lys	Asp	Lys	Lys	Met	
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	Thr	Ser	Asp	Tyr	Val	Leu	Thr	Leu	Asp	Glu	Ile	Gln	Gly	Trp	Gln	Gly	
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20	Tyr Thr Cys Lys Tyr Gln Asn Asp Trp Tyr Ser Gln Cys Leu 515 520 525		
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45	Asp Lys Tyr Thr Asn Val Val Leu Asn Asn Ala Ala Gly Ser Gln Val 65 70 75 80		
50	Ser Leu Pro Ala Thr Thr Ala Trp Thr Thr Val Pro Ala Gly Gln Val 85 90 95		
55	Leu Leu Asn Ala Gly Ala Asn Thr Ile Gln Ile Gln Asn Asn Trp Gly 100 105 110		
	Trp Tyr Leu Val Asp Ser Ile Ser Leu Lys Pro Ala Ala Thr Arg Gly 115 120 125		
	Ala His Gln Ile Thr Thr Lys Pro Val Asn Lys Asn Ala Asn Ser Asp 130 135 140		
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15	Ile	Ala	Phe	Ala	Lys	Lys	Gly	Gly	Ile	Val	Thr	Phe	Cys	Trp	His	Trp
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	Gly	Phe	Tyr	Thr	Asp	Ala	Thr	Asp	Phe	Asn	Ile	Glu	Thr	Ala	Leu	Lys
					245					250					255	
25	Asp	Thr	Thr	Asn	Ala	Asn	Tyr	Thr	Leu	Leu	Met	Lys	Asp	Ile	Asp	Thr
				260					265					270		
30	Ile	Ala	Val	Gln	Leu	Lys	Lys	Leu	Gln	Asp	Ala	Gly	Val	Pro	Val	Ile
			275					280					285			
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	Lys	Gly	Pro	Glu	Pro	Ala	Lys	Lys	Leu	Trp	Lys	Ile	Met	Tyr	Asp	Arg
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					325					330					335	
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 45 Val Ile His Lys Thr Asn Gly Asp Glu
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Claims

1. A cleaning composition comprising an amylase enzyme and an enzyme having glycoside hydrolase activity and selected from glycoside hydrolase family GH 39.
2. A cleaning composition comprising an amylase enzyme and a glycoside hydrolase enzyme having glycoside hydrolase activity and 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 to SEQ ID NO:1.
3. A cleaning composition according to claim 1 or claim 2 wherein the glycoside hydrolase comprises PslGh.
4. A cleaning composition according to any preceding claim wherein the glycoside hydrolase enzyme is obtainable from *Pseudomonas*, preferably from *Pseudomonas aeruginosa*.
5. A cleaning composition according to any preceding claim wherein the glycoside hydrolase enzyme is an isolated glycoside hydrolase.
6. A cleaning composition according to any preceding claim wherein the composition further comprises additional enzyme selected from galactanases, mannanases, nucleases, and mixtures thereof.

7. A cleaning composition according to claim 6 wherein the composition additionally comprises a nuclease enzyme, preferably a deoxyribonuclease enzyme.
8. A cleaning composition according to any preceding claim wherein the composition further comprises one or preferably two or more, preferably three or more additional enzymes selected from lipases, proteases, pectate lyases, cellulases, cutinases, and mixtures thereof.
9. A cleaning composition according to any preceding claim wherein the composition further comprises a β -N-acetylglucosaminidase enzyme from E.C. 3.2.1.52, preferably an enzyme having at least 70% identity to SEQ ID NO: 26.
10. 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.
11. A cleaning composition according to claim 10 wherein the surfactant system additionally comprises a nonionic surfactant, preferably the weight ratio of the anionic to nonionic surfactant is from 25:1 to 1:2.
12. A cleaning composition according to claim 10 or claim 11 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.
13. 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 500pom.
14. Use of an enzyme having glycoside hydrolase activity and belonging to the GH family 39, 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.
15. Use according to claim 14 wherein the glycoside hydrolase enzyme has 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 to SEQ ID NO:1.



EUROPEAN SEARCH REPORT

Application Number
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A	US 2010/125047 A1 (LANT NEIL JOSEPH [GB]) 20 May 2010 (2010-05-20) * claims; examples *	6-9,11, 12	
A	SHAN YU ET AL: "PslG, a self-produced glycosyl hydrolase, triggers biofilm disassembly by disrupting exopolysaccharide matrix", CELL RESEARCH - XIBAO YANJIU, vol. 25, no. 12, 27 November 2015 (2015-11-27), pages 1352-1367, XP055310902, GB, CN ISSN: 1001-0602, DOI: 10.1038/cr.2015.129 * the whole document *	1-14	
			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 22 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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ON EUROPEAN PATENT APPLICATION NO.**

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The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

22-01-2018

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15			JP 2017524344 A	31-08-2017
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