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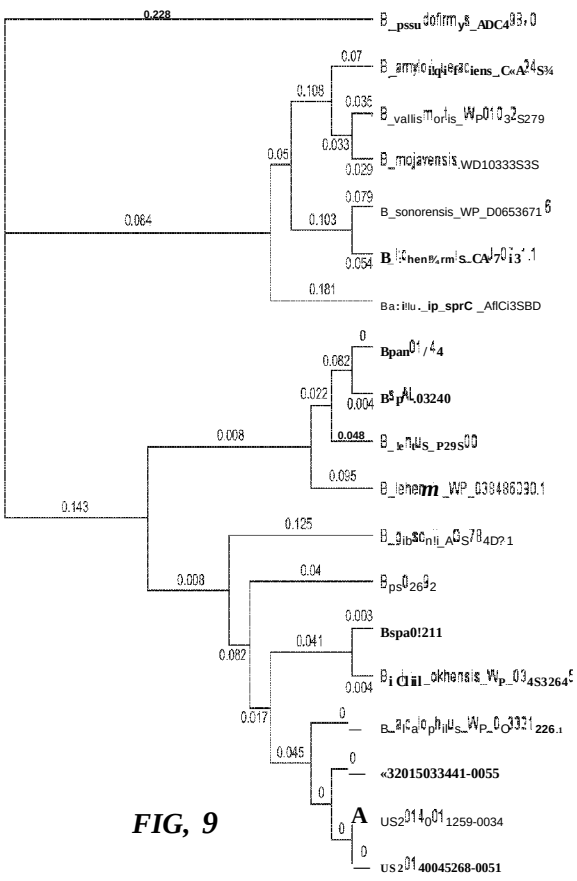
[Continued on nextpage]

(54) Title: SERINE PROTEASES

(57) Abstract: The present disclosure relates to serine proteases and variants thereof. Compositions containing the serine proteases are suitable for use in cleaning fabrics and hard surfaces, as well as in a variety of industrial applications.



FIG, 9

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SERINE PROTEASES

[001] The present disclosure relates to serine proteases cloned from *Bacillus spp.,* and variants thereof. Compositions containing the serine proteases are suitable for use in cleaning fabrics and hard surfaces, as well as in a variety of industrial applications.

[002] Serine proteases are enzymes (EC No. 3.4.21) possessing an active site serine that initiates hydrolysis of peptide bonds of proteins. There are two broad categories of serine proteases, based on their structure: chymotrypsin-like (trypsin-like) and subtilisin-like. The prototypical subtilisin (EC No. 3.4.21.62) was initially obtained from *Bacillus subtilis.* Subtilisins and their homologues are members of the S8 peptidase family of the MEROPS classification scheme. Members of family S8 have a catalytic triad in the order Asp, His and Ser in their amino acid sequence.

[003] Although serine proteases have long been known in the art of industrial enzymes, there remains a need for further serine proteases that are suitable for particular conditions and uses.

[004] The present compositions and methods relate to recombinant serine proteases cloned from *Bacillus spp.,* and variants thereof. Compositions containing the serine proteases are suitable for use in cleaning fabrics and hard surfaces, as well as in a variety of industrial applications.

[005] In some embodiments, the invention is a recombinant polypeptide or active fragment thereof comprising an amino acid sequence having at least 93% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:3, 6, 9, and 12. In some embodiments, the recombinant polypeptide has cleaning activity in a detergent composition, including an automatic dish washing detergent and a laundry detergent.

[006] In some embodiments, the invention is a composition comprising a surfactant and the recombinant polypeptide stated above. In some embodiments, the surfactant is selected from the group consisting of a non-ionic surfactant, an anionic surfactant, a cationic surfactant, a zwitterionic surfactant, an ampholytic surfactant, a semi-polar non-ionic surfactant, and a combination thereof. In some embodiments, the composition is a detergent composition, such as a laundry detergent, a fabric softening detergent, a dishwashing detergent, and a hard-surface cleaning detergent. In some embodiments, the composition further comprises at least one calcium ion and/or zinc ion, at least one stabilizer, at least one bleaching agent, phosphate, or

borate. In some embodiments the composition is phosphate-free and/or borate-free. In some embodiments, the composition is a granular, powder, solid, bar, liquid, tablet, gel, paste or unit dose composition. In some embodiments, the composition further comprising one or more additional enzymes or enzyme derivatives selected from the group consisting of acyl transferases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinosidases, aryl esterases, beta-galactosidases, carrageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1, 4-glucanases, endo-beta-mannanases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipoxygenases, mannanases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases, peroxidases, phenoloxidases, phosphatases, phospholipases, phytases, polygalacturonases, proteases, pullulanases, reductases, rhamnogalacturonases, beta-glucanases, tannases, transglutaminases, xylan acetyl-esterases, xylanases, xyloglucanases, xylosidases, metalloproteases, additional serine proteases, and combinations thereof.

[007] In some embodiments, the invention is a method of cleaning, comprising contacting a surface or an item with a composition listed above. In some embodiments, the invention is a method for producing a recombinant polypeptide comprising stably transforming a host cell with an expression vector comprising a polynucleotide encoding the recombinant polypeptide above.

BRIEF DESCRIPTION OF THE DRAWINGS

[008] Figure 1 provides a plasmid map for expression of Bpan01744 protease.

[009] Figure 2 provides a plot of the cleaning performance of Bpan01744, BspAL03240, Bps02592 and BspQ01211 in HDL OMO detergent.

[0010] Figure 3 provides a plot of the cleaning performance of Bpan01744, BspAL03240, Bps02592 and BspQ01211 in HDD OMO detergent.

[0011] Figure 4 provides a plot of the cleaning performance of Bpan01744, BspAL03240, Bps02592 and BspQ01211 in ADW GSMB-pH9 detergent, unrinsed.

[0012] Figure 5 provides a plot of the cleaning performance of Bpan01744, BspAL03240, Bps02592 and BspQ01211 in ADW GSMB-pH9 detergent, rinsed.

[0013] Figure 6 provides a plot of the cleaning performance of Bpan01744, BspAL03240, Bps02592 and BspQ01211 in ADW GSMB-pHIO detergent, unrinsed.

[0014] Figure 7 provides a plot of the cleaning performance of Bpan01744, BspAL03240, Bps02592 and BspQ01211 in ADW GSMB-pHIO detergent, rinsed.

[0015] Figure 8.1 to 8.3 provide MUSCLE multiple sequence alignment of subtilisins including Bpan01744, BspAL03240, Bps02592 and BspQ01211.

[0016] Figure 9 provides phylogenetic tree of subtilisins including Bpan01744, BspAL03240, Bps02592 and BspQ01211.

[0017] Described are compositions and methods relating to recombinant serine proteases from *Bacillus* species. The compositions and methods are based, in part, on the observation that recombinant Bpan01744, BspAL03240, Bps02592 and BspQ01211, among others, have protease activity in the presence of a surfactant, in basic reaction conditions, and at elevated temperatures. These features of Bpan01744, BspAL03240, Bps02592 and BspQ01211 make these proteases well suited for use in cleansing fabrics and hard surfaces, as well as in textile, leather and feather processing. The new proteases are also well suited to inclusion in compositions for protein degradation, including but not limited to laundry and dish washing detergents.

[0018] Prior to describing the present compositions and methods in detail, the following terms are defined for clarity. Terms and abbreviations not defined should be accorded their ordinary meaning as used in the art. Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Unless otherwise indicated, the practice of the present disclosure involves conventional techniques commonly used in molecular biology, protein engineering, and microbiology. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present disclosure, some suitable methods and materials are described herein. The terms defined immediately below are more fully described by reference to the Specification as a whole.

[0019] As used herein, the singular "a," "an" and "the" includes the plural unless the context clearly indicates otherwise. Unless otherwise indicated, nucleic acid sequences are written left to right in 5' to 3' orientation; and amino acid sequences are written left to right in amino to carboxy orientation. It is to be understood that this disclosure is not limited to the particular methodology, protocols, and reagents described herein, absent an indication to the contrary.

[0020] It is intended 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.

[0021] As used herein in connection with a numerical value, the term "about" refers to a range of +/- 0.5 of the numerical value, unless the term is otherwise specifically defined in context. For instance, the phrase a "pH value of about 6" refers to pH values of from 5.5 to 6.5, unless the pH value is specifically defined otherwise.

[0022] As used herein, the terms "protease" and "proteinase" refer to an enzyme that has the ability to break down proteins and peptides. A protease has the ability to conduct "proteolysis," by hydrolysis of peptide bonds that link amino acids together in a peptide or polypeptide chain forming the protein. This activity of a protease as a protein-digesting enzyme is referred to as "proteolytic activity." Many well-known procedures exist for measuring proteolytic activity. For example, proteolytic activity may be ascertained by comparative assays that analyze the respective protease' s ability to hydrolyze a suitable substrate. Exemplary substrates useful in the analysis of protease or proteolytic activity, include, but are not limited to, di-methyl casein (Sigma C-9801), bovine collagen (Sigma C-9879), bovine elastin (Sigma E-1625), and bovine keratin (ICN Biomedical 902111). Colorimetric assays utilizing these substrates are well known in the art (See e.g., WO 99/34011 and U.S. Pat. No. 6,376,450). The pNA peptidyl assay (See e.g., Del Mar et al., Anal Biochem, 99:316-320, 1979) also finds use in determining the active enzyme concentration. This assay measures the rate at which p-nitroaniline is released as the enzyme hydrolyzes a soluble synthetic substrate, such as succinyl-alanine-alanine-proline-phenylalanine-p-nitroanilide (suc-AAPF-pNA). The rate of production of yellow color from the hydrolysis reaction is measured at 410 nm on a spectrophotometer and is proportional to the active enzyme concentration. In addition, absorbance measurements at 280 nanometers (nm) can be used to determine the total protein concentration in a sample of purified protein. The activity on substrate/protein concentration gives the enzyme specific activity.

[0023] The term "variant," with respect to a polypeptide, refers to a polypeptide that differs from a specified wild-type, parental, or reference polypeptide in that it includes one or more naturally-occurring or man-made substitutions, insertions, or deletions of an amino acid. Similarly, the term "variant," with respect to a polynucleotide, refers to a polynucleotide that differs in nucleotide sequence from a specified wild-type, parental, or reference polynucleotide.

The identity of the wild-type, parental, or reference polypeptide or polynucleotide will be apparent from context.

[0024] As used herein, "the genus Bacillus" includes all species within the genus "Bacillus," as known to those of skill in the art, including but not limited to *B. subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. clausii, B. halodurans, B. megaterium, B. coagulans, B. circulans, B. gibsonii,* and *B. thuringiensis*. It is recognized that the genus *Bacillus* continues to undergo taxonomical reorganization. Thus, it is intended that the genus include species that have been reclassified, including but not limited to such organisms as *B. stearothermophilus,* which is now named *"Geobacillus stearothermophilus",* or *B. polymyxa,* which is now *"Paenibacillus polymyxa".* The production of resistant endospores under stressful environmental conditions is considered the defining feature of the genus *Bacillus,* although this characteristic also applies to the recently named *Alicyclobacillus, Amphibacillus, Aneurinibacillus, Anoxybacillus, Brevibacillus, Filobacillus, Gracilibacillus, Halobacillus, Paenibacillus, Salibacillus, Thermobacillus, Ureibacillus,* and *Virgibacillus.*

[0025] As used herein, the term "mutation" refers to changes made to a reference amino acid or nucleic acid sequence. It is intended that the term encompass substitutions, insertions and deletions.

[0026] As used herein, the term "vector" refers to a nucleic acid construct used to introduce or transfer nucleic acid(s) into a target cell or tissue. A vector is typically used to introduce foreign DNA into a cell or tissue. Vectors include plasmids, cloning vectors, bacteriophages, viruses (e.g., viral vector), cosmids, expression vectors, shuttle vectors, and the like. A vector typically includes an origin of replication, a multicloning site, and a selectable marker. The process of inserting a vector into a target cell is typically referred to as transformation. The present invention includes, in some embodiments, a vector that comprises a DNA sequence encoding a serine protease polypeptide (e.g., precursor or mature serine protease polypeptide) that is operably linked to a suitable prosequence (e.g., secretory, signal peptide sequence, etc.) capable of effecting the expression of the DNA sequence in a suitable host, and the folding and translocation of the recombinant polypeptide chain.

[0027] As used herein in the context of introducing a nucleic acid sequence into a cell, the term "introduced" refers to any method suitable for transferring the nucleic acid sequence into

the cell. Such methods for introduction include but are not limited to protoplast fusion, transfection, transformation, electroporation, conjugation, and transduction. Transformation refers to the genetic alteration of a cell which results from the uptake, optional genomic incorporation, and expression of genetic material (e.g., DNA).

[0028] As used herein, a nucleic acid is "operably linked" with another nucleic acid sequence when it is placed into a functional relationship with another nucleic acid sequence. For example, a promoter or enhancer is operably linked to a nucleotide coding sequence if the promoter affects the transcription of the coding sequence. A ribosome binding site may be operably linked to a coding sequence if it is positioned so as to facilitate translation of the coding sequence. Typically, "operably linked" DNA sequences are contiguous. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers may be used in accordance with conventional practice.

[0029] As used herein the term "gene" refers to a polynucleotide (e.g., a DNA segment), that encodes a polypeptide and includes regions preceding and following the coding regions. In some instances a gene includes intervening sequences (introns) between individual coding segments (exons).

[0030] As used herein, "recombinant" when used with reference to a cell typically indicates that the cell has been modified by the introduction of a foreign nucleic acid sequence or that the cell is derived from a cell so modified. For example, a recombinant cell may comprise a gene not found in identical form within the native (non-recombinant) form of the cell, or a recombinant cell may comprise a native gene (found in the native form of the cell) that has been modified and re-introduced into the cell. A recombinant cell may comprise a nucleic acid endogenous to the cell that has been modified without removing the nucleic acid from the cell; such modifications include those obtained by gene replacement, site-specific mutation, and related techniques known to those of ordinary skill in the art. Recombinant DNA technology includes techniques for the production of recombinant DNA in vitro and transfer of the recombinant DNA into cells where it may be expressed or propagated, thereby producing a recombinant polypeptide. "Recombination" and "recombining" of polynucleotides or nucleic acids refer generally to the assembly or combining of two or more nucleic acid or polynucleotide strands or fragments to generate a new polynucleotide or nucleic acid.

[0031] A nucleic acid or polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods known to those of skill in the art, it can be transcribed and/or translated to produce the polypeptide or a fragment thereof. The anti-sense strand of such a nucleic acid is also said to encode the sequence.

[0032] The terms "host strain" and "host cell" refer to a suitable host for an expression vector comprising a DNA sequence of interest.

[0033] A "protein" or "polypeptide" comprises a polymeric sequence of amino acid residues. The terms "protein" and "polypeptide" are used interchangeably herein. The single and 3-letter code for amino acids as defined in conformity with the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) is used throughout this disclosure. The single letter X refers to any of the twenty amino acids. It is also understood that a polypeptide may be coded for by more than one nucleotide sequence due to the degeneracy of the genetic code. Mutations can be named by the one letter code for the parent amino acid, followed by a position number and then the one letter code for the variant amino acid. For example, mutating glycine (G) at position 87 to serine (S) is represented as "G087S" or "G87S". When describing modifications, a position followed by amino acids listed in parentheses indicates a list of substitutions at that position by any of the listed amino acids. For example, 6(L,I) means position 6 can be substituted with a leucine or isoleucine. At times, in a sequence, a slash (/) is used to define substitutions, e.g. F/V, indicates that the particular position may have a phenylalanine or valine at that position.

[0034] A "prosequence" or "propeptide sequence" refers to an amino acid sequence between the signal peptide sequence and mature protease sequence that is necessary for the proper folding and secretion of the protease; they are sometimes referred to as intramolecular chaperones. Cleavage of the prosequence or propeptide sequence results in a mature active protease. Bacterial serine proteases are often expressed as pro-enzymes.

[0035] The terms "signal sequence" and "signal peptide" refer to a sequence of amino acid residues that may participate in the secretion or direct transport of the mature or precursor form of a protein. The signal sequence is typically located N-terminal to the precursor or mature protein sequence. The signal sequence may be endogenous or exogenous. A signal sequence is normally absent from the mature protein. A signal sequence is typically cleaved from the protein by a signal peptidase after the protein is transported.

[0036] The term "mature" form of a protein, polypeptide, or peptide refers to the functional

form of the protein, polypeptide, or peptide without the signal peptide sequence and propeptide sequence.

[0037] The term "precursor" form of a protein or peptide refers to a mature form of the protein having a prosequence operably linked to the amino or carbonyl terminus of the protein. The precursor may also have a "signal" sequence operably linked to the amino terminus of the prosequence. The precursor may also have additional polypeptides that are involved in post-translational activity (e.g., polypeptides cleaved therefrom to leave the mature form of a protein or peptide).

[0038] The term "wild-type" in reference to an amino acid sequence or nucleic acid sequence indicates that the amino acid sequence or nucleic acid sequence is a native or naturally-occurring sequence. As used herein, the term "naturally-occurring" refers to anything (e.g., proteins, amino acids, or nucleic acid sequences) that is found in nature. Conversely, the term "non-naturally occurring" refers to anything that is not found in nature (e.g., recombinant nucleic acids and protein sequences produced in the laboratory or modification of the wild-type sequence).

[0039] As used herein with regard to amino acid residue positions, "corresponding to" or "corresponds to" or "corresponds" refers to an amino acid residue at the enumerated position in a protein or peptide, or an amino acid residue that is analogous, homologous, or equivalent to an enumerated residue in a protein or peptide. As used herein, "corresponding region" generally refers to an analogous position in a related proteins or a reference protein.

[0040] The terms "derived from" and "obtained from" refer to not only a protein produced or producible by a strain of the organism in question, but also a protein encoded by a DNA sequence isolated from such strain and produced in a host organism containing such DNA sequence. Additionally, the term refers to a protein which is encoded by a DNA sequence of synthetic and/or cDNA origin and which has the identifying characteristics of the protein in question. To exemplify, "proteases derived from *Bacillus"* refers to those enzymes having proteolytic activity that are naturally produced by *Bacillus,* as well as to serine proteases like those produced by *Bacillus* sources but which through the use of genetic engineering techniques are produced by other host cells transformed with a nucleic acid encoding the serine proteases.

[0041] The term "identical" in the context of two polynucleotide or polypeptide sequences refers to the nucleic acids or amino acids in the two sequences that are the same when aligned for maximum correspondence, as measured using sequence comparison or analysis algorithms

described below and known in the art.

[0042] As used herein, "% identity" or percent identity" or "PID" refers to protein sequence identity. Percent identity may be determined using standard techniques known in the art. Useful algorithms include the BLAST algorithms (See, Altschul et al., J Mol Biol, 215:403-410, 1990; and Karlin and Altschul, Proc Natl Acad Sci USA, 90:5873-5787, 1993). The BLAST program uses several search parameters, most of which are set to the default values. The NCBI BLAST algorithm finds the most relevant sequences in terms of biological similarity but is not recommended for query sequences of less than 20 residues (Altschul et al., Nucleic Acids Res, 25:3389-3402, 1997; and Schaffer et al., Nucleic Acids Res, 29:2994-3005, 2001). Exemplary default BLAST parameters for a nucleic acid sequence searches include: Neighboring words threshold = 11; E-value cutoff = 10; Scoring Matrix = NUC.3.1 (match = 1, mismatch = -3);Gap Opening = 5; and Gap Extension = 2. Exemplary default BLAST parameters for amino acid sequence searches include: Word size = 3; E-value cutoff = 10; Scoring Matrix = BLOSUM62; Gap Opening = 11; and Gap extension = 1. A percent (%) amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "reference" sequence including any gaps created by the program for optimal/maximum alignment. BLAST algorithms refer to the "reference" sequence as the "query" sequence.

[0043] As used herein, "homologous proteins" or "homologous proteases" refers to proteins that have distinct similarity in primary, secondary, and/or tertiary structure. Protein homology can refer to the similarity in linear amino acid sequence when proteins are aligned. Homologous search of protein sequences can be done using BLASTP and PSI-BLAST from NCBI BLAST with threshold (E-value cut-off) at 0.001. (Altschul SF, Madde TL, Shaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI BLAST a new generation of protein database search programs. Nucleic Acids Res 1997 Set 1; 25(17):3389-402). Using this information, proteins sequences can be grouped. A phylogenetic tree can be built using the amino acid sequences. Amino acid sequences can be entered in a program such as the Vector NTI Advance suite and a Guide Tree can be created using the Neighbor Joining (NJ) method (Saitou and Nei, Mol Biol Evol, 4:406-425, 1987). The tree construction can be calculated using Kimura's correction for sequence distance and ignoring positions with gaps. A program such as AlignX can display the calculated distance values in parenthesis following the molecule name displayed on the phylogenetic tree.

[0044] Understanding the homology between molecules can reveal the evolutionary history of the molecules as well as information about their function; if a newly sequenced protein is homologous to an already characterized protein, there is a strong indication of the new protein's biochemical function. The most fundamental relationship between two entities is homology; two molecules are said to be homologous if they have been derived from a common ancestor. Homologous molecules, or homologs, can be divided into two classes, paralogs and orthologs. Paralogs are homologs that are present within one species. Paralogs often differ in their detailed biochemical functions. Orthologs are homologs that are present within different species and have very similar or identical functions. A protein superfamily is the largest grouping (clade) of proteins for which common ancestry can be inferred. Usually this common ancestry is based on sequence alignment and mechanistic similarity. Superfamilies typically contain several protein families which show sequence similarity within the family. The term "protein clan" is commonly used for protease superfamilies based on the MEROPS protease classification system.

[0045] The CLUSTAL W algorithm is another example of a sequence alignment algorithm (See, Thompson et al., Nucleic Acids Res, 22:4673-4680, 1994). Default parameters for the CLUSTAL W algorithm include: Gap opening penalty = 10.0; Gap extension penalty = 0.05; Protein weight matrix = BLOSUM series; DNA weight matrix = IUB; Delay divergent sequences % = 40; Gap separation distance = 8; DNA transitions weight = 0.50; List hydrophilic residues = GPSNDQEKR; Use negative matrix = OFF; Toggle Residue specific penalties = ON; Toggle hydrophilic penalties = ON; and Toggle end gap separation penalty = OFF. In CLUSTAL algorithms, deletions occurring at either terminus are included. For example, a variant with a five amino acid deletion at either terminus (or within the polypeptide) of a polypeptide of 500 amino acids would have a percent sequence identity of 99% (495/500 identical residues x 100) relative to the "reference" polypeptide. Such a variant would be encompassed by a variant having "at least 99% sequence identity" to the polypeptide.

[0046] A nucleic acid or polynucleotide is "isolated" when it is at least partially or completely separated from other components, including but not limited to for example, other proteins, nucleic acids, cells, etc. Similarly, a polypeptide, protein or peptide is "isolated" when it is at least partially or completely separated from other components, including but not limited to for example, other proteins, nucleic acids, cells, etc. On a molar basis, an isolated species is more abundant than are other species in a composition. For example, an isolated species may

comprise at least about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% (on a molar basis) of all macromolecular species present. Preferably, the species of interest is purified to essential homogeneity (i.e., contaminant species cannot be detected in the composition by conventional detection methods). Purity and homogeneity can be determined using a number of techniques well known in the art, such as agarose or polyacrylamide gel electrophoresis of a nucleic acid or a protein sample, respectively, followed by visualization upon staining. If desired, a high-resolution technique, such as high performance liquid chromatography (HPLC) or a similar means can be utilized for purification of the material.

[0047] The term "purified" as applied to nucleic acids or polypeptides generally denotes a nucleic acid or polypeptide that is essentially free from other components as determined by analytical techniques well known in the art (e.g., a purified polypeptide or polynucleotide forms a discrete band in an electrophoretic gel, chromatographic eluate, and/or a media subjected to density gradient centrifugation). For example, a nucleic acid or polypeptide that gives rise to essentially one band in an electrophoretic gel is "purified." A purified nucleic acid or polypeptide is at least about 50% pure, usually at least about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.5%, about 99.6%, about 99.7%, about 99.8% or more pure (e.g., percent by weight on a molar basis). In a related sense, a composition is enriched for a molecule when there is a substantial increase in the concentration of the molecule after application of a purification or enrichment technique. The term "enriched" refers to a compound, polypeptide, cell, nucleic acid, amino acid, or other specified material or component that is present in a composition at a relative or absolute concentration that is higher than a starting composition.

[0048] As used herein, the term "functional assay" refers to an assay that provides an indication of a protein's activity. In some embodiments, the term refers to assay systems in which a protein is analyzed for its ability to function in its usual capacity. For example, in the case of a protease, a functional assay involves determining the effectiveness of the protease to hydrolyze a proteinaceous substrate.

[0049] The term "cleaning activity" refers to a cleaning performance achieved by a serine

protease polypeptide or reference protease under conditions prevailing during the proteolytic, hydrolyzing, cleaning, or other process of the disclosure. In some embodiments, cleaning performance of a serine protease polypeptide or reference protease may be determined by using various assays for cleaning one or more various enzyme sensitive stains on an item or surface (e.g., a stain resulting from food, grass, blood, ink, milk, oil, and/or egg protein). Cleaning performance of a variant or reference protease can be determined by subjecting the stain on the item or surface to standard wash condition(s) and assessing the degree to which the stain is removed by using various chromatographic, spectrophotometric, or other quantitative methodologies. Exemplary cleaning assays and methods are known in the art and include, but are not limited to those described in WO99/34011 and US 6,605,458, both of which are herein incorporated by reference, as well as those cleaning assays and methods included in the Examples provided below.

[0050] The term "cleaning effective amount" of a serine protease polypeptide or reference protease refers to the amount of protease that achieves a desired level of enzymatic activity in a specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and are based on many factors, such as the particular protease used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, tablet, bar) composition is required, etc.

[0051] The term "cleaning adjunct material" refers to any liquid, solid, or gaseous material included in cleaning composition other than a serine protease polypeptide of the disclosure. In some embodiments, the cleaning compositions of the present disclosure include one or more cleaning adjunct materials. Each cleaning adjunct material is typically selected depending on the particular type and form of cleaning composition (e.g., liquid, granule, powder, bar, paste, spray, tablet, gel, foam, or other composition). Preferably, each cleaning adjunct material is compatible with the protease enzyme used in the composition.

[0052] Cleaning compositions and cleaning formulations include any composition that is suited for cleaning, bleaching, disinfecting, and/or sterilizing any object, item, and/or surface. Such compositions and formulations include, but are not limited to for example, liquid and/or solid compositions, including cleaning or detergent compositions (e.g., liquid, tablet, gel, bar, granule, and/or solid laundry cleaning or detergent compositions and fine fabric detergent compositions; hard surface cleaning compositions and formulations, such as for glass, wood,

ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; fabric fresheners; fabric softeners; and textile, laundry booster cleaning or detergent compositions, laundry additive cleaning compositions, and laundry pre-spotter cleaning compositions; dishwashing compositions, including hand or manual dishwashing compositions (e.g., "hand" or "manual" dishwashing detergents) and automatic dishwashing compositions (e.g., "automatic dishwashing detergents"). Single dosage unit forms also find use with the present invention, including but not limited to pills, tablets, gelcaps, or other single dosage units such as pre-measured powders or liquids.

[0053] Cleaning composition or cleaning formulations, as used herein, include, unless otherwise indicated, granular or powder-form all-purpose or heavy-duty washing agents, especially cleaning detergents; liquid, granular, gel, solid, tablet, paste, or unit dosage form all-purpose washing agents, especially the so-called heavy-duty liquid (HDL) detergent or heavy-duty dry (HDD) detergent types; liquid fine-fabric detergents; hand or manual dishwashing agents, including those of the high-foaming type; hand or manual dishwashing, automatic dishwashing, or dishware or tableware washing agents, including the various tablet, powder, solid, granular, liquid, gel, and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, including antibacterial hand-wash types, cleaning bars, mouthwashes, denture cleaners, car shampoos, carpet shampoos, bathroom cleaners; hair shampoos and/or hair-rinses for humans and other animals; shower gels and foam baths and metal cleaners; as well as cleaning auxiliaries, such as bleach additives and "stain-stick" or pre-treat types. In some embodiments, granular compositions are in "compact" form; in some embodiments, liquid compositions are in a "concentrated" form.

[0054] As used herein, "fabric cleaning compositions" include hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics (e.g., clothes, linens, and other textile materials).

[0055] As used herein, "non-fabric cleaning compositions" include non-textile (i.e., non-fabric) surface cleaning compositions, including, but not limited to for example, hand or manual or automatic dishwashing detergent compositions, oral cleaning compositions, denture cleaning compositions, contact lens cleaning compositions, wound debridement compositions, and personal cleansing compositions.

[0056] As used herein, the term "detergent composition" or "detergent formulation" is used in reference to a composition intended for use in a wash medium for the cleaning of soiled or dirty objects, including particular fabric and/or non-fabric objects or items. Such compositions of the present disclosure are not limited to any particular detergent composition or formulation. Indeed, in some embodiments, the detergents of the disclosure comprise at least one serine protease polypeptide of the disclosure and, in addition, one or more surfactants, transferase(s), hydrolytic enzymes, oxido reductases, builders (e.g., a builder salt), bleaching agents, bleach activators, bluing agents, fluorescent dyes, caking inhibitors, masking agents, enzyme activators, antioxidants, and/or solubilizers. In some instances, a builder salt is a mixture of a silicate salt and a phosphate salt, preferably with more silicate (e.g., sodium metasilicate) than phosphate (e.g., sodium tripolyphosphate). Some compositions of the disclosure, such as, but not limited to, cleaning compositions or detergent compositions, do not contain any phosphate (e.g., phosphate salt or phosphate builder).

[0057] As used herein, the term "bleaching" refers to the treatment of a material (e.g., fabric, laundry, pulp, etc.) or surface for a sufficient length of time and/or under appropriate pH and/or temperature conditions to effect a brightening (i.e., whitening) and/or cleaning of the material. Examples of chemicals suitable for bleaching include, but are not limited to, for example, $C10_2$, H_20_2, peracids, $N0_2$, etc.

[0058] As used herein, "wash performance" of a protease (e.g., a serine protease polypeptide of the disclosure) refers to the contribution of a serine protease polypeptide to washing that provides additional cleaning performance to the detergent as compared to the detergent without the addition of the serine protease polypeptide to the composition. Wash performance is compared under relevant washing conditions. In some test systems, other relevant factors, such as detergent composition, sud concentration, water hardness, washing mechanics, time, pH, and/or temperature, can be controlled in such a way that condition(s) typical for household application in a certain market segment (e.g., hand or manual dishwashing, automatic dishwashing, dishware cleaning, tableware cleaning, fabric cleaning, etc.) are imitated.

[0059] The term "relevant washing conditions" is used herein to indicate the conditions, particularly washing temperature, time, washing mechanics, sud concentration, type of detergent and water hardness, actually used in households in a hand dishwashing, automatic dishwashing, or laundry detergent market segment.

[0060] As used herein, the term "disinfecting" refers to the removal of contaminants from the surfaces, as well as the inhibition or killing of microbes on the surfaces of items. It is not intended that the present disclosure be limited to any particular surface, item, or contaminant(s) or microbes to be removed.

[0061] The "compact" form of the cleaning compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt. Inorganic filler salts are conventional ingredients of detergent compositions in powder form. In conventional detergent compositions, the filler salts are present in substantial amounts, typically about 17 to about 35% by weight of the total composition. In contrast, in compact compositions, the filler salt is present in amounts not exceeding about 15% of the total composition. In some embodiments, the filler salt is present in amounts that do not exceed about 10%, or more preferably, about 5%, by weight of the composition. In some embodiments, the inorganic filler salts are selected from the alkali and alkaline-earth-metal salts of sulfates and chlorides. In some embodiments, the filler salt is sodium sulfate.

[0062] The present disclosure provides novel serine protease enzymes. The serine protease polypeptides of the present disclosure include isolated, recombinant, substantially pure, or non-naturally occurring polypeptides. In some embodiments, the polypeptides are useful in cleaning applications and can be incorporated into cleaning compositions that are useful in methods of cleaning an item or a surface in need thereof.

[0063] In some embodiments, the polypeptide of the present invention is a polypeptide having a specified degree of amino acid sequence homology to the exemplified polypeptides, e.g., 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 6, 9, and 12. In some embodiments, the polypeptide of the present invention is a polypeptide having a specified degree of amino acid sequence homology to the exemplified polypeptides, e.g., 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 6, 9, and 12. Other embodiments are directed to a recombinant polypeptide or an active fragment thereof comprising an amino acid sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO: 3, 6, or 9. Some embodiments are directed to a recombinant polypeptide or an active

fragment thereof comprising an amino acid sequence having at least 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO: 3, 6, 9, or 12. In further embodiments, the recombinant polypeptide or active fragment thereof comprises an amino acid sequence having at least 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to the amino acid sequence of SEQ ID NO: 3, 6, or 12, with the proviso that the polypeptide or active fragment thereof does not comprise WP_034632645. Some embodiments are directed to a recombinant polypeptide or an active fragment thereof comprising an amino acid sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:3 or 6. Homology can be determined by amino acid sequence alignment, e.g., using a program such as BLAST, ALIGN, MUSCLE, or CLUSTAL, as described herein. In some embodiments, the polypeptide is an isolated, recombinant, substantially pure, or non-naturally occurring enzyme having protease activity, such as subtilisin activity, or casein hydrolysis activity (for example, dimethylcasein hydrolysis activity).

[0064] Also provided is a polypeptide enzyme of the present invention, having protease activity, such as alkaline protease activity, said enzyme comprising an amino acid sequence which differs from the amino acid sequence of SEQ ID NO:3, 6, 9, or 12 by no more than 50, no more than 40, no more than 30, no more than 25, no more than 20, no more than 15, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 amino acid residue(s), when aligned using any of the previously described alignment methods.

[0065] As noted above, the variant enzyme polypeptides of the invention have enzymatic activities (e.g., protease activities) and thus are useful in cleaning applications, including but not limited to, methods for cleaning dishware items, tableware items, fabrics, and items having hard surfaces (e.g., the hard surface of a table, table top, wall, furniture item, floor, ceiling, etc.). Exemplary cleaning compositions comprising one or more variant serine protease enzyme polypeptides of the invention are described infra. The enzymatic activity (e.g., protease enzyme activity) of an enzyme polypeptide of the invention can be determined readily using procedures well known to those of ordinary skill in the art. The Examples presented infra describe methods for evaluating the enzymatic activity and cleaning performance. The performance of polypeptide enzymes of the invention in removing stains (e.g., a protein stain such as blood/milk/ink or egg

yolk), cleaning hard surfaces, or cleaning laundry, dishware or tableware item(s) can be readily determined using procedures well known in the art and/or by using procedures set forth in the Examples.

[0066] The serine protease polypeptides of the present invention can have protease activity over a broad range of pH conditions. In some embodiments, the serine protease polypeptides have protease activity on dimethylcasein as a substrate, as demonstrated in Examples below. In some embodiments, the serine protease polypeptides have protease activity at a pH of from about 4.0 to about 12.0. In some embodiments, the serine protease polypeptides have protease activity at a pH of from about 6.0 to about 12.0. In some embodiments, the serine protease polypeptides have at least 50%, 60%, 70%, 80% or 90% of maximal protease activity at a pH of from about 6.0 to about 12.0, or from about 7.0 to about 12.0. In some embodiments, the serine protease polypeptides have protease activity at a pH above 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0 or 11.5. In some embodiments, the serine protease polypeptides have protease activity at a pH below 12.0, 11.5, 11.0, 10.5, 10.0, 9.5, 9.0, 8.5, 8.0, 7.5, 7.0, or 6.5.

[0067] In some embodiments, the serine protease polypeptides of the present invention have protease activity at a temperature range from about 10°C to about 90°C, or from about 30°C to about 80°C. In some embodiments, the serine protease polypeptides of the present invention have protease activity at a temperature range of from about 55°C to about 75°C. In some embodiments, the serine protease polypeptides have at least 50%, 60%, 70%, 80% or 90% of maximal protease activity at a temperature of from about 55°C to about 75°C. In some embodiments, the serine proteases have activity at a temperature above 50°C, 55°C, 60°C, 65°C, or 70°C. In some embodiments, the serine proteases have activity at a temperature below 80°C, 75°C, 70°C, 65°C, 60°C, or 55°C.

[0068] In some embodiments, the serine protease polypeptides of the present invention demonstrate cleaning performance in a cleaning composition. Cleaning compositions often include ingredients harmful to the stability and performance of enzymes, making cleaning compositions a harsh environment for enzymes, e.g. serine proteases, to retain function. Thus, it is not trivial for an enzyme to be put in a cleaning composition and expect enzymatic function (e.g. serine protease activity, such as demonstrated by cleaning performance). In some embodiments, the serine protease polypeptides of the present invention demonstrate cleaning performance in automatic dishwashing (ADW) detergent compositions. In some embodiments,

the cleaning performance in ADW detergent compositions includes cleaning of egg yolk stains. In some embodiments, the serine protease polypeptides of the present invention demonstrate cleaning performance in laundry detergent compositions. In some embodiments, the cleaning performance in laundry detergent compositions includes cleaning of blood/milk/ink stains. In each of the cleaning compositions, the serine protease polypeptides of the present invention demonstrate cleaning performance with or without a bleach component.

[0069] In some embodiments, the serine protease polypeptides of the present invention have stability in detergent compositions. In some embodiments, the serine protease polypeptides of the present invention have a thermostability $T_{50\%}$ value of at least 60°C.

[0070] A polypeptide of the invention can be subject to various changes, such as one or more amino acid insertions, deletions, and/or substitutions, either conservative or non-conservative, including where such changes do not substantially alter the enzymatic activity of the polypeptide. Similarly, a nucleic acid of the invention can also be subject to various changes, such as one or more substitutions of one or more nucleotides in one or more codons such that a particular codon encodes the same or a different amino acid, resulting in either a silent variation (e.g., when the encoded amino acid is not altered by the nucleotide mutation) or non-silent variation, one or more deletions of one or more nucleic acids (or codons) in the sequence, one or more additions or insertions of one or more nucleic acids (or codons) in the sequence, and/or cleavage of or one or more truncations of one or more nucleic acids (or codons) in the sequence. Many such changes in the nucleic acid sequence may not substantially alter the enzymatic activity of the resulting encoded polypeptide enzyme compared to the polypeptide enzyme encoded by the original nucleic acid sequence. A nucleic acid sequence of the invention can also be modified to include one or more codons that provide for optimum expression in an expression system (e.g., bacterial expression system), while, if desired, said one or more codons still encode the same amino acid(s).

[0071] The invention provides isolated, non-naturally occurring, or recombinant nucleic acids which may be collectively referred to as "nucleic acids of the invention" or "polynucleotides of the invention", which encode polypeptides of the invention. Nucleic acids of the invention, including all described below, are useful in recombinant production (e.g., expression) of polypeptides of the invention, typically through expression of a plasmid expression vector comprising a sequence encoding the polypeptide of interest or fragment

thereof. As discussed above, polypeptides include serine protease polypeptides having enzymatic activity (e.g., proteolytic activity) which are useful in cleaning applications and cleaning compositions for cleaning an item or a surface (e.g., surface of an item) in need of cleaning.

[0072] In some embodiments, the polynucleotide of the present invention is a polynucleotide having a specified degree of nucleic acid homology to the exemplified polynucleotide. In some embodiments, the polynucleotide comprises a nucleic acid sequence having at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the nucleic acid sequence of SEQ ID NO:l, 4, 7, or 10. In some embodiments, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:l, 4, 7, and 10. In other embodiments, the polynucleotide of the present invention may also have a complementary nucleic acid sequence to a nucleic acid sequence selected from the group consisting of SEQ ID NOs:l, 4 and 8. In some embodiments, the polynucleotide comprises a nucleic acid sequence encoding a recombinant polypeptide or an active fragment thereof, comprising an amino acid sequence having at least 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity to the amino acid sequence selected from the group consisting of SEQ ID NOs:3, 6, 9, and 12. In some embodiments, the polynucleotide comprises a nucleic acid sequence encoding a recombinant polypeptide or an active fragment thereof, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3, 6, 9, and 12. Homology can be determined by amino acid sequence alignment, e.g., using a program such as BLAST, ALIGN, MUSCLE, or CLUSTAL, as described herein.

[0073] In some embodiments, the invention provides an isolated, recombinant, substantially pure, synthetically derived, or non-naturally occurring nucleic acid comprising a nucleotide sequence encoding any polypeptide (including any fusion protein, etc.) of the invention described above in the section entitled "Polypeptides of the Invention" and elsewhere herein. The invention also provides an isolated, recombinant, substantially pure, synthetically derived, or non-naturally-occurring nucleic acid comprising a nucleotide sequence encoding a combination of two or more of any polypeptides of the invention described above and elsewhere herein. The present invention provides nucleic acids encoding a serine protease polypeptide of the present invention, wherein the serine protease polypeptide is a mature form having proteolytic activity. In some embodiments, the serine protease is expressed recombinantly with a homologous pro-

peptide sequence. In other embodiments, the serine protease is expressed recombinantly with a heterologous pro-peptide sequence (e.g., GG36 pro-peptide sequence).

[0074] Nucleic acids of the invention can be generated by using any suitable synthesis, manipulation, and/or isolation techniques, or combinations thereof. For example, a polynucleotide of the invention may be produced using standard nucleic acid synthesis techniques, such as solid-phase synthesis techniques that are well-known to those skilled in the art. In such techniques, fragments of up to 50 or more nucleotide bases are typically synthesized, then joined (e.g., by enzymatic or chemical ligation methods) to form essentially any desired continuous nucleic acid sequence. The synthesis of the nucleic acids of the invention can be also facilitated by any suitable method known in the art, including but not limited to chemical synthesis using the classical phosphoramidite method (See e.g., Beaucage et al. Tetrahedron Letters 22:1859-69 [1981]); or the method described by Matthes et al. (See, Matthes et al., EMBO J. 3:801-805 [1984], as is typically practiced in automated synthetic methods. Nucleic acids of the invention also can be produced by using an automatic DNA synthesizer. Customized nucleic acids can be ordered from a variety of commercial sources (e.g., The Midland Certified Reagent Company, the Great American Gene Company, Operon Technologies Inc., and DNA2.0). Other techniques for synthesizing nucleic acids and related principles are known in the art (See e.g., Itakura et al., Ann. Rev. Biochem. 53:323 [1984]; and Itakura et al., Science 198:1056 [1984]).

[0075] As indicated above, recombinant DNA techniques useful in modification of nucleic acids are well known in the art. For example, techniques such as restriction endonuclease digestion, ligation, reverse transcription and cDNA production, and polymerase chain reaction (e.g., PCR) are known and readily employed by those of skill in the art. Nucleotides of the invention may also be obtained by screening cDNA libraries using one or more oligonucleotide probes that can hybridize to or PCR-amplify polynucleotides which encode a serine protease polypeptide polypeptide(s) of the invention. Procedures for screening and isolating cDNA clones and PCR amplification procedures are well known to those of skill in the art and described in standard references known to those skilled in the art. Some nucleic acids of the invention can be obtained by altering a naturally occurring polynucleotide backbone (e.g., that encodes an enzyme or parent protease) by, for example, a known mutagenesis procedure (e.g., site-directed mutagenesis, site saturation mutagenesis, and in vitro recombination). A variety of

methods are known in the art that are suitable for generating modified polynucleotides of the invention that encode serine protease polypeptides of the invention, including, but not limited to, for example, site-saturation mutagenesis, scanning mutagenesis, insertional mutagenesis, deletion mutagenesis, random mutagenesis, site-directed mutagenesis, and directed-evolution, as well as various other recombinatorial approaches.

[0076] The present invention provides vectors comprising at least one serine protease polynucleotide of the invention described herein (e.g., a polynucleotide encoding a serine protease polypeptide of the invention described herein), expression vectors or expression cassettes comprising at least one nucleic acid or polynucleotide of the invention, isolated, substantially pure, or recombinant DNA constructs comprising at least one nucleic acid or polynucleotide of the invention, isolated or recombinant cells comprising at least one polynucleotide of the invention, and compositions comprising one or more such vectors, nucleic acids, expression vectors, expression cassettes, DNA constructs, cells, cell cultures, or any combination or mixtures thereof.

[0077] In some embodiments, the invention provides recombinant cells comprising at least one vector (e.g., expression vector or DNA construct) of the invention which comprises at least one nucleic acid or polynucleotide of the invention. Some such recombinant cells are transformed or transfected with such at least one vector, although other methods are available and known in the art. Such cells are typically referred to as host cells. Some such cells comprise bacterial cells, including, but are not limited to *Bacillus sp.* cells, such as *B. subtilis* cells. The invention also provides recombinant cells (e.g., recombinant host cells) comprising at least one serine protease polypeptide of the invention.

[0078] In some embodiments, the invention provides a vector comprising a nucleic acid or polynucleotide of the invention. In some embodiments, the vector is an expression vector or expression cassette in which a polynucleotide sequence of the invention which encodes a serine protease polypeptide of the invention is operably linked to one or additional nucleic acid segments required for efficient gene expression (e.g., a promoter operably linked to the polynucleotide of the invention which encodes a serine protease polypeptide of the invention). A vector may include a transcription terminator and/or a selection gene, such as an antibiotic resistance gene, that enables continuous cultural maintenance of plasmid-infected host cells by growth in antimicrobial-containing media.

[0079] An expression vector may be derived from plasmid or viral DNA, or in alternative embodiments, contains elements of both. Exemplary vectors include, but are not limited to pC194, pJHIOl, pE194, pHP13 (See, Harwood and Cutting [eds.], Chapter 3, Molecular Biological Methods for Bacillus, John Wiley & Sons [1990]; suitable replicating plasmids for B. subtilis include those listed on p. 92). See also, Perego, Integrational Vectors for Genetic Manipulations in Bacillus subtilis, in Sonenshein et al., [eds.] Bacillus subtilis and Other Gram-Positive Bacteria: Biochemistry, Physiology and Molecular Genetics, American Society for Microbiology, Washington, D.C. [1993], pp. 615-624), and p2JM103BBI.

[0080] For expression and production of a protein of interest (e.g., serine protease polypeptide) in a cell, at least one expression vector comprising at least one copy of a polynucleotide encoding the serine protease polypeptide, and in some instances comprising multiple copies, is transformed into the cell under conditions suitable for expression of the serine protease. In some embodiments of the present invention, a polynucleotide sequence encoding the serine protease polypeptide (as well as other sequences included in the vector) is integrated into the genome of the host cell, while in other embodiments, a plasmid vector comprising a polynucleotide sequence encoding the serine protease polypeptide remains as autonomous extra-chromosomal element within the cell. The invention provides both extrachromosomal nucleic acid elements as well as incoming nucleotide sequences that are integrated into the host cell genome. The vectors described herein are useful for production of the serine protease polypeptides of the invention. In some embodiments, a polynucleotide construct encoding the serine protease polypeptide is present on an integrating vector that enables the integration and optionally the amplification of the polynucleotide encoding the serine protease polypeptide into the host chromosome. Examples of sites for integration are well known to those skilled in the art. In some embodiments, transcription of a polynucleotide encoding a serine protease polypeptide of the invention is effectuated by a promoter that is the wild-type promoter for the selected precursor protease. In some other embodiments, the promoter is heterologous to the precursor protease, but is functional in the host cell. Specifically, examples of suitable promoters for use in bacterial host cells include, but are not limited to, for example, the amyE, amyQ, amyL, pstS, sacB, pSPAC, pAprE, pVeg, pHpall promoters, the promoter of the B. *stearothermophilus* maltogenic amylase gene, the *B. amyloliquefaciens* (BAN) amylase gene, the *B. subtilis* alkaline protease gene, the *B. clausii* alkaline protease gene the *B. pumilis* xylosidase

gene, the *B. thuringiensis* cryIIIA, and the *B. Ucheniformis* alpha-amylase gene. Additional promoters include, but are not limited to the A4 promoter, as well as phage Lambda PR or PL promoters, and the *E. coli* lac, trp or tac promoters.

[0081] Serine protease polypeptides of the present invention can be produced in host cells of any suitable microorganism, including bacteria and fungi. In some embodiments, serine protease polypeptides of the present invention can be produced in Gram-positive bacteria. In some embodiments, the host cells are *Bacillus spp., Streptomyces spp., Escherichia spp., Aspergillus spp., Trichoderma spp., Pseudomonas spp., Corynebacterium spp., Saccharomyces spp.,* or *Pichia spp.* In some embodiments, the serine protease polypeptides are produced by *Bacillus sp.* host cells. Examples of *Bacillus sp.* host cells that find use in the production of the serine protease polypeptides of the invention include, but are not limited to *B. Ucheniformis, B. lentus, B. subtilis, B. amyloliquefaciens, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. coagulans, B. circulans, B. pumilis, B. thuringiensis, B. clausii,* and *B. megaterium,* as well as other organisms within the genus *Bacillus.* In some embodiments, *B. subtilis* host cells are used for production of serine protease polypeptides. US 5,264,366 and 4,760,025 (RE 34,606) describe various *Bacillus* host strains that can be used for producing serine protease polypeptide of the invention, although other suitable strains can be used.

[0082] Several bacterial strains that can be used to produce serine protease polypeptides of the invention include non-recombinant (i.e., wild-type) *Bacillus sp.* strains, as well as variants of naturally-occurring strains and/or recombinant strains. In some embodiments, the host strain is a recombinant strain, wherein a polynucleotide encoding a polypeptide of interest has been introduced into the host. In some embodiments, the host strain is a *B. subtilis* host strain and particularly a recombinant *B. subtilis* host strain. Numerous *B. subtilis* strains are known, including, but not limited to for example, 1A6 (ATCC 39085), 168 (1A01), SB19, W23, Ts85, B637, PB1753 through PB1758, PB3360, JH642, 1A243 (ATCC 39,087), ATCC 21332, ATCC 6051, Mil 13, DE100 (ATCC 39,094), GX4931, PBT 110, and PEP 211strain (See e.g., Hoch et al., Genetics 73:215-228 [1973]; See also, US 4,450,235 and 4,302,544, and EP 0134048, each of which is incorporated by reference in its entirety). The use of *B. subtilis* as an expression host cells is well known in the art (See e.g., Palva et al., Gene 19:81-87 [1982]; Fahnestock and Fischer, J. Bacterid. , 165:796-804 [1986]; and Wang et al., Gene 69:39-47 [1988]).

[0083] In some embodiments, the *Bacillus* host cell is a *Bacillus sp.* that includes a mutation

or deletion in at least one of the following genes, degU, degS, degR and degQ. In some embodiments, the mutation is in a degU gene, and in some embodiments the mutation is degU(Hy)32 (See e.g., Msadek et al., J. Bacteriol. 172:824-834 [1990]; and Olmos et al., Mol. Gen. Genet. 253:562-567 [1997]). In some embodiments, the *Bacillus* host comprises a mutation or deletion in scoC4 (See e.g., Caldwell et al., J. Bacteriol. 183:7329-7340 [2001]); spoIIE (See e.g., Arigoni et al., Mol. Microbiol. 31:1407-1415 [1999]); and/or oppA or other genes of the opp operon (See e.g., Perego et al., Mol. Microbiol. 5:173-185 [1991]). Indeed, it is contemplated that any mutation in the opp operon that causes the same phenotype as a mutation in the oppA gene will find use in some embodiments of the altered *Bacillus* strain of the invention. In some embodiments, these mutations occur alone, while in other embodiments, combinations of mutations are present. In some embodiments, an altered *Bacillus* host cell strain that can be used to produce a serine protease polypeptide of the invention is a *Bacillus* host strain that already includes a mutation in one or more of the above-mentioned genes. In addition, *Bacillus sp.* host cells that comprise mutation(s) and/or deletions of endogenous protease genes find use. In some embodiments, the *Bacillus* host cell comprises a deletion of the aprE and the nprE genes. In other embodiments, the *Bacillus sp.* host cell comprises a deletion of 5 protease genes, while in other embodiments, the *Bacillus sp.* host cell comprises a deletion of 9 protease genes (See e.g., US 2005/0202535, incorporated herein by reference).

[0084] Host cells are transformed with at least one nucleic acid encoding at least one serine protease polypeptide of the invention using any suitable method known in the art. Methods for introducing a nucleic acid (e.g., DNA) into *Bacillus* cells or *E. coli* cells utilizing plasmid DNA constructs or vectors and transforming such plasmid DNA constructs or vectors into such cells are well known. In some embodiments, the plasmids are subsequently isolated from *E. coli* cells and transformed into *Bacillus* cells. However, it is not essential to use intervening microorganisms such as *E. coli*, and in some embodiments, a DNA construct or vector is directly introduced into a *Bacillus* host.

[0085] Those of skill in the art are well aware of suitable methods for introducing nucleic acid sequences of the invention into *Bacillus* cells (See e.g., Ferrari et al., "Genetics," in Harwood et al. [eds.], Bacillus, Plenum Publishing Corp. [1989], pp. 57-72; Saunders et al., J. Bacteriol. 157:718-726 [1984]; Hoch et al., J. Bacteriol. 93:1925 -1937 [1967]; Mann et al., Current Microbiol. 13:131-135 [1986]; Holubova, Folia Microbiol. 30:97 [1985]; Chang et al.,

Mol. Gen. Genet. 168:11-115 [1979]; Vorobjeva et al., FEMS Microbiol. Lett. 7:261-263 [1980]; Smith et al., Appl. Env. Microbiol. 51:634 [1986]; Fisher et al., Arch. Microbiol. 139:213-217 [1981]; and McDonald, J. Gen. Microbiol. 130:203 [1984]). Indeed, such methods as transformation, including protoplast transformation and transfection, transduction, and protoplast fusion are well known and suited for use in the present invention. Methods known in the art to transform *Bacillus* cells include such methods as plasmid marker rescue transformation, which involves the uptake of a donor plasmid by competent cells carrying a partially homologous resident plasmid (See, Contente et al., Plasmid 2:555-571 [1979]; Haima et al., Mol. Gen. Genet. 223:185-191 [1990]; Weinrauch et al., J. Bacteriol. 154:1077-1087 [1983]; and Weinrauch et al., J. Bacteriol. 169:1205-1211 [1987]). In this method, the incoming donor plasmid recombines with the homologous region of the resident "helper" plasmid in a process that mimics chromosomal transformation.

[0086] In addition to commonly used methods, in some embodiments, host cells are directly transformed with a DNA construct or vector comprising a nucleic acid encoding a serine protease polypeptide of the invention (i.e., an intermediate cell is not used to amplify, or otherwise process, the DNA construct or vector prior to introduction into the host cell). Introduction of the DNA construct or vector of the invention into the host cell includes those physical and chemical methods known in the art to introduce a nucleic acid sequence (e.g., DNA sequence) into a host cell without insertion into the host genome. Such methods include, but are not limited to calcium chloride precipitation, electroporation, naked DNA, liposomes and the like. In additional embodiments, DNA constructs or vector are co-transformed with a plasmid, without being inserted into the plasmid. In further embodiments, a selective marker is deleted from the altered *Bacillus* strain by methods known in the art (See, Stahl et al., J. Bacteriol. 158:411-418 [1984]; and Palmeros et al., Gene 247:255 -264 [2000]).

[0087] In some embodiments, the transformed cells of the present invention are cultured in conventional nutrient media. The suitable specific culture conditions, such as temperature, pH and the like are known to those skilled in the art and are well described in the scientific literature. In some embodiments, the invention provides a culture (e.g., cell culture) comprising at least one serine protease polypeptide or at least one nucleic acid of the invention.

[0088] In some embodiments, host cells transformed with at least one polynucleotide sequence encoding at least one serine protease polypeptide of the invention are cultured in a

suitable nutrient medium under conditions permitting the expression of the present protease, after which the resulting protease is recovered from the culture. In some embodiments, the protease produced by the cells is recovered from the culture medium by conventional procedures, including, but not limited to for example, separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt (e.g., ammonium sulfate), chromatographic purification (e.g., ion exchange, gel filtration, affinity, etc.).

[0089] In some embodiments, a serine protease polypeptide produced by a recombinant host cell is secreted into the culture medium. A nucleic acid sequence that encodes a purification facilitating domain may be used to facilitate purification of proteins. A vector or DNA construct comprising a polynucleotide sequence encoding a serine protease polypeptide may further comprise a nucleic acid sequence encoding a purification facilitating domain to facilitate purification of the serine protease polypeptide (See e.g., Kroll et al., DNA Cell Biol. 12:441-53 [1993]). Such purification facilitating domains include, but are not limited to, for example, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (See, Porath, Protein Expr. Purif. 3:263-281 [1992]), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system. The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (e.g., sequences available from Invitrogen, San Diego, CA) between the purification domain and the heterologous protein also find use to facilitate purification.

[0090] Assays for detecting and measuring the enzymatic activity of an enzyme, such as a serine protease polypeptide of the invention, are well known. Various assays for detecting and measuring activity of proteases (e.g., serine protease polypeptides of the invention), are also known to those of ordinary skill in the art. In particular, assays are available for measuring protease activity that are based on the release of acid-soluble peptides from casein or hemoglobin, measured as absorbance at 280 nm or colorimetrically using the Folin method. Other exemplary assays involve the solubilization of chromogenic substrates (See e.g., Ward, "Proteinases," in Fogarty (ed.)., Microbial Enzymes and Biotechnology, Applied Science, London, [1983], pp. 251-317). Other exemplary assays include, but are not limited to succinyl-Ala-Ala-Pro-Phe-para nitroanilide assay (suc-AAPF-pNA) and the 2,4,6-trinitrobenzene sulfonate sodium salt assay (TNBS assay). Numerous additional references known to those in

the art provide suitable methods (See e.g., Wells et al., Nucleic Acids Res. 11:7911-7925 [1983]; Christianson et al., Anal. Biochem. 223:119 -129 [1994]; and Hsia et al., Anal Biochem. 242:221-227 [1999]).

[0091] A variety of methods can be used to determine the level of production of a mature protease (e.g., mature serine protease polypeptides of the present invention) in a host cell. Such methods include, but are not limited to, for example, methods that utilize either polyclonal or monoclonal antibodies specific for the protease. Exemplary methods include, but are not limited to enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), fluorescent immunoassays (FIA), and fluorescent activated cell sorting (FACS). These and other assays are well known in the art (See e.g., Maddox et al., J. Exp. Med. 158:1211 [1983]).

[0092] In some other embodiments, the invention provides methods for making or producing a mature serine protease polypeptide of the invention. A mature serine protease polypeptide does not include a signal peptide or a propeptide sequence. Some methods comprise making or producing a serine protease polypeptide of the invention in a recombinant bacterial host cell, such as for example, a *Bacillus sp.* cell (e.g., a *B. subtilis* cell). In some embodiments, the invention provides a method of producing a serine protease polypeptide of the invention, the method comprising cultivating a recombinant host cell comprising a recombinant expression vector comprising a nucleic acid encoding a serine protease polypeptide of the invention under conditions conducive to the production of the serine protease polypeptide. Some such methods further comprise recovering the serine protease polypeptide from the culture.

[0093] In some embodiments the invention provides methods of producing a serine protease polypeptide of the invention, the methods comprising: (a) introducing a recombinant expression vector comprising a nucleic acid encoding a serine protease polypeptide of the invention into a population of cells (e.g., bacterial cells, such as *B. subtilis* cells); and (b) culturing the cells in a culture medium under conditions conducive to produce the serine protease polypeptide encoded by the expression vector. Some such methods further comprise: (c) isolating the serine protease polypeptide from the cells or from the culture medium.

[0094] Unless otherwise noted, all component or composition levels provided herein are made in reference to the active level 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. Enzyme components weights are based on total active protein. All percentages

and ratios are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated. Compositions of the invention include cleaning compositions, such as detergent compositions. In the exemplified detergent compositions, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions.

[0095] While not essential for the purposes of the present invention, the non-limiting list of adjuncts illustrated hereinafter are suitable for use in the instant cleaning compositions. In some embodiments, these adjuncts are incorporated for example, to assist or enhance cleaning performance, for treatment of the substrate to be cleaned, or to modify the aesthetics of the cleaning composition as is the case with perfumes, colorants, dyes or the like. It is understood that such adjuncts are in addition to the serine protease polypeptides of the present invention. The precise nature of these additional components, and levels of incorporation thereof, will depend on the physical form of the composition and the nature of the cleaning operation for which it is to be used. Suitable adjunct materials include, but are not limited to, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dispersants, suds suppressors, dyes, perfumes, colorants, filler salts, , photoactivators, fluorescers, fabric conditioners, hydrolyzable surfactants, preservatives, anti-oxidants, anti-shrinkage agents, anti-wrinkle agents, germicides, fungicides, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents, alkalinity sources, solubilizing agents, carriers, processing aids, pigments, and pH control agents, surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, additional enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids and/or pigments. In addition to the disclosure below, suitable examples of such other adjuncts and levels of use are found in US 5,576,282; 6,306,812; 6,326,348; 6,610,642; 6,605,458; 5,705,464; 5,710,115; 5,698,504; 5,695,679; 5,686,014; and 5,646,101 all of which are incorporated herein by reference. In embodiments in which the cleaning adjunct materials are not compatible with the serine protease polypeptides of the present invention in the cleaning compositions, then suitable methods of

keeping the cleaning adjunct materials and the protease(s) separated (i.e., not in contact with each other) until combination of the two components is appropriate are used. Such separation methods include any suitable method known in the art (e.g., gelcaps, encapsulation, tablets, physical separation, etc.). The aforementioned adjunct ingredients may constitute the balance of the cleaning compositions of the present invention.

[0096] The cleaning compositions of the present invention are advantageously employed for example, in laundry applications, hard surface cleaning applications, dishwashing applications, including automatic dishwashing and hand dishwashing, as well as cosmetic applications such as dentures, teeth, hair and skin cleaning. The enzymes of the present invention are also suited for use in contact lens cleaning and wound debridement applications. In addition, due to the unique advantages of increased effectiveness in lower temperature solutions, the enzymes of the present invention are ideally suited for laundry applications. Furthermore, the enzymes of the present invention find use in granular and liquid compositions.

[0097] The serine protease polypeptides of the present invention also find use in cleaning additive products. In some embodiments, low temperature solution cleaning applications find use. In some embodiments, the present invention provides cleaning additive products including at least one enzyme of the present invention is ideally suited for inclusion in a wash process when additional bleaching effectiveness is desired. Such instances include, but are not limited to low temperature solution cleaning applications. In some embodiments, the additive product is in its simplest form, one or more proteases. In some embodiments, the additive is packaged in dosage form for addition to a cleaning process. In some embodiments, the additive is packaged in dosage form for addition to a cleaning process where a source of peroxygen is employed and increased bleaching effectiveness is desired. Any suitable single dosage unit form finds use with the present invention, including but not limited to pills, tablets, gelcaps, or other single dosage units such as pre-measured powders or liquids. In some embodiments, filler(s) or carrier material(s) are included to increase the volume of such compositions. Suitable filler or carrier materials include, but are not limited to, various salts of sulfate, carbonate and silicate as well as talc, clay and the like. Suitable filler or carrier materials for liquid compositions include, but are not limited to water or low molecular weight primary and secondary alcohols including polyols and diols. Examples of such alcohols include, but are not limited to, methanol, ethanol, propanol and isopropanol. In some embodiments, the compositions contain from about 5% to about 90%

of such materials. Acidic fillers find use to reduce pH. Alternatively, in some embodiments, the cleaning additive includes adjunct ingredients, as more fully described below.

[0098] The present cleaning compositions and cleaning additives require an effective amount of at least one of the serine protease polypeptides provided herein, alone or in combination with other proteases and/or additional enzymes. The required level of enzyme is achieved by the addition of one or more serine protease polypeptides of the present invention. Typically the present cleaning compositions comprise at least about 0.0001 weight percent, from about 0.0001 to about 10, from about 0.001 to about 1, or from about 0.01 to about 0.1 weight percent of at least one of the serine protease polypeptides of the present invention.

[0099] The cleaning compositions herein are typically formulated such that, during use in aqueous cleaning operations, the wash water will have a pH of from about 4.0 to about 11.5, or even from about 5.0 to about 11.5, or even from about 5.0 to about 8.0, or even from about 7.5 to about 10.5. Liquid product formulations are typically formulated to have a pH from about 3.0 to about 9.0 or even from about 3 to about 5. Granular laundry products are typically formulated to have a pH from about 9 to about 11. In some embodiments, the cleaning compositions of the present invention can be formulated to have an alkaline pH under wash conditions, such as a pH of from about 8.0 to about 12.0, or from about 8.5 to about 11.0, or from about 9.0 to about 11.0. In some embodiments, the cleaning compositions of the present invention can be formulated to have a neutral pH under wash conditions, such as a pH of from about 5.0 to about 8.0, or from about 5.5 to about 8.0, or from about 6.0 to about 8.0, or from about 6.0 to about 7.5. In some embodiments, the neutral pH conditions can be measured when the cleaning composition is dissolved 1:100 (wt:wt) in de-ionised water at 20°C, measured using a conventional pH meter. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

[00100] In some embodiments, when the serine protease polypeptide (s) is/are employed in a granular composition or liquid, it is desirable for the serine protease polypeptide to be in the form of an encapsulated particle to protect the serine protease polypeptide from other components of the granular composition during storage. In addition, encapsulation is also a means of controlling the availability of the serine protease polypeptide during the cleaning process. In some embodiments, encapsulation enhances the performance of the serine protease polypeptide (s) and/or additional enzymes. In this regard, the serine protease polypeptides of the

present invention are encapsulated with any suitable encapsulating material known in the art. In some embodiments, the encapsulating material typically encapsulates at least part of the serine protease polypeptide (s) of the present invention. Typically, the encapsulating material is water-soluble and/or water-dispersible. In some embodiments, the encapsulating material has a glass transition temperature (Tg) of 0°C or higher. Glass transition temperature is described in more detail in W097/11151. The encapsulating material is typically selected from consisting of carbohydrates, natural or synthetic gums, chitin, chitosan, cellulose and cellulose derivatives, silicates, phosphates, borates, polyvinyl alcohol, polyethylene glycol, paraffin waxes, and combinations thereof. When the encapsulating material is a carbohydrate, it is typically selected from monosaccharides, oligosaccharides, polysaccharides, and combinations thereof. In some typical embodiments, the encapsulating material is a starch (See e.g., EP0922499; US 4,977,252; US 5,354,559; and US 5,935,826). In some embodiments, the encapsulating material is a microsphere made from plastic such as thermoplastics, acrylonitrile, methacrylonitrile, polyacrylonitrile, polymethacrylonitrile and mixtures thereof. Commercially available microspheres that find use include, but are not limited to those supplied by EXPANCEL ® (Stockviksverken, Sweden), and PM6545, PM6550, PM7220, PM7228, EXTENDOSPHERES ®, LUXSIL ®, Q-CEL ®, and SPHERICEL ® (PQ Corp., Valley Forge, PA).

[00101] There are a variety of wash conditions including varying detergent formulations, wash water volumes, wash water temperatures, and lengths of wash time, to which proteases involved in washing are exposed. A low detergent concentration system includes detergents where less than about 800 ppm of the detergent components are present in the wash water. A medium detergent concentration includes detergents where between about 800 ppm and about 2000ppm of the detergent components are present in the wash water. A high detergent concentration system includes detergents where greater than about 2000 ppm of the detergent components are present in the wash water. In some embodiments, the "cold water washing" of the present invention utilizes "cold water detergent" suitable for washing at temperatures from about 10°C to about 40°C, or from about 20°C to about 30°C, or from about 15°C to about 25°C, as well as all other combinations within the range of about 15°C to about 35°C, and all ranges within 10°C to 40°C.

[00102] Different geographies typically have different water hardness. Water hardness is usually described in terms of the grains per gallon mixed Ca^{2+}/Mg^{2+}. Hardness is a measure of

the amount of calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the water. Most water in the United States is hard, but the degree of hardness varies. Moderately hard (60-120 ppm) to hard (121-181 ppm) water has 60 to 181 parts per million.

Table I. Water Hardness

Water	Grains per gallon	Parts per million
Soft	less than 1.0	less than 17
Slightly hard	1.0 to 3.5	17 to 60
Moderately hard	3.5 to 7.0	60 to 120
Hard	7.0 to 10.5	120 to 180
Very hard	greater than 10.5	greater than 180

[00103] Accordingly, in some embodiments, the present invention provides serine protease polypeptides that show surprising wash performance in at least one set of wash conditions (e.g., water temperature, water hardness, and/or detergent concentration). In some embodiments, the serine protease polypeptides of the present invention are comparable in wash performance to other serine protease polypeptide proteases. In some embodiments of the present invention, the serine protease polypeptides provided herein exhibit enhanced oxidative stability, enhanced thermal stability, enhanced cleaning capabilities under various conditions, and/or enhanced chelator stability. In addition, the serine protease polypeptides of the present invention find use in cleaning compositions that do not include detergents, again either alone or in combination with builders and stabilizers.

[00104] In some embodiments of the present invention, the cleaning compositions comprise at least one serine protease polypeptide of the present invention at a level from about 0.00001 to about 10% by weight of the composition and the balance (e.g., about 99.999 to about 90.0%) comprising cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention comprises at least one serine protease polypeptide at a level of about 0.0001 to about 10%, about 0.001 to about 5%, about 0.001 to about 2%, about 0.005 to about 0.5% by weight of the composition and the balance of the cleaning composition (e.g., about 99.9999 to about 90.0%, about 99.999 to about 98%, about 99.995 to about 99.5% by weight) comprising cleaning adjunct materials.

[00105] In some embodiments, the cleaning compositions of the present invention comprise one or more additional detergent enzymes, which provide cleaning performance and/or fabric care and/or dishwashing benefits. Examples of suitable enzymes include, but are not limited to, acyl transferases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinosidases, aryl

esterases, beta-galactosidases, carrageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1, 4-glucanases, endo-beta-mannanases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipoxygenases, mannanases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases, peroxidases, phenoloxidases, phosphatases, phospholipases, phytases, polygalacturonases, proteases, pullulanases, reductases, rhamnogalacturonases, beta-glucanases, tannases, transglutaminases, xylan acetyl-esterases, xylanases, xyloglucanases, and xylosidases, or any combinations or mixtures thereof. In some embodiments, a combination of enzymes is used (i.e., a "cocktail") comprising conventional applicable enzymes like protease, lipase, cutinase and/or cellulase in conjunction with amylase is used.

[00106] In addition to the serine protease polypeptides provided herein, any other suitable protease finds use in the compositions of the present invention. Suitable proteases include those of animal, vegetable or microbial origin. In some embodiments, microbial proteases are used. In some embodiments, chemically or genetically modified mutants are included. In some embodiments, the protease is a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases include subtilisins, especially those derived from *Bacillus* (e.g., subtilisin, *lentus, amyloliquefaciens*, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168). Additional examples include those mutant proteases described in US RE34,606; 5,955,340; 5,700,676; 6,312,936; and 6,482,628, all of which are incorporated herein by reference. Additional protease examples include, but are not limited to trypsin (e.g., of porcine or bovine origin), and the *Fusarium* protease described in WO89/06270. In some embodiments, commercially available protease enzymes that find use in the present invention include, but are not limited to MAXATASE ®, MAXACAL™, MAXAPEM™, OPTICLEAN ®, OPTIMASE ®, PROPERASE ®, PURAFECT ®, PURAFECT ® OXP, PURAMAX™, EXCELLASE™, PREFERENZ™ proteases (e.g. P100, PI 10, P280), EFFECTENZ™ proteases (e.g. P1000, P1050, P2000), EXCELLENZ™ proteases (e.g. P1000), ULTIMASE ®, and PURAFAST™ (Genencor); ALCALASE ®, SAVINASE ®, PRIMASE ®, DURAZYM™, POLARZYME ®, OVOZYME ®, KANNASE ®, LIQUANASE ®, NEUTRASE ®, RELASE ® and ESPERASE ® (Novozymes); BLAP™ and BLAP™ (Henkel Kommanditgesellschaft auf Aktien, Duesseldorf, Germany), and KAP *(B. alkalophilus* subtilisin; Kao Corp., Tokyo, Japan). Various proteases are described in W095/23221, WO92/21760, WO09/149200, WO09/149144, WO

09/149145, WOl 1/072099, WOlO/056640, WOlO/056653, WOl 1/140364, W012/151534, US 2008/0090747, and US 5,801,039; 5,340,735; 5,500,364; 5,855,625; RE34,606; 5,955,340; 5,700,676; 6,312,936; 6,482,628; 8,530,219; and various other patents. In some further embodiments, neutral metalloproteases find use in the present invention, including but not limited to the neutral metalloproteases described in WO1999014341, WO1999033960, WO 1999014342, WO1999034003, WO2007044993, WO2009058303, WO2009058661, WO 2014/071410, WO2014/194032, WO2014/194034, WO2014/194054, and WO2014/194117. Exemplary metalloproteases include nprE, the recombinant form of neutral metalloprotease expressed in *B. subtilis* (See e.g., WO07/044993), and PMN, the purified neutral metalloprotease from *B. amyloliquefacients*.

[00107] In addition, any suitable lipase finds use in the present invention. Suitable lipases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are encompassed by the present invention. Examples of useful lipases include *H. lanuginosa* lipase (See e.g., EP258068, and EP305216), *Rhizomucor miehei* lipase (See e.g., EP238023), *Candida* lipase, such as *C. antarctica* lipase (e.g., *C. antarctica* lipase A or B; See e.g., EP214761), *Pseudomonas* lipases such as *P. alcaligenes* lipase and *P. pseudoalcaligenes* lipase (See e.g., EP218272), *P. cepacia* lipase (See e.g., EP331376), *P. stutzeri* lipase (See e.g., GB 1,372,034), *P. fluorescens* lipase, *Bacillus* lipase (e.g., *B. subtilis* lipase [Dartois et al., Biochem. Biophys. Acta 1131:253-260 [1993]); *B. stearothermophilus* lipase [See e.g., JP64/744992]; and *B. pumilus* lipase [See e.g., W091/16422]).

[00108] Furthermore, a number of cloned lipases find use in some embodiments of the present invention, including but not limited to *Penicillium camembertii* lipase (See, Yamaguchi et al., Gene 103:61-67 [1991]), *Geotricum candidum* lipase (See, Schimada et al., J. Biochem., 106:383-388 [1989]), and various *Rhizopus* lipases such as *R. delemar* lipase (See, Hass et al., Gene 109:117-113 [1991]), *a R. niveus* lipase (Kugimiya et al., Biosci. Biotech. Biochem. 56:716-719 [1992]) and *R. oryzae* lipase.

[00109] Other types of lipase polypeptide enzymes such as cutinases also find use in some embodiments of the present invention, including but not limited to the cutinase derived from *Pseudomonas mendocina* (See, WO88/09367), and the cutinase derived from *Fusarium solani pisi* (See, WO90/09446).

[00110] Additional suitable lipases include lipases such as M1 LIPASE™, LUMA FAST™,

and LIPOMAX™ (Genencor); LIPEX®, LIPC-LASE® and LIPOLASE*^ ULTRA (Novozymes); and LIPASE P™ "Amano" (Amano Pharmaceutical Co. Ltd., Japan).

[00111] In some embodiments of the present invention, the cleaning compositions of the present invention further comprise lipases at a level from about 0.00001 to about 10% of additional lipase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention also comprise lipases at a level of about 0.0001 to about 10%, about 0.001 to about 5%, about 0.001 to about 2%, about 0.005 to about 0.5% lipase by weight of the composition.

[00112] In some embodiments of the present invention, any suitable amylase finds use in the present invention. In some embodiments, any amylase (e.g., alpha and/or beta) suitable for use in alkaline solutions also find use. Suitable amylases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Amylases that find use in the present invention, include, but are not limited to a-amylases obtained from *B. licheniformis* (See e.g., GB 1,296,839). Additional suitable amylases include those found in W09510603, W09526397, W09623874, W09623873, W09741213, WO 9919467, WO0060060, WO0029560, W09923211, W09946399, WO0060058, WO0060059, W09942567, WO0114532, WO02092797, WO0166712, WO0188107, WO0196537, WO 0210355, WO9402597, WO0231124, W09943793, W09943794, WO20041 13551, WO 2005001064, WO2005003311, WO0164852, WO2006063594, WO2006066594, WO 2006066596, WO2006012899, WO2008092919, WO2008000825, WO2005018336, WO 005066338, WO2009140504, WO2005019443, WO2010091221, WO2010088447, WO 0134784, WO2006012902, WO2006031554, WO2006136161, WO2008101894, WO 2010059413, WO201 1098531, WO201 1080352, WO201 1080353, WO201 1080354, WO 2011082425, WO201 1082429, WO201 1076123, WO201 1087836, WO201 1076897, WO 94183314, W09535382, WO9909183, WO9826078, WO9902702, W09743424, W09929876, WO9100353, WO9605295, WO9630481, WO9710342, WO2008088493, WO2009149419, WO 2009061381, WO2009100102, WO2010104675, WO20101 17511, and WO20101 15021. Commercially available amylases that find use in the present invention include, but are not limited to DURAMYL®, TERMAMYL®, FUNGAMYL®, STAINZYME®, STAINZYME PLUS®, STAINZYME ULTRA®, and BAN™ (Novozymes), as well as POWERASE™,

RAPIDASE® and MAXAMYL ® P (Genencor).

[00113] In some embodiments of the present invention, the cleaning compositions of the present invention further comprise amylases at a level from about 0.00001 to about 10% of additional amylase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention also comprise amylases at a level of about 0.0001 to about 10%, about 0.001 to about 5%, about 0.001 to about 2%, about 0.005 to about 0.5% amylase by weight of the composition.

[00114] In some further embodiments, any suitable cellulase finds used in the cleaning compositions of the present invention. Suitable cellulases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Suitable cellulases include, but are not limited to *H. insolens* cellulases (See e.g., US 4,435,307). Especially suitable cellulases are the cellulases having color care benefits (See e.g., EP0495257). Commercially available cellulases that find use in the present include, but are not limited to CELLUZYME ®, CAREZYME ® (Novozymes), REVITALENZ™ 100 (Danisco US Inc) and KAC-500(B)™ (Kao Corporation). In some embodiments, cellulases are incorporated as portions or fragments of mature wild-type or variant cellulases, wherein a portion of the N-terminus is deleted (See e.g., US 5,874,276). Additional suitable cellulases include those found in WO2005054475, WO2005056787, and US 7,449,318 and 7,833,773. In some embodiments, the cleaning compositions of the present invention further comprise cellulases at a level from about 0.00001 to about 10% of additional cellulase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention also comprise cellulases at a level of about 0.0001 to about 10%, about 0.001 to about 5%, about 0.001 to about 2%, about 0.005 to about 0.5% cellulase by weight of the composition.

[00115] Any mannanase suitable for use in detergent compositions also finds use in the present invention. Suitable mannanases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Various mannanases are known which find use in the present invention (See e.g., US 6,566,114; 6,602,842; 6,440,991, all of which are incorporated herein by reference). Commercially available mannanases that find use in the present invention include, but are not limited to

MANNASTAR®, PURABRITE™, and MANNAWAY*. In some embodiments, the cleaning compositions of the present invention further comprise mannanases at a level from about 0.00001 to about 10% of additional mannanase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some embodiments of the present invention, the cleaning compositions of the present invention also comprise mannanases at a level of about 0.0001 to about 10%, about 0.001 to about 5%, about 0.001 to about 2%, about 0.005 to about 0.5% mannanase by weight of the composition.

[00116] In some embodiments, peroxidases are used in combination with hydrogen peroxide or a source thereof (e.g., a percarbonate, perborate or persulfate) in the compositions of the present invention. In some alternative embodiments, oxidases are used in combination with oxygen. Both types of enzymes are used for "solution bleaching" (i.e., to prevent transfer of a textile dye from a dyed fabric to another fabric when the fabrics are washed together in a wash liquor), preferably together with an enhancing agent (See e.g., W094/12621 and WO95/01426). Suitable peroxidases/oxidases include, but are not limited to those of plant, bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. In some embodiments, the cleaning compositions of the present invention further comprise peroxidase and/or oxidase enzymes at a level from about 0.00001 to about 10% of additional peroxidase and/or oxidase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In some other embodiments of the present invention, the cleaning compositions of the present invention also comprise peroxidase and/or oxidase enzymes at a level of about 0.0001 to about 10%, about 0.001 to about 5%, about 0.001 to about 2%, about 0.005 to about 0.5% peroxidase and/or oxidase enzymes by weight of the composition.

[00117] In some embodiments, additional enzymes find use, including but not limited to perhydrolases (See e.g., WO2005056782, WO2007106293, WO2008063400, WO2008106214, and WO2008106215). In addition, in some embodiments, mixtures of the above mentioned enzymes are encompassed herein, in particular one or more additional protease, amylase, lipase, mannanase, and/or at least one cellulase. Indeed, it is contemplated that various mixtures of these enzymes will find use in the present invention. It is also contemplated that the varying levels of the serine protease polypeptide(s) and one or more additional enzymes may both independently range to about 10%, the balance of the cleaning composition being cleaning adjunct materials. The specific selection of cleaning adjunct materials are readily made by

considering the surface, item, or fabric to be cleaned, and the desired form of the composition for the cleaning conditions during use (e.g., through the wash detergent use).

[00118] In some embodiments, an effective amount of one or more serine protease polypeptide(s) provided herein is included in compositions useful for cleaning a variety of surfaces in need of proteinaceous stain removal. Such cleaning compositions include cleaning compositions for such applications as cleaning hard surfaces, fabrics, and dishes. Indeed, in some embodiments, the present invention provides fabric cleaning compositions, while in other embodiments, the present invention provides non-fabric cleaning compositions. Notably, the present invention also provides cleaning compositions suitable for personal care, including oral care (including dentrifices, toothpastes, mouthwashes, etc., as well as denture cleaning compositions), skin, and hair cleaning compositions. It is intended that the present invention encompass detergent compositions in any form (i.e., liquid, granular, bar, semi-solid, gels, emulsions, tablets, capsules, etc.).

[00119] By way of example, several cleaning compositions wherein the serine protease polypeptides of the present invention find use are described in greater detail below. In some embodiments in which the cleaning compositions of the present invention are formulated as compositions suitable for use in laundry machine washing method(s), the compositions of the present invention preferably contain at least one surfactant and at least one builder compound, as well as one or more cleaning adjunct materials preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. In some embodiments, laundry compositions also contain softening agents (i.e., as additional cleaning adjunct materials). The compositions of the present invention also find use in detergent additive products in solid or liquid form. Such additive products are intended to supplement and/or boost the performance of conventional detergent compositions and can be added at any stage of the cleaning process. In some embodiments, the density of the laundry detergent compositions herein ranges from about 400 to about 1200 g/liter, while in other embodiments, it ranges from about 500 to about 950 g/liter of composition measured at 20°C.

[00120] In embodiments formulated as compositions for use in manual dishwashing methods, the compositions of the invention preferably contain at least one surfactant and preferably at least one additional cleaning adjunct material selected from organic polymeric compounds, suds

enhancing agents, group II metal ions, solvents, hydrotropes and additional enzymes.

[00121] In some embodiments, various cleaning compositions such as those provided in US 6,605,458 find use with the serine protease polypeptides of the present invention. Thus, in some embodiments, the compositions comprising at least one serine protease polypeptide of the present invention is a compact granular fabric cleaning composition, while in other embodiments, the composition is a granular fabric cleaning composition useful in the laundering of colored fabrics, in further embodiments, the composition is a granular fabric cleaning composition which provides softening through the wash capacity, in additional embodiments, the composition is a heavy duty liquid fabric cleaning composition. In some embodiments, the compositions comprising at least one serine protease polypeptide of the present invention are fabric cleaning compositions such as those described in US 6,610,642 and 6,376,450. In addition, the serine protease polypeptides of the present invention find use in granular laundry detergent compositions of particular utility under European or Japanese washing conditions (See e.g., US 6,610,642).

[00122] In some alternative embodiments, the present invention provides hard surface cleaning compositions comprising at least one serine protease polypeptide provided herein. Thus, in some embodiments, the compositions comprising at least one serine protease polypeptide of the present invention is a hard surface cleaning composition such as those described in US 6,610,642; 6,376,450; and 6,376,450.

[00123] In yet further embodiments, the present invention provides dishwashing compositions comprising at least one serine protease polypeptide provided herein. Thus, in some embodiments, the compositions comprising at least one serine protease polypeptide of the present invention is a hard surface cleaning composition such as those in US 6,610,642 and 6,376,450. In some still further embodiments, the present invention provides dishwashing compositions comprising at least one serine protease polypeptide provided herein. In some further embodiments, the compositions comprising at least one serine protease polypeptide of the present invention comprise oral care compositions such as those in US 6,376,450 and 6,376,450. The formulations and descriptions of the compounds and cleaning adjunct materials contained in the aforementioned US 6,376,450; 6,605,458; 6,605,458; and 6,610,642 find use with the serine protease polypeptides provided herein.

[00124] The cleaning compositions of the present invention are formulated into any suitable

form and prepared by any process chosen by the formulator, non-limiting examples of which are described in US 5,879,584; 5,691,297; 5,574,005; 5,569,645; 5,565,422; 5,516,448; 5,489,392; and 5,486,303, all of which are incorporated herein by reference. When a low pH cleaning composition is desired, the pH of such composition is adjusted via the addition of a material such as monoethanolamine or an acidic material such as HC1.

[00125] In some embodiments, the cleaning compositions according to the present invention comprise an acidifying particle or an amino carboxylic builder. Examples of an amino carboxylic builder include aminocarboxylic acids, salts and derivatives thereof. In some embodiment, the amino carboxylic builder is an aminopolycarboxylic builder, such as glycine-N,N-diacetic acid or derivative of general formula MOOC-CHR-N($CH_2COOM)_2$ where R is Ci_{-i_2}alkyl and M is alkali metal. In some embodiments, the amino carboxylic builder can be methyl glycine diacetic acid (MGDA), GLDA (glutamic-N,N-diacetic acid), iminodisuccinic acid (IDS), carboxymethyl inulin and salts and derivatives thereof, aspartic acid-N-monoacetic acid (ASMA), aspartic acid-N,N-diacetic acid (ASDA), aspartic acid-N-monopropionic acid (ASMP), iminodisuccinic acid (IDA), N-(2-sulfomethyl) aspartic acid (SMAS), N-(2-sulfoethyl)aspartic acid (SEAS), N-(2-sulfomethyl) glutamic acid (SMGL), N-(2-sulfoethyl) glutamic acid (SEGL), IDS (iminodiacetic acid) and salts and derivatives thereof such as N-methyliminodiacetic acid (MIDA), alpha-alanine-N,N-diacetic acid (alpha-ALDA), serine-N,N-diacetic acid (SEDA), isoserine-N,Ndiacetic acid (ISDA) , phenylalanine-N,N-diacetic acid (PHDA), anthranilic acid-N,N-diacetic acid (ANDA), sulfanilic acid-N,N-diacetic acid (SLDA), taurine-N,N-diacetic acid (TUDA) and sulfomethyl-N,N-diacetic acid (SMDA) and alkali metal salts and derivative thereof. In some embodiments, the acidifying particle has a weight geometric mean particle size of from about 400 μ to about 1200 μ and a bulk density of at least 550 g/L. In some embodiments, the acidifying particle comprises at least about 5% of the builder.

[00126] In some embodiments, the acidifying particle can comprise any acid, including organic acids and mineral acids. Organic acids can have one or two carboxyls and in some instances up to 15 carbons, especially up to 10 carbons, such as formic, acetic, propionic, capric, oxalic, succinic, adipic, maleic, fumaric, sebacic, malic, lactic, glycolic, tartaric and glyoxylic acids. In some embodiments, the acid is citric acid. Mineral acids include hydrochloric and sulphuric acid. In some instances, the acidifying particle of the invention is a highly active particle comprising a high level of amino carboxylic builder. Sulphuric acid has been found to

further contribute to the stability of the final particle.

[00127] In some embodiments, the cleaning compositions according to the present invention comprise at least one surfactant and/or a surfactant system wherein the surfactant is selected from nonionic surfactants, anionic surfactants, cationic surfactants, ampholytic surfactants, zwitterionic surfactants, semi-polar nonionic surfactants and mixtures thereof. In some embodiments, the surfactant is present at a level of from about 0.1 to about 60%, while in alternative embodiments the level is from about 1 to about 50%, while in still further embodiments the level is from about 5 to about 40%, by weight of the cleaning composition.

[00128] In some embodiments, the cleaning compositions of the present invention comprise one or more detergent builders or builder systems. In some embodiments incorporating at least one builder, the cleaning compositions comprise at least about 1%, from about 3 to about 60% or even from about 5 to about 40% builder by weight of the cleaning composition. Builders include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates, alkali metal silicates, alkaline earth and alkali metal carbonates, aluminosilicates, polycarboxylate compounds, ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1,3,5-trihydroxy benzene-2,4,6-trisulphonic acid, and carboxymethyloxysuccinic acid, the various alkali metal, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic acid and nitrilotriacetic acid, as well as polycarboxylates such as mellitic acid, succinic acid, citric acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof. Indeed, it is contemplated that any suitable builder will find use in various embodiments of the present invention.

[00129] In some embodiments, the builders form water-soluble hardness ion complexes (e.g., sequestering builders), such as citrates and polyphosphates (e.g., sodium tripolyphosphate and sodium tripolyphospate hexahydrate, potassium tripolyphosphate, and mixed sodium and potassium tripolyphosphate, etc.). It is contemplated that any suitable builder will find use in the present invention, including those known in the art (See e.g., EP2100949).

[00130] In some embodiments, builders for use herein include phosphate builders and non-phosphate builders. In some embodiments, the builder is a phosphate builder. In some embodiments, the builder is a non-phosphate builder. If present, builders are used in a level of from 0.1 to 80%, or from 5 to 60%, or from 10 to 50% by weight of the composition. In some

embodiments the product comprises a mixture of phosphate and non-phosphate builders. Suitable phosphate builders include mono-phosphates, di-phosphates, tri-polyphosphates or oligomeric-poylphosphates, including the alkali metal salts of these compounds, including the sodium salts. In some embodiments, a builder can be sodium tripolyphosphate (STPP). Additionally, the composition can comprise carbonate and/or citrate, preferably citrate that helps to achieve a neutral pH composition of the invention. Other suitable non-phosphate builders include homopolymers and copolymers of polycarboxylic acids and their partially or completely neutralized salts, monomeric polycarboxylic acids and hydroxycarboxylic acids and their salts. In some embodiments, salts of the above mentioned compounds include the ammonium and/or alkali metal salts, i.e. the lithium, sodium, and potassium salts, including sodium salts. Suitable polycarboxylic acids include acyclic, alicyclic, hetero-cyclic and aromatic carboxylic acids, wherein in some embodiments, they can contain at least two carboxyl groups which are in each case separated from one another by, in some instances, no more than two carbon atoms.

[00131] In some embodiments, the cleaning compositions of the present invention contain at least one chelating agent. Suitable chelating agents include, but are not limited to copper, iron and/or manganese chelating agents and mixtures thereof. In embodiments in which at least one chelating agent is used, the cleaning compositions of the present invention comprise from about 0.1 to about 15% or even from about 3.0 to about 10% chelating agent by weight of the subject cleaning composition.

[00132] In some still further embodiments, the cleaning compositions provided herein contain at least one deposition aid. Suitable deposition aids include, but are not limited to, polyethylene glycol, polypropylene glycol, polycarboxylate, soil release polymers such as polytelephthalic acid, clays such as kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite, and mixtures thereof.

[00133] As indicated herein, in some embodiments, anti-redeposition agents find use in some embodiments of the present invention. In some embodiments, non-ionic surfactants find use. For example, in automatic dishwashing embodiments, non-ionic surfactants find use for surface modification purposes, in particular for sheeting, to avoid filming and spotting and to improve shine. These non-ionic surfactants also find use in preventing the re-deposition of soils. In some embodiments, the anti-redeposition agent is a non-ionic surfactant as known in the art (See e.g., EP2100949). In some embodiments, the non-ionic surfactant can be ethoxylated nonionic

surfactants, epoxy-capped poly(oxyalkylated) alcohols and amine oxides surfactants.

[00134] In some embodiments, the cleaning compositions of the present invention include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. In embodiments in which at least one dye transfer inhibiting agent is used, the cleaning compositions of the present invention comprise from about 0.0001 to about 10%, from about 0.01 to about 5%, or even from about 0.1 to about 3% by weight of the cleaning composition.

[00135] In some embodiments, silicates are included within the compositions of the present invention. In some such embodiments, sodium silicates (e.g., sodium disilicate, sodium metasilicate, and crystalline phyllosilicates) find use. In some embodiments, silicates are present at a level of from about 1 to about 20%. In some embodiments, silicates are present at a level of from about 5 to about 15% by weight of the composition.

[00136] In some still additional embodiments, the cleaning compositions of the present invention also contain dispersants. Suitable water-soluble organic materials include, but are not limited to the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

[00137] In some further embodiments, the enzymes used in the cleaning compositions are stabilized by any suitable technique. In some embodiments, the enzymes employed herein are stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions that provide such ions to the enzymes. In some embodiments, the enzyme stabilizers include oligosaccharides, polysaccharides, and inorganic divalent metal salts, including alkaline earth metals, such as calcium salts, such as calcium formate. It is contemplated that various techniques for enzyme stabilization will find use in the present invention. For example, in some embodiments, the enzymes employed herein are stabilized by the presence of water-soluble sources of zinc (II), calcium (II) and/or magnesium (II) ions in the finished compositions that provide such ions to the enzymes, as well as other metal ions (e.g., barium (II), scandium (II), iron (II), manganese (II), aluminum (III), Tin (II), cobalt (II), copper (II), nickel (II), and oxovanadium (IV). Chlorides and sulfates also find use in some

embodiments of the present invention. Examples of suitable oligosaccharides and polysaccharides (e.g., dextrins) are known in the art (See e.g., WO07/145964). In some embodiments, reversible protease inhibitors also find use, such as boron-containing compounds (e.g., borate, 4-formyl phenyl boronic acid) and/or a tripeptide aldehyde find use to further improve stability, as desired.

[00138] In some embodiments, bleach, bleach activators and/or bleach catalysts are present in the compositions of the present invention. In some embodiments, the cleaning compositions of the present invention comprise inorganic and/or organic bleaching compound(s). Inorganic bleaches include, but are not limited to perhydrate salts (e.g., perborate, percarbonate, perphosphate, persulfate, and persilicate salts). In some embodiments, inorganic perhydrate salts are alkali metal salts. In some embodiments, inorganic perhydrate salts are included as the crystalline solid, without additional protection, although in some other embodiments, the salt is coated. Any suitable salt known in the art finds use in the present invention (See e.g., EP2100949).

[00139] In some embodiments, bleach activators are used in the compositions of the present invention. Bleach activators are typically organic peracid precursors that enhance the bleaching action in the course of cleaning at temperatures of 60°C and below. Bleach activators suitable for use herein include compounds which, under perhydrolysis conditions, give aliphatic peroxoycarboxylic acids having preferably from about 1 to about 10 carbon atoms, in particular from about 2 to about 4 carbon atoms, and/or optionally substituted perbenzoic acid. Additional bleach activators are known in the art and find use in the present invention (See e.g., EP21 00949).

[00140] In addition, in some embodiments and as further described herein, the cleaning compositions of the present invention further comprise at least one bleach catalyst. In some embodiments, the manganese triazacyclononane and related complexes find use, as well as cobalt, copper, manganese, and iron complexes. Additional bleach catalysts find use in the present invention (See e.g., US 4,246,612; 5,227,084; 4,810,410; and WO99/06521 and EP2100949).

[00141] In some embodiments, the cleaning compositions of the present invention contain one or more catalytic metal complexes. In some embodiments, a metal-containing bleach catalyst finds use. In some embodiments, the metal bleach catalyst comprises a catalyst system

comprising a transition metal cation of defined bleach catalytic activity, (e.g., copper, iron, titanium, ruthenium, tungsten, molybdenum, or manganese cations), an auxiliary metal cation having little or no bleach catalytic activity (e.g., zinc or aluminum cations), and a sequestrate having defined stability constants for the catalytic and auxiliary metal cations, particularly ethylenediaminetetraacetic acid, ethylenediaminetetra (methylenephosphonic acid) and water-soluble salts thereof are used (See e.g., US 4,430,243). In some embodiments, the cleaning compositions of the present invention are catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art (See e.g., US 5,576,282). In additional embodiments, cobalt bleach catalysts find use in the cleaning compositions of the present invention. Various cobalt bleach catalysts are known in the art (See e.g., US 5,597,936 and 5,595,967) and are readily prepared by known procedures.

[00142] In some additional embodiments, the cleaning compositions of the present invention include a transition metal complex of a macropolycyclic rigid ligand (MRL). As a practical matter, and not by way of limitation, in some embodiments, the compositions and cleaning processes provided by the present invention are adjusted to provide on the order of at least one part per hundred million of the active MRL species in the aqueous washing medium, and in some embodiments, provide from about 0.005 to about 25 ppm, more preferably from about 0.05 to about 10 ppm, and most preferably from about 0.1 to about 5 ppm of the MRL in the wash liquor.

[00143] In some embodiments, transition-metals in the instant transition-metal bleach catalyst include, but are not limited to manganese, iron and chromium. MRLs also include, but are not limited to special ultra-rigid ligands that are cross-bridged (e.g., 5,12-diethyl-l,5,8,12-tetraazabicyclo[6.6.2]hexadecane). Suitable transition metal MRLs are readily prepared by known procedures (See e.g., WO2000/32601 and US 6,225,464).

[00144] In some embodiments, the cleaning compositions of the present invention comprise metal care agents. Metal care agents find use in preventing and/or reducing the tarnishing, corrosion, and/or oxidation of metals, including aluminum, stainless steel, and non-ferrous metals (e.g., silver and copper). Suitable metal care agents include those described in EP2100949, WO9426860 and W094/26859). In some embodiments, the metal care agent is a zinc salt. In some further embodiments, the cleaning compositions of the present invention comprise from about 0.1 to about 5% by weight of one or more metal care agent.

[00145] In some embodiments, the cleaning composition is a high density liquid (HDL) composition having a variant serine protease polypeptide protease. The HDL liquid laundry detergent can comprise a detersive surfactant (10%-40%) comprising anionic detersive surfactant (selected from a group of linear or branched or random chain, substituted or unsubstituted alkyl sulphates, alkyl sulphonates, alkyl alkoxylated sulphate, alkyl phosphates, alkyl phosphonates, alkyl carboxylates, and/or mixtures thereof); and optionally non-ionic surfactant (selected from a group of linear or branched or random chain, substituted or unsubstituted alkyl alkoxylated alcohol, for example a Cg-Cigalkyl ethoxylated alcohol and/or C_6-Ci_2alkyl phenol alkoxylates), optionally wherein the weight ratio of anionic detersive surfactant (with a hydrophilic index (HIc) of from 6.0 to 9) to non-ionic detersive surfactant is greater than 1:1.

[00146] The composition can comprise optionally, a surfactancy boosting polymer consisting of amphiphilic alkoxylated grease cleaning polymers (selected from a group of alkoxylated polymers having branched hydrophilic and hydrophobic properties, such as alkoxylated polyalkylenimines in the range of 0.05wt%-10wt%) and/or random graft polymers (typically comprising of hydrophilic backbone comprising monomers selected from the group consisting of: unsaturated Ci-Cecarboxylic acids, ethers, alcohols, aldehydes, ketones, esters, sugar units, alkoxy units, maleic anhydride, saturated polyalcohols such as glycerol, and mixtures thereof; and hydrophobic side chain(s) selected from the group consisting of: C_4-C_{25}alkyl group, polypropylene, polybutylene, vinyl ester of a saturated C_2-C_6mono-carboxylic acid, C_1-C_6alkyl ester of acrylic or methacrylic acid, and mixtures thereof.

[00147] The composition can comprise additional polymers such as soil release polymers (include anionically end-capped polyesters, for example SRPl, polymers comprising at least one monomer unit selected from saccharide, dicarboxylic acid, polyol and combinations thereof, in random or block configuration, ethylene terephthalate-based polymers and co-polymers thereof in random or block configuration, for example Repel-o-tex SF, SF-2 and SRP6, Texcare SRA100, SRA300, SRN100, SRN170, SRN240, SRN300 and SRN325, Marloquest SL), anti-redeposition polymers (0.1 to 10wt%, include carboxylate polymers, such as polymers comprising at least one monomer selected from acrylic acid, maleic acid (or maleic anhydride), fumaric acid, itaconic acid, aconitic acid, mesaconic acid, citraconic acid, methylenemalonic acid, and any mixture thereof, vinylpyrrolidone homopolymer, and/or polyethylene glycol, molecular weight in the range of from 500 to 100,000 Da); cellulosic polymer (including those

selected from alkyl cellulose, alkyl alkoxyalkyl cellulose, carboxyalkyl cellulose, alkyl carboxyalkyl cellulose examples of which include carboxymethyl cellulose, methyl cellulose, methyl hydroxyethyl cellulose, methyl carboxymethyl cellulose, and mixtures thereof) and polymeric carboxylate (such as maleate/acrylate random copolymer or polyacrylate homopolymer).

[00148] The composition can further comprise saturated or unsaturated fatty acid, preferably saturated or unsaturated C_{12}-C_{24} fatty acid (0 to 10 wt%); deposition aids (examples for which include polysaccharides, preferably cellulosic polymers, poly diallyl dimethyl ammonium halides (DADMAC), and co-polymers of DADMAC with vinyl pyrrolidone, acrylamides, imidazoles, imidazolinium halides, and mixtures thereof, in random or block configuration, cationic guar gum, cationic cellulose such as cationic hydoxyethyl cellulose, cationic starch, cationic polyacylamides, and mixtures thereof.

[00149] The composition can further comprise dye transfer inhibiting agents examples of which include manganese phthalocyanine, peroxidases, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles and/or mixtures thereof; chelating agents examples of which include ethylene-diamine-tetraacetic acid (EDTA); diethylene triamine penta methylene phosphonic acid (DTPMP); hydroxy-ethane diphosphonic acid (HEDP); ethylenediamine N,N'-disuccinic acid (EDDS); methyl glycine diacetic acid (MGDA); diethylene triamine penta acetic acid (DTPA); propylene diamine tetracetic acid (PDT A); 2-hydroxypyridine-N-oxide (HPNO); or methyl glycine diacetic acid (MGDA); glutamic acid N,N-diacetic acid (N,N-dicarboxymethyl glutamic acid tetrasodium salt (GLDA); nitrilotriacetic acid (NTA); 4,5-dihydroxy-m-benzenedisulfonic acid; citric acid and any salts thereof; N-hydroxyethylethylenediaminetri-acetic acid (HEDTA), triethylenetetraaminehexaacetic acid (TTHA), N-hydroxyethyliminodiacetic acid (HEIDA), dihydroxyethylglycine (DHEG), ethylenediaminetetrapropionic acid (EDTP) and derivatives thereof.

[00150] The composition may comprise an enzyme stabilizer (examples of which include polyols such as propylene glycol or glycerol, sugar or sugar alcohol, lactic acid, reversible protease inhibitor, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid).

[00151] The composition can further comprise silicone or fatty-acid based suds suppressors;

heuing dyes, calcium and magnesium cations, visual signaling ingredients, anti-foam (0.001 to about 4.0wt%), and/or structurant/thickener (0.01 to 5wt%, selected from the group consisting of diglycerides and triglycerides, ethylene glycol distearate, microcrystalline cellulose, cellulose based materials, microfiber cellulose, biopolymers, xanthan gum, gellan gum, and mixtures thereof).

[00152] Suitable detersive surfactants also include cationic detersive surfactants (selected from a group of alkyl pyridinium compounds, alkyl quarternary ammonium compounds, alkyl quarternary phosphonium compounds, alkyl ternary sulphonium compounds, and/or mixtures thereof); zwitterionic and/or amphoteric detersive surfactants (selected from a group of alkanolamine sulpho-betaines); ampholytic surfactants; semi-polar non-ionic surfactants and mixtures thereof.

[00153] The composition can be any liquid form, for example a liquid or gel form, or any combination thereof. The composition may be in any unit dose form, for example a pouch.

[00154] In some embodiments, the cleaning composition is a high density powder (HDD) composition having a variant serine protease polypeptide protease. The HDD powder laundry detergent can comprise a detersive surfactant including anionic detersive surfactants (selected from a group of linear or branched or random chain, substituted or unsubstituted alkyl sulphates, alkyl sulphonates, alkyl alkoxylated sulphate, alkyl phosphates, alkyl phosphonates, alkyl carboxylates and/or mixtures thereof), non-ionic detersive surfactant (selected from a group of linear or branched or random chain, substituted or unsubstituted Cg-Cigalkyl ethoxylates, and/or C_{6}-Ci_{2}alkyl phenol alkoxylates), cationic detersive surfactants (selected from a group of alkyl pyridinium compounds, alkyl quaternary ammonium compounds, alkyl quaternary phosphonium compounds, alkyl ternary sulphonium compounds, and mixtures thereof), zwitterionic and/or amphoteric detersive surfactants (selected from a group of alkanolamine sulpho-betaines); ampholytic surfactants; semi-polar non-ionic surfactants and mixtures thereof; builders (phosphate free builders [for example zeolite builders examples of which include zeolite A, zeolite X, zeolite P and zeolite MAP in the range of 0wt% to less than 10wt%]; phosphate builders [examples of which include sodium tri-polyphosphate in the range of 0wt% to less than 10wt%]; citric acid, citrate salts and nitrilotriacetic acid or salt thereof in the range of less than 15 wt%); silicate salt (sodium or potassium silicate or sodium meta-silicate in the range of 0 to less than 10wt%, or layered silicate (SKS-6)); carbonate salt (sodium carbonate and/or sodium

bicarbonate in the range of 0 to less than 10 wt%); and bleaching agents (photobleaches, examples of which include sulfonated zinc phthalocyanines, sulfonated aluminum phthalocyanines, xanthenes dyes, and mixtures thereof; hydrophobic or hydrophilic bleach activators (examples of which include dodecanoyl oxybenzene sulfonate, decanoyl oxybenzene sulfonate, decanoyl oxybenzoic acid or salts thereof, 3,5,5-trimethy hexanoyl oxybenzene sulfonate, tetraacetyl ethylene diamine-TAED, and nonanoyloxybenzene sulfonate-NOBS, nitrile quats, and mixtures thereof; hydrogen peroxide; sources of hydrogen peroxide (inorganic perhydrate salts examples of which include mono or tetra hydrate sodium salt of perborate, percarbonate, persulfate, perphosphate, or persilicate); preformed hydrophilic and/or hydrophobic peracids (selected from a group consisting of percarboxylic acids and salts, percarbonic acids and salts, perimidic acids and salts, peroxymono sulfuric acids and salts) & mixtures thereof and/or bleach catalyst (such as imine bleach boosters examples of which include iminium cations and polyions; iminium zwitterions; modified amines; modified amine oxides; N-sulphonyl imines; N-phosphonyl imines; N-acyl imines; thiadiazole dioxides; perfluoroimines; cyclic sugar ketones and mixtures thereof; metal-containing bleach catalyst for example copper, iron, titanium, ruthenium, tungsten, molybdenum, or manganese cations along with an auxiliary metal cations such as zinc or aluminum and a sequestrate such as ethylenediaminetetraacetic acid, ethylenediaminetetra(methylenephosphonic acid) and water-soluble salts thereof).

[00155] The composition can further comprise additional detergent ingredients including perfume microcapsules, starch encapsulated perfume accord, hueing agents, additional polymers including fabric integrity and cationic polymers, dye lock ingredients, fabric-softening agents, brighteners (for example C.I. Fluorescent brighteners), flocculating agents, chelating agents, alkoxylated polyamines, fabric deposition aids, and/or cyclodextrin.

[00156] In some embodiments, the cleaning composition is an automatic dishwashing (ADW) detergent composition having a serine protease of the present invention. The ADW detergent composition can comprise two or more non-ionic surfactants selected from a group of ethoxylated non-ionic surfactants, alcohol alkoxylated surfactants, epoxy-capped poly(oxyalkylated) alcohols, or amine oxide surfactants present in amounts from 0 to 10% by weight; builders in the range of 5-60% comprising either phosphate (mono-phosphates, di-phosphates, tri-polyphosphates or oligomeric-poylphosphates, preferred sodium

tripolyphosphate-STPP or phosphate-free builders [amino acid based compounds, examples of which include MGDA (methyl-glycine-diacetic acid), and salts and derivatives thereof, GLDA (glutamic-N,Ndiacetic acid) and salts and derivatives thereof, IDS (iminodisuccinic acid) and salts and derivatives thereof, carboxy methyl inulin and salts and derivatives thereof and mixtures thereof, nitrilotriacetic acid (NTA), diethylene triamine penta acetic acid (DTPA), B-alaninediacetic acid (B-ADA) and their salts], homopolymers and copolymers of poly-carboxylic acids and their partially or completely neutralized salts, monomeric polycarboxylic acids and hydroxycarboxylic acids and their salts in the range of 0.5 to 50% by weight; sulfonated/carboxylated polymers (provide dimensional stability to the product) in the range of about 0.1 to about 50% by weight; drying aids in the range of about 0.1 to about 10% by weight (selected from polyesters, especially anionic polyesters optionally together with further monomers with 3 to 6 functionalities which are conducive to polycondensation, specifically acid, alcohol or ester functionalities, polycarbonate-, polyurethane- and/or polyurea-polyorganosiloxane compounds or precursor compounds thereof of the reactive cyclic carbonate and urea type); silicates in the range from about 1 to about 20% by weight (sodium or potassium silicates for example sodium disilicate, sodium meta-silicate and crystalline phyllosilicates); bleach-inorganic (for example perhydrate salts such as perborate, percarbonate, perphosphate, persulfate and persilicate salts) and organic (for example organic peroxyacids including diacyl and tetraacylperoxides, especially diperoxydodecanedioc acid, diperoxytetradecanedioc acid, and diperoxyhexadecanedioc acid); bleach activators- organic peracid precursors in the range from about 0.1 % to about 10% by weight; bleach catalysts (selected from manganese triazacyclononane and related complexes, Co, Cu, Mn and Fe bispyridylamine and related complexes, and pentamine acetate cobalt(III) and related complexes); metal care agents in the range from about 0.1% to 5% by weight (selected from benzatriazoles, metal salts and complexes, and/or silicates); enzymes in the range from about 0.01 to 5.0mg of active enzyme per gram of automatic dishwashing detergent composition (acyl transferases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinosidases, aryl esterases, beta-galactosidases, carrageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1, 4-glucanases, endo-beta-mannanases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipoxygenases, mannanases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases,

peroxidases, phenoloxidases, phosphatases, phospholipases, phytases, polygalacturonases, proteases, pullulanases, reductases, rhamnogalacturonases, beta-glucanases, tannases, transglutaminases, xylan acetyl-esterases, xylanases, xyloglucanases, and xylosidases, and any mixture thereof); and enzyme stabilizer components (selected from oligosaccharides, polysaccharides and inorganic divalent metal salts).

[00157] In some embodiments, the cleaning composition is borate-free. In some embodiments, the cleaning composition is phosphate-free.

[00158] Representative detergent formulations that beneficially include a serine protease polypeptide of the present invention include the detergent formulations found in WO2013063460, pages 78-152, and in particular the tables of pages 94 to 152 are hereby incorporated by reference. The serine proteases are normally incorporated into the detergent composition at a level of from 0.00001 to 10% of enzyme protein by weight of the composition. In some embodiments, the detergent composition comprises more than 0.0001%, 0.001%, 0.01%, or 0.1% of the serine protease by weight of the composition. In some embodiments, the detergent composition comprises less than 1%, 0.1%, 0.01%, or 0.001% of the serine protease by weight of the composition.

[00159] Also provided are compositions and methods of treating fabrics (***e.g.,*** to desize a textile) using a serine protease polypeptide of the present invention. Fabric-treating methods are well known in the art (see, e.g., US 6,077,316). For example, the feel and appearance of a fabric can be improved by a method comprising contacting the fabric with a serine protease in a solution. The fabric can be treated with the solution under pressure.

[00160] A serine protease of the present invention can be applied during or after the weaving of a textile, or during the desizing stage, or one or more additional fabric processing steps. During the weaving of textiles, the threads are exposed to considerable mechanical strain. Prior to weaving on mechanical looms, warp yarns are often coated with sizing starch or starch derivatives to increase their tensile strength and to prevent breaking. A serine protease of the present invention can be applied during or after the weaving to remove these sizing starch or starch derivatives. After weaving, the serine protease can be used to remove the size coating before further processing the fabric to ensure a homogeneous and wash-proof result.

[00161] A serine protease of the present invention can be used alone or with other desizing chemical reagents and/or desizing enzymes to desize fabrics, including cotton-containing fabrics,

as detergent additives, *e.g.,* in aqueous compositions. An amylase also can be used in compositions and methods for producing a stonewashed look on indigo-dyed denim fabric and garments. For the manufacture of clothes, the fabric can be cut and sewn into clothes or garments, which are afterwards finished. In particular, for the manufacture of denim jeans, different enzymatic finishing methods have been developed. The finishing of denim garment normally is initiated with an enzymatic desizing step, during which garments are subjected to the action of proteolytic enzymes to provide softness to the fabric and make the cotton more accessible to the subsequent enzymatic finishing steps. The serine protease can be used in methods of finishing denim garments *(e.g.,* a "bio-stoning process"), enzymatic desizing and providing softness to fabrics, and/or finishing process.

[00162] The serine protease polypeptides described herein find further use in the enzyme aided removal of proteins from animals and their subsequent degradation or disposal, such as feathers, skin, hair, hide, and the like. In some instances, immersion of the animal carcass in a solution comprising a serine protease polypeptide of the present invention can act to protect the skin from damage in comparison to the traditional immersion in scalding water or the defeathering process. In one embodiment, feathers can be sprayed with an isolated serine protase polypeptide of the present invention under conditions suitable for digesting or initiating degradation of the plumage. In some embodiments, a serine protease of the present invention can be used, as above, in combination with an oxidizing agent.

[00163] In some embodiments, removal of the oil or fat associated with raw feathers is assisted by using a serine protease polypeptide of the present invention. In some embodiments, the serine protease polypeptides are used in compositions for cleaning the feathers as well as to sanitize and partially dehydrate the fibers. In yet other embodiments, the disclosed serine protease polypeptides find use in recovering protein from plumage. In some other embodiments, the serine protease polypeptides are applied in a wash solution in combination with 95% ethanol or other polar organic solvent with or without a surfactant at about 0.5% (v/v).

[00164] In a further aspect of the invention, the serine protease polypeptides of the present invention can be used as a component of an animal feed composition, animal feed additive and/or pet food comprising a serine protease and variants thereof. The present invention further relates to a method for preparing such an animal feed composition, animal feed additive composition and/or pet food comprising mixing the serine protease polypeptide with one or more

animal feed ingredients and/or animal feed additive ingredients and/or pet food ingredients. Furthermore, the present invention relates to the use of the serine protease polypeptide in the preparation of an animal feed composition and/or animal feed additive composition and/or pet food.

[00165] The term "animal" includes all non-ruminant and ruminant animals. In a particular embodiment, the animal is a non-ruminant animal, such as a horse and a mono-gastric animal. Examples of mono-gastric animals include, but are not limited to, pigs and swine, such as piglets, growing pigs, sows; poultry such as turkeys, ducks, chicken, broiler chicks, layers; fish such as salmon, trout, tilapia, catfish and carps; and crustaceans such as shrimps and prawns. In a further embodiment the animal is a ruminant animal including, but not limited to, cattle, young calves, goats, sheep, giraffes, bison, moose, elk, yaks, water buffalo, deer, camels, alpacas, llamas, antelope, pronghorn and nilgai.

[00166] In the present context, it is intended that the term "pet food" is understood to mean a food for a household animal such as, but not limited to, dogs, cats, gerbils, hamsters, chinchillas, fancy rats, guinea pigs; avian pets, such as canaries, parakeets, and parrots; reptile pets, such as turtles, lizards and snakes; and aquatic pets, such as tropical fish and frogs.

[00167] The terms "animal feed composition," "feedstuff and "fodder" are used interchangeably and can comprise one or more feed materials selected from the group comprising a) cereals, such as small grains (e.g., wheat, barley, rye, oats and combinations thereof) and/or large grains such as maize or sorghum; b) by products from cereals, such as corn gluten meal, Distillers Dried Grain Solubles (DDGS) (particularly corn based Distillers Dried Grain Solubles (cDDGS), wheat bran, wheat middlings, wheat shorts, rice bran, rice hulls, oat hulls, palm kernel, and citrus pulp; c) protein obtained from sources such as soya, sunflower, peanut, lupin, peas, fava beans, cotton, canola, fish meal, dried plasma protein, meat and bone meal, potato protein, whey, copra, sesame; d) oils and fats obtained from vegetable and animal sources; and e) minerals and vitamins.

The protease polypeptides described herein find further use in the enzyme aided bleaching of paper pulps such as chemical pulps, semi-chemical pulps, kraft pulps, mechanical pulps or pulps prepared by the sulfite method. In general terms, paper pulps are incubated with a protease polypeptide of the present invention under conditions suitable for bleaching the paper pulp.

[00168] In some embodiments, the pulps are chlorine free pulps bleached with oxygen, ozone, peroxide or peroxyacids. In some embodiments, the protease polypeptides are used in enzyme aided bleaching of pulps produced by modified or continuous pulping methods that exhibit low lignin contents. In some other embodiments, the protease polypeptides are applied alone or preferably in combination with xylanase and/or endoglucanase and/or alpha-galactosidase and/or cellobiohydrolase enzymes.

[00169] The protease polypeptides described herein find further use in the enzyme aided removal of proteins from animals and their subsequent degradation or disposal, such as feathers, skin, hair, hide, and the like. In some instances, immersion of the animal carcass in a solution comprising a protease polypeptide of the present invention can act to protect the skin from damage in comparison to the traditional immersion in scalding water or the defeathering process. In one embodiment, feathers can be sprayed with an isolated protease polypeptide of the present invention under conditions suitable for digesting or initiating degradation of the plumage. In some embodiments, a protease of the present invention can be used, as above, in combination with an oxidizing agent.

[00170] In some embodiments, removal of the oil or fat associated with raw feathers is assisted by using a protease polypeptide of the present invention. In some embodiments, the protease polypeptides are used in compositions for cleaning the feathers as well as to sanitize and partially dehydrate the fibers. In some other embodiments, the protease polypeptides are applied in a wash solution in combination with 95% ethanol or other polar organic solvent with or without a surfactant at about 0.5% (v/v). In yet other embodiments, the disclosed protease polypeptides find use in recovering protein from plumage. The disclosed protease polypeptides may be used alone or in combination in suitable feather processing and proteolytic methods, such as those disclosed in PCT/EP2013/065362, PCT/EP2013/065363, and PCT/EP2013/065364, which are hereby incorporated by reference. In some embodiments, the recovered protein can be subsequently used in animal or fish feed.

EXAMPLES

[00171] The following examples are provided to demonstrate and illustrate certain preferred embodiments and aspects of the present disclosure and should not be construed as limiting. In the experimental disclosure which follows, the following abbreviations apply: ADW (automatic

dish washing); BMI (blood/milk/ink); BSA (bovine serum albumin); CAPS (*N*-cyclohexyl-3-aminopropanesulfonic acid); CHES (N-cyclohexyl-2-aminoethanesulfonic acid); DMC (dimethyl casein); HDD (heavy duty dry/powder); HDL (heavy duty liquid); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); MTP (microtiter plate); ND (not done); OD (optical density); PCR (polymerase chain reaction); ppm (parts per million); QS (quantity sufficient); rpm (revolutions per minute); AAPF (succinyl-Ala-Ala-Pro-Phe-p-nitroanilide); TNBSA (2,4,6-trinitrobenzene sulfonic acid); v/v (volume to volume); and w/v (weight to volume).

EXAMPLE 1

Discovery and Identification of *Bacillus* serine proteases

[00172] *B. patagoniensis* DSM 16117, *Bacillus* sp. DSM 8714, *B. pseudalcaliphilus* DSM 8725, and *Bacillus* sp. ATPhlO were selected as a potential source for enzymes useful in industrial applications. The DSM strains were obtained from Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. *Bacillus sp.* ATPhlO is from the Dupont Culture Collection.

[00173] To identify enzymes produced by these strains and the genes that encode these enzymes, the genomes of these strains were sequenced using Illumina® sequencing by synthesis (SBS) technology. Genome sequencing and assembly of the sequence data was performed by BaseClear (Leiden, The Netherlands). Contigs were annotated by BioXpr (Namur, Belgium). One of genes identified this way in strain *B. patagoniensis* DSM 16117 encodes a protein that shows homology to serine proteases of various other bacteria. The nucleotide sequence of this gene, *Bpan01744.n*, is depicted in SEQ ID NO:l: TTGAATAAGAAAATGGGGAAGGTTGT CGCTAGTACCGCATTACTAATCTCACTAGCTTTTAGTTCATCAATTGCACAGGCAGC AGAGGAAGCGAAAGAGAAATATTTAATTGGTTTCACAGAGCAGGAAGCGGTATCTA CTTTTGTAGAACAAATTGAAGAAGAAGAGGTTAGTATTTCAGAAGTCGATGACGTTG AAATTGATCTTTTATATGAATTTGAAACGATTCCAGTTTTATCAGTAGAATTAAATCC TGAAGATGTGGCTTCTTTGGAATCAGACCCAGCAATTTCTTATATTGAAGAAGATGC TGAAGTGACTACAATGGCTCAATCAGTTCCTTGGGGGATAAGCCGTGTTCAAGCTCA ATCTGCTCATAATCGAGGTATAACAGGATCAGGAGTGAAGGTGGCGGTTCTTGATAC AGGTATTTCAACACATGAAGATTTAAATGTACGGGGAGGGGCAAGCTTCGTAGCAG GTGAACCTGGTTATCAAGATGGGAATGGACACGGGACACATGTAGCAGGAACGATA GCCGCTCTAAATAATTCAATAGGCGTACTTGGTGTTGCACCGAATGCAGAATTATAT

GCAGTTAAAGTACTTGGAGCTAGTGGTTCTGGATCAATTAGTGGAATTGCACAAGGG
TTGCAATGGGCTGGCAATAATGGAATGCATATAGCTAATATGAGCCTTGGTACTTCT
GCACCGAGCGCAACTCTTGAACAAGCTGTTAACGCGGCGACATCTCGTGGTGTACTT
GTTATCGCAGCCTCTGGTAATTCTGGTGCTGGCTCAGTTGGTTATCCTGCACGTTACG
CGAATGCGATGGCAGTAGGTGCAACTGATCAAAATAACAACCGTGCAAGCTTCTCT
CAATATGGTGCAGGTCTTGATATTGTCGCTCCTGGCGTAGGTGTTCAAAGCACATAT
CCAGGGAACCGTTATGCGAGTTTGAATGGTACTTCAATGGCAACTCCTCACGTAGCT
GGTGTTGCAGCACTTGTTAAACAGAAAAACCCTTCATGGTCTAATGTACAAGTTAGA
AATCACTTGAAAAATACTGCAACTAATCTTGGCAATACGAATCTTTATGGTAGCGGA
CTAGTAAACGCAGAAGCAGCAACACGT.

[00174] The preproenzyme encoded by the ***Bpan01744.n*** gene is depicted in SEQ ID NO:2. At the N-terminus, the protein has a signal peptide with a length of 27 amino acids as predicted by SignalP-NN (Emanuelsson et al., Nature Protocols (2007) 2: 953-971). This signal peptide sequence is underlined and in bold in SEQ ID NO:2. The presence of a signal peptide indicates that this serine protease is a secreted enzyme. The enzyme has a pro sequence which is predicted to be 82 amino acids. The sequence of the predicted, fully processed mature chain (Bpan01744, 269 amino acids) is depicted in SEQ ID NO:3.

[00175] SEQ ID NO:2 sets forth the amino acid sequence of the serine protease precursor Bpan01744: **MNKK*MG*KVV*AS*TALL*lS*L***AFSSSIAQAA*** *EEAKEKYLIGFTEQEAVSTFVEQIE EEEVSISEVDDVEIDLLYEFETIPVLSVELNPEDVASLESDPAISYIEEDAEVTTMAQSYPWG1S* RVQAQSAHNRGITGSGVKVAVLDTGISTHEDLNVRGGASFVAGEPGYQDGNGHGTHV AGTIAALNNSIGVLGVAPNAELYAVKVLGASGSGSISGIAQGLQWAGNNGMHIANMSL GTSAPSATLEQAVNAATSRGVLVIAASGNSGAGSVGYPARYANAMAVGATDQNNNRA SFSQYGAGLDIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQKNPSWSNV QVRNHLKNTATNLGNTNLYGSGLVNAEAATR.

[00176] SEQ ID NO:3 sets forth the amino acid sequence of the mature protease Bpan01744: AQSVPWGISRVQAQSAHNRGITGSGVKVAVLDTGISTHEDLNVRGGASFVAGEPGYQD GNGHGTHVAGTIAALNNSIGVLGVAPNAELYAVKVLGASGSGSISGIAQGLQWAGNNG MHIANMSLGTSAPSATLEQAVNAATSRGVLVIAASGNSGAGSVGYPARYANAMAVGA TDQNNNRASFSQYGAGLDIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQ KNPSWSNVQVRNHLKNTATNLGNTNLYGSGLVNAEAATR.

[00177] A second serine protease gene was identified by mining the genome of *Bacillus* sp. DSM 8714. The nucleotide sequence of this gene, *BspAL03240.n*, is depicted in SEQ ID NO:4:

TTGAATAAGAAAATGGGGAAGGTTGTCGCTAGTACCGCATTACTAATCTCACTAGCT
TTTAGTTCATCAATTGCACAGGCAGCAGAGGAAGCGAAAGAGAAATATTTAATTGG
TTTCACAGAGCAGGAAGCAGTATCTACTTTTGTAGAGCAAATTGAAGAAGAAGAGG
TTAGTATTTCAGAAGTCGACGACGTTGAAATTGATCTTTTATATGAATTTGAAACAA
TTCCAGTTTTATCAGTAGAAATAAATCCTGAAGATGTTGCTTCTTTGGAATCAGACC
CAGCAATTTCTTATATTGAAGAAGATGCTGAAGTGACTACAATGGCTCAATCAGTTC
CATGGGGGATAAGCCGTGTTCAAGCTCAATCTGCTCATAATCGAGGTATAACAGGTT
CAGGAGTGAAGGTGGCTGTTCTTGATACAGGTATTTCAACACATGAAGATTTAAATG
TACGGGGAGGGGCAAGCTTTGTAGCAGGTGAACCTGGTTATCAAGATGGGAATGGA
CACGGGACACATGTAGCAGGAACGATAGCCGCTCTAAACAATTCATTAGGCGTACT
TGGTGTTGCACCGAATGCAGAATTATATGCAGTTAAAGTACTTGGAGCTAGTGGCTC
TGGATCAATCAGTGGAATTGCACAAGGGTTGCAATGGGCTGGTAATAATGGAATGC
ATATAGCTAATATGAGCCTTGGTACTTCTGCACCAAGCGCAACTCTTGAACAAGCTG
TTAACGCAGCGACATCTCGTGGTGTACTTGTTATCGCAGCCTCTGGTAATTCTGGTGC
TGGATCAGTTGGTTATCCTGCACGTTACGCGAATGCGATGGCAGTAGGTGCAACTGA
TCAAAATAACAACCGTGCAAGCTTCTCTCAATACGGTGCAGGTCTTGATATTGTCGC
TCCTGGCGTAGGTGTTCAAAGCACATATCCAGGGAACCGTTATGCGAGCTTGAATGG
TACTTCAATGGCAACTCCTCACGTAGCAGGTGTTGCAGCACTTGTTAAACAGAAAAA
CCCTTCATGGTCTAATGTACAAGTTAGGAATCACTTGAAAAATACTGCAACTAATCT
TGGCAATACGAATCTTTATGGTAGCGGACTAGTAAATGCAGAAGCAGCAACACGT.

[00178] The preproenzyme encoded by the *BspAL03240.n* gene is depicted in SEQ ID NO:5. At the N-terminus, the protein has a signal peptide with a length of 27 amino acids as predicted by SignalP-NN (Emanuelsson et al., Nature Protocols (2007) 2: 953-971). This signal peptide sequence is underlined and in bold in SEQ ID NO:5. The presence of a signal peptide indicates that this serine protease is a secreted enzyme. The enzyme has a pro sequence which is predicted to be 82 amino acids. The sequence of the predicted, fully processed mature chain (BspAL03240, 269 amino acids) is depicted in SEQ ID NO:6.

[00179] SEQ ID NO:5 sets forth the amino acid sequence of the serine protease precursor BspAL03240: **MNKK*MG*KVV*A*S**TALL*I*S**LAF***SSS**I*A*Q***AAEEAKEKYLIGFTEQEAVSTFVEQ**

*IEEEEVSISEVDDVEIDLLYEFETIPVLSVEINPEDVASLESDPAISYIEEDAEVTTM*AQSVPWGI
SRVQAQSAHNRGITGSGVKVAVLDTGISTHEDLNVRGGASFVAGEPGYQDGNGHGTHV
AGTIAALNNSLGVLGVAPNAELYAVKVLGASGSGSISGIAQGLQWAGNNGMHIANMSL
GTSAPSATLEQAVNAATSRGVLVIAASGNSGAGSVGYPARYANAMAVGATDQNNNRA
SFSQYGAGLDIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQKNPSWSNV
QVRNHLKNTATNLGNTNLYGSGLVNAEAATR.

[00180] SEQ ID NO:6 sets forth the amino acid sequence of the mature protease BspAL03240: AQSVPWGISRVQAQSAHNRGITGSGVKVAVLDTGISTHEDLNVRGGASF
VAGEPGYQDGNGHGTHVAGTIAALNNSLGVLGVAPNAELYAVKVLGASGSGSISGIAQ
GLQWAGNNGMHIANMSLGTSAPSATLEQAVNAATSRGVLVIAASGNSGAGSVGYPAR
YANAMAVGATDQNNNRASFSQYGAGLDIVAPGVGVQSTYPGNRYASLNGTSMATPHV
AGVAALVKQKNPSWSNVQVRNHLKNTATNLGNTNLYGSGLVNAEAATR.

[00181] A third serine protease gene was identified by mining the genome of *B. pseudalcaliphilus* DSM 8725. The nucleotide sequence of this gene, *Bps02592.n,* is depicted in SEQ ID NO:7: TTGAAGAAAATGTGGACAAAGTTCATAGCTGGTGCTGCTTTATTTTTA
TCCATTTCATTAACTTCTTCCGTCGTATCTGCAGAGGAGATCAAAAAGCAATATCTG
ATTGGGTTTGAGAATCAGCTTCAAGTAACCGAATTTCTTGAGGCAACCGAAAAAGG
AAACGATCAAGTCTCGTTATTTGCAGAGGTTAATAATGATACCGTTGAAATGGAACT
CTTATACGAATTTGAAGAAATTCCAGTTGTATCGGTTGAATTAAGTCCTGAAGATGT
TCAAAGCCTCAAAAAAGATCCTTCCATTGCTTACGTTGAAGAGGATGTAGAGGTCAA
AATAGCTAACCAAACGACACCTTGGGGAATTACACGTGTACAAGCTCCAACGGCGT
TGAATAGAGGCTTTACTGGTTCGGGCGTACGTGTAGCAGTCCTTGATACAGGTATTG
CCACTCATTCCGACTTAAATATTCGCGGTGGTGTTAGTTTTGTCAGTGGTGAACCTGG
TTATCAAGATGGCAACGGTCACGGCACCCACGTTGCCGGAACAATTGCAGCCTTAA
ATAATTCAATTGGTGTTATTGGTGTAGCTCCTAATGCTGAGTTATATGCCGTGAAAG
TTCTTGGTGCTAATGGCTCTGGCTCCGTTAGTGCTATTGCACAAGGCCTACAATGGTC
CGCACAAAATAATATGCATATTGCAAACCTTAGCTTAGGAAGTCCAACTGGAAGCC
AAACATTAGAACTTGCTGTGAATCAAGCTAATAGTGCCGGTGTATTAGTCGTTGCCG
CTTCAGGTAATAATGGTTCAGGAACAGTCTCTTACCCAGCTCGTTATACGAATGCAT
TGGCTGTTGGAGCGACTGATCAAAATAACAACCGTGCCAGCTTTTCTCAATATGGGA
CAGGCTTAAACATTGTGGCACCAGGTGTTGGTGTACAAAGCACATACCCTGGAAATC

GCTATGCTAGTTTAAACGGTACGTCCATGGCAACACCACATGTTGCTGGAGTTGCGG
CTCTAGTTAAACAGAAGAACCCTAGTTGGTCAAACACGCAAATTCGAAATCACCTCT
TGAATACAGCTACTTCATTAGGTAGTTCAACTCAGTTCGGTAGCGGACTCGTTAACG
CTGAAGCAGCTACAAGA.

[00182] The preproenzyme encoded by the *Bps02592.n* gene is depicted in SEQ ID NO:8. At the N-terminus, the protein has a signal peptide with a length of 27 amino acids as predicted by SignalP-NN (Emanuelsson et al., Nature Protocols (2007) 2: 953-971). This signal peptide sequence is underlined and in bold in SEQ ID NO:8. The presence of a signal peptide indicates that this serine protease is a secreted enzyme. The enzyme has a pro sequence which is predicted to be 86 amino acids. The sequence of the predicted, fully processed mature chain (Bps02592, 269 amino acids) is depicted in SEQ ID NO:9.

[00183] SEQ ID NO: 8 sets forth the amino acid sequence of the serine protease precursor Bps02592:

[00184] **MKKMWTKFIAGAALFLSISLTSSVVSA** ***EE/KKOYL/GFENOLOVIEFLEATEK*** *GNDQVSLFAEVNNDTVEMELLYEFEEIPWSVELSPEDVQSLKKDPSIAYVEEDVEVKIANQTT* PWGITRVQAPTALNRGFTGSGVRVAVLDTGIATHSDLNIRGGVSFVSGEPGYQDGNGHG THVAGTIAALNNSIGVIGVAPNAELYAVKVLGANGSGSVSAIAQGLQWSAQNNMHIAN LSLGSPTGSQTLELAVNQANSAGVLVVAASGNNGSGTVSYPARYTNALAVGATDQNN NRASFSQYGTGLNIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQKNPSW SNTQIRNHLLNTATSLGSSTQFGSGLVNAEAATR

[00185] SEQ ID NO: 9 sets forth the amino acid sequence of the mature protease Bps02592: NQTTPWGITRVQAPTALNRGFTGSGVRVAVLDTGIATHSDLNIRGGVSFVSGEPGYQDG NGHGTHVAGTIAALNNSIGVIGVAPNAELYAVKVLGANGSGSVSAIAQGLQWSAQNNM HIANLSLGSPTGSQTLELAVNQANSAGVLVVAASGNNGSGTVSYPARYTNALAVGATD QNNNRASFSQYGTGLNIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQKN PSWSNTQIRNHLLNTATSLGSSTQFGSGLVNAEAATR.

[00186] A fourth serine protease gene was identified by mining the genome of *Bacillus* sp. ATPhlO. The nucleotide sequence of this gene, *BspQ01211.n,* is depicted in SEQ ID NO: 10: TTGAAAAAGTTATTTACGAAAGTAGTTGCCGGTGCGGCGTTGTTATTGTCCATCTCC CTTTCTACCACATCGATCTCTGCCGAGGAGCAGAAAAAGCAATATCTAATTGGGTTT GAAAATCAAGTAAGCGTAACTGAATTTGTAGAAAGTAGCGAAAAAGGAAAAGATG

AATTTTCTATTTTTGCTGAAATAAATGATGAAACCATCGAAATGGACCTTCTCTATG
AATTCGAGGATATTCCGGTCGTTTCAGTTGAAGTAAGTCCAGAGGATGTGAAGGATT
TAGAAGGAGACCCTTCTATTGCTTTCATTGAGGAAGACATTGAGGTTAGTATTTTTA
ACCAAACGATTCCTTGGGGAATTACACGTGTACAAGCCCCCGCTGCCATTAACAGA
GGATTCACTGGAGCAGGGGTTCGCGTGGCTGTTCTTGACACAGGGATTTCAAATCAT
CCTGATCTAAATATTCGCGGTGGGGTAAGTTTTGTTCCTGGTGAATCTACTTATCAAG
ATGGAAATGGTCATGGTACTCATGTTGCTGGTACGATTGCTGCATTAAACAATTCAA
TCGGTGTTGTTGGAGTGGCCCCAAACACAGAGCTTTATGCTGTAAAGGTATTAGGTG
CAAATGGCTCAGGGTCGATTAGTTCCATTGCTCAAGGACTACAATGGACAGCTCAAA
ATAATATTCATGTTGCCAATTTAAGTTTAGGGAGTTCAACAGGAAGTCAAACATTAG
AGTTAGCTGTCAATCAAGCGACAAGCGCAGGGGTGTTAGTCGTTGCTGCATCAGGG
AATAATGGGTCTGGTACAATCTCTTATCCAGCGCGTTATGCCAATGCACTTGCCGTA
GGTGCAACAGACCAAAATAATAATCGTGCAAGCTTTTCACAATATGGGACAGGCTT
AAACATTGTAGCACCAGGAGTCGGAGTTCAAAGTACGTACCCTGGTAATCGCTATGC
AAGCTTAAGCGGAACATCAATGGCGACTCCTCATGTTGCTGGTGTTGCTGCACTTGT
GAAACAAAAGAACCCAAGCTGGTCTAACACGCAAATTAGACAGCATCTTCTCAATA
CAGCTACTCCACTAGGAAGCTCGAACCAATACGGAAGTGGACTTGTTAATGCAGAA
GCTGCCACAAGA.

[00187] The preproenzyme encoded by the *BspQ01211.n* gene is depicted in SEQ ID NO: 11. At the N-terminus, the protein has a signal peptide with a length of 27 amino acids as predicted by SignalP-NN (Emanuelsson et al., Nature Protocols (2007) 2: 953-971). This signal peptide sequence is underlined and in bold in SEQ ID NO:ll. The presence of a signal peptide indicates that this serine protease is a secreted enzyme. The enzyme has a pro sequence which is predicted to be 86 amino acids. The sequence of the predicted, fully processed mature chain (BspQ01211, 269 amino acids) is depicted in SEQ ID NO: 12.

[00188] SEQ ID NO: 11 sets forth the amino acid sequence of the serine protease precursor BspQ0121 1: *MKKhFTKVYAGAALhhSlShSTTSlSAEEOKKOYLIGFENOVSVTEFVESSE KGKDEFSIFAEINDETIEMDLLYEFEDIPWSVEVSPEDVKDLEGDPSIAFIEEDIEVSIFNQTJP* WGITRVQAPAAINRGFTGAGVRVAVLDTGISNHPDLNIRGGVSFVPGESTYQDGNGHGT HVAGTIAALNNSIGVVGVAPNTELYAVKVLGANGSGSISSIAQGLQWTAQNNIHVANLS LGSSTGSQTLELAVNQATSAGVLVVAASGNNGSGTISYPARYANALAVGATDQNNNRA

SFSQYGTGLNIVAPGVGVQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPSWSNTQ IRQHLLNTATPLGSSNQYGSGLVNAEAATR.

[00189] SEQ ID NO: 12 sets forth the amino acid sequence of the mature protease BspQ01211 (269 amino acids): NQTIPWGITRVQAPAAINRGFTGAGVRVAVLDTGISNHPDLNIRGGVS FVPGESTYQDGNGHGTHVAGTIAALNNSIGVVGVAPNTELYAVKVLGANGSGSISSIAQ GLQWTAQNNIHVANLSLGSSTGSQTLELAVNQATSAGVLVVAASGNNGSGTISYPARY ANALAVGATDQNNNRASFSQYGTGLNIVAPGVGVQSTYPGNRYASLSGTSMATPHVA GVAALVKQKNPSWSNTQIRQHLLNTATPLGSSNQYGSGLVNAEAATR.

EXAMPLE 2

Heterologous Expression of Bpan01744, BspAL03240, Bps02592, and BspQ01211

[00190] Bpan01744, BspAL03240, Bps02592 and BspQ01211 protease was produced in *B. subtilis* using an expression cassette consisting of the pro-mature sequence of Bpan01744, BspAL03240, Bps02592 or BspQ01211, preceded by the *B. subtilis aprE* promoter, the *B. subtilis aprE* signal peptide sequence, and ending with the BPN' transcription terminator sequence. The four different cassettes were cloned into the pHYT replicating shuttle vector and transformed into a suitable *B. subtilis* strain. The pHYT vector was derived from pHY300PLK (Takara) by adding a terminator after the tetracycline resistance gene using the *BstEII* and *Eco*RI sites (terminator sequence, GGTTACCTTGAATGTATATAAACATTCTCAAAGGGATTTC T AATAAAAAACGCTCGGTTGCCGCCGGGCGTTTTTTATGCATCGATGGAATTCXSEQ ID NO: 14). The *Hin*dIII site in pHY300PLK was also removed using a linker cloned into the BamHI and Hindlll sites (new linker sequence, GGATCCTGACTGCCTGAGCTT) (SEQ ID NO:15).

[00191] A map of the pHYT vector containing the Bpan01744 gene (pHYT-Bpan01744) is shown in Figure 1. The expression plasmids for BspAL03240, Bps02592 and BspQ01211 (not shown) are similar and differ only in the sequence encoding the pro-mature parts of the respective proteases.

[00192] To produce Bpan01744, BspAL03240, Bps02592 and BspQ01211, *B. subtilis* transformants containing pHYT-Bpan01744, pHYT-BspAL03240, pHYT-Bps02592 or pHYT-BspQ01211, respectively, were cultivated in an enriched semi-defined media based on MOPs buffer, with urea as major nitrogen source, glucose as the main carbon source, and supplemented with 1% soytone for robust cell growth. The media was supplemented with 25 ppm tetracycline.

After incubation (2 days at 32°C), protease was detected in the growth medium of all four strains. After centrifugation and filtration, culture supernatants with Bpan01744, BspAL03240, Bps02592 or BspQ01211 protease were used for assays and purification.

EXAMPLE 3

Cleaning Performance of Bpan01744, BspAL03240, Bps02592 and BspQ01211

[00193] The cleaning performance of serine proteases Bpan01744, BspAL03240, Bps02592 and BspQ01211 were tested on BMI (blood/milk/ink on cotton) microswatches (EMPA-116, Center for Testmaterials, The Netherlands) for laundry based applications, and on egg yolk (egg yolk on polyacryl fabric, aged and colored with carbon black dye) microswatches (PAS-38, Center for Testmaterials, The Netherlands) for dish based applications.

[00194] Automatic dishwashing (ADW) cleaning assays in GSM-B detergent at pH 9 and 10.5, were carried out at 40°C for 30 min. Following incubation, lOOuL of supernatant was transferred to a fresh MTP (Costar 9017) and absorbance was read at 405 nm for PAS-38 swatches, using the SpectraMax plate reader. The absorbance from a buffer-only control was subtracted and the resulting OD values at 405nm were plotted as a function of protease concentration. The data was fitted to a Sigmoidal fit.

[00195] To prepare rinsed PAS38 swatches, 180µ1lOmM CAPS buffer of pHl 1 was added to micro plates containing PAS38 µswatches. The plates were sealed and incubated in an iEMS incubator for 30 min at 60°C and 1100 rpm shaking. After incubation the buffer was removed using a Biotek plate washer, and the swatches were rinsed with demi water to remove any residual CAPS buffer. The plates were air dried prior to usage in the performance assay.

[00196] Laundry cleaning assay with commercially available HDL (OMO Klein & Krachtig, Unilever) or HDD (OMO Color, Unilever) detergent formulas was carried out at 25°C for 15 min. Following incubation, lOOuL of supernatant was transferred to a fresh MTP (Costar 9017) and absorbance was read at 405 nm for BMI swatches (Center for Testmaterials, The Netherlands) using the SpectraMax plate reader. The absorbance from a buffer-only control was subtracted and the resulting OD values at 405nm were plotted as a function of protease concentration. Two swatches per well were used for assays with HDL detergent, while HDD detergent assay was done using one microswatch per well. The data was fitted to a Langmuir equation.

[00197] The cleaning performances for serine proteases Bpan01744, BspAL03240, Bps02592

and BspQ01211 are shown in Figures 2-7.

EXAMPLE 4

Stability of Bpan01744, BspAL03240, Bps02592 and BspQ01211

[00198] Serine proteases Bpan01744, BspAL03240, Bps02592 and BspQ0121 1 were tested for stability in 50mM Tris pH9; 2mM CaC12 over a temperature range from 40-80°C by measuring the residual activity following incubation at elevated temperatures. Another *Bacillus* subtilisin, Bgi02446 SEQ ID NO: 13, was used for comparison. Diluted enzyme sample was mixed in stressor and stressed protease activity was measured. The diluted sample in stressor was incubated at elevated temperatures and after incubation the stressed protease activity was measured. For the unstressed condition, enzyme was assayed immediately for activity by the DMC method (as described below). For the stressed condition, the PCR plate was sealed and incubated at elevated temperatures for 5 min using an Eppendorf 384 Thermocycler, then assayed for activity. Stressed and unstressed activity was measured by the DMC method.

[00199] The amino acid sequence of the processed mature enzyme, Bgi02446 (268 amino acids), is set forth as SEQ ID NO: 13: QTVPWGITRVQ APAVHNRGITGS GVRV AILDS GIS A HSDLNIRGG ASFVPGEPTT ADLNGHGTH VAGTV AALNNS IGVIGV APN AELY AVKVLG A NGS GSV SGIAQGLEW AATNNMHIANMS LGS DFPS STLERA VNYATS RDVLVIA ATGNN GSGSVGYPARY ANAMAVGATDQNNRRANFS QYGTGIDIV APG VNVQS TYPGNR YVSM NGTS MATPHV AGAAALVKQR YPSWNATQIRNHLKNTATNLGNS SQFGS GLVN AEAAT R.

[00200] For the DMC assay, the reagent solutions used were: 2.5% Dimethylcasein (DMC, Sigma) in 100 mM Sodium Carbonate pH 9.5, 0.075% TNBSA (2,4,6-trinitrobenzene sulfonic acid, Thermo Scientific) in 100 mM Sodium Carbonate pH 9.5. Dilution Solution: 10 mM NaCl, 0.1 mM $CaCl_2$, 0.005% Tween-80, 0.02% Na-azide. MTPs (Greiner PS-microwell 384) were filled with 27.5 uL DMC substrate following the addition of 5 uL of 20 ppm protease supernatant. 27.5 uL of TNBSA solution was then added with slow mixing. Activity was measured at 405 nm over 5 min using a SpectraMax plate reader in kinetic mode at RT and activity was expressed as mOD/min.

[00201] The absorbance from a buffer-only control was subtracted and the resulting OD values at 405nm were plotted as a function of temperature. Where possible, the data was fitted to a 4 parameter logistic function (y=a+b/(l+(x/c) ^d)). The temperature at which 50% activity

for Bpan01744, BspAL03240, Bps02592, BspQ01211, and Bgi02446 proteases was retained was calculated and is shown in Table 1.

Table 1: Stability of serine proteases Bpan01744, BspAL03240, Bps02592, BspQ01211 and Bgi02446

Enzyme	$T_{50\%}$ (°C)
Bpan01744	67
BspAL03240	65
Bps02592	60
BspQ01211	62
Bgi02446	63.5

EXAMPLE 5

Comparison of Bpan01744, BspAL03240, Bps02592 and BspQ01211 to Related Molecules

Identification of Homologous Proteases

[00202] Homologs were identified by a BLAST search (Altschul et al., Nucleic Acids Res, 25:3389-402, 1997) against the NCBI non-redundant protein database and the Genome Quest Patent database with search parameters set to default values using the mature protein amino acid sequences for Bpan01744 (SEQ ID NO:3), BspAL03240 (SEQ ID NO:6), Bps02592 (SEQ ID NO:9), and BspQ01211 (SEQID NO: 12) as the query sequences. Percent identity (PID) for both search sets is defined as the number of identical residues divided by the number of aligned residues in the pairwise alignment. Value labeled "sequence length" on tables corresponds to the length (in amino acids) for the proteins referenced with the listed Accession numbers, while "aligned length" refers to sequence used for alignment and PID calculation. Tables 2-5 provide a list of sequences with the percent identity from the NCBI non-redundant protein database and Tables 6-9 provide a list of sequences from the Genome Quest patent database with the percent identity to Bpan01744, BspAL03240, Bps02592 and BspQ01211, respectively.

Table 2. List of sequences with percent identity to Bpan01744 protein identified from the NCBI non-redundant protein database

Accession #	PID	Organism	Sequence Length	Alignment Length
P27693	90.0	*Bacillus alcalophilus*	380	269
P41362	89.6	*Bacillus alcalophilus*	380	269
AFR78140	89.2	*synthetic construct*	269	269
P29599	88	*Bacillus lentus*	269	269
BAA25184	86.2	*Bacillus sp.AprN*	379	268

Table 2. List of sequences with percent identity to Bpan01744 protein identified from the NCBI non-redundant protein database

Accession #	PID	Organism	Sequence Length	Alignment Length
AFK08970	85.4	*Bacillus lehensis*	378	268
P20724	85.4	*Bacillus subtilis*	378	268
AGS78407	80.6	*Bacillus gibsonii*	375	268
WP_003321226	79.5	*Bacillus alcalophilus ATCC 27647*	382	268
WP_003323709	76.1	*Bacillus alcalophilus ATCC 27647*	187	180
ADK62564	69.5	*Bacillus sp. B001*	375	269
BAA02443	64.7	*Bacillus halodurans*	361	269
AAC43580	64.5	*Bacillus sp.*	378	273
BAA05540	64.3	*Bacillus sp.*	361	269
NP_241721	64.3	*Bacillus halodurans C-125*	361	269
ABI26631	63.6	*Bacillus clausii*	361	269
ADD64465	63.6	*Bacillus sp. JB99*	361	269
WP_010192403	63.4	*Bacillus sp. m.3-13*	381	273
BAD02409	63.0	*Bacillus sp. KSM-LD1*	404	273
YP_003426762	61.9	*Bacillus pseudofirmus OF4*	374	273
BAA06158	61.9	*Bacillus sp.*	374	273
WP_022628745	61.9	*Bacillus marmarensis DSM 21297*	374	273
BAF34115	61.5	*Alkaliphilus transvaalensis*	376	273
ABD33463	61.2	*Bacillus sp. hr08*	355	255
BAD11988	60.8	*Bacillus sp. KSM-LD1*	376	273
WP_017729072	60.8	*Bacillus sp. LI (2012)*	362	273
YP_003972439	60.7	*Bacillus atrophaeus UCMB-5137*	382	275
WP_010192405	60.6	*Bacillus sp. m.3-13*	379	274
WP_021 837258	60.6	*Bacillus licheniformis CG-B52*	379	274
AAG31027	60.4	*Bacillus licheniformis*	374	268
AAG31028	60.4	*Bacillus licheniformis*	374	268
AAC43581	60.2	*Bacillus sp.*	379	274
WP_023856571	60.2	*Bacillus sp. CPSM8*	378	274
AAG00494	60.2	*Bacillus licheniformis*	310	274
WP_003 180337	60.2	*Bacillus licheniformis WX-02*	379	274
ABY65723	60.2	*Bacillus subtilis*	354	274
CAJ70731	60.2	*Bacillus licheniformis*	379	274
AAT75303	60.2	*Bacillus mojavensis*	379	274
AAG00493	60.2	*Bacillus licheniformis*	310	274
YP_078307	60.2	*Bacillus licheniformis*	379	274
ADK11044	60.2	*Bacillus licheniformis*	379	274
ADI24411	60.2	*Bacillus subtilis*	347	241
AAG31026	60.1	*Bacillus licheniformis*	374	268

Table 2. List of sequences with percent identity to Bpan01744 protein identified from the NCBI non-redundant protein database

Accession #	PID	Organism	Sequence Length	Alignment Length
YP_005556107	60.0	*B. subtilis subsp. subtilis str. RO-NN-1*	381	275
AEQ38580	60.0	*Bacillus Ucheniformis*	370	275

Table 3. List of sequences with percent identity to BspAL03240 protein identified from the NCBI non-redundant protein database

Accession #	PID	*Organism*	Sequence Length	Alignment Length
P27693	89.6	*Bacillus alcalophilus*	380	269
P41362	89.2	*Bacillus alcalophilus*	380	269
AFR78140	88.8	*synthetic construct*	269	269
P29599	88	*Bacillus lentus*	269	269
BAA25184	85.8	*Bacillus sp.AprN*	379	268
AFK08970	85.1	*Bacillus lehensis*	378	268
P20724	85.1	*Bacillus subtilis*	378	268
AGS78407	80.2	*Bacillus gibsonii*	375	268
WP_003321226	79.1	*Bacillus alcalophilus ATCC 27647*	382	268
WP_003323709	76.1	*Bacillus alcalophilus ATCC 27647*	187	180
ADK62564	69.5	*Bacillus sp. B001*	375	269
AAC43580	64.5	*Bacillus sp.*	378	273
BAA02443	64.3	*Bacillus halodurans*	361	269
BAA05540	63.9	*Bacillus sp.*	361	269
NP_241721	63.9	*Bacillus halodurans C-125*	361	269
WP_010192403	63.4	*Bacillus sp. m3-13*	381	273
ABI26631	63.2	*Bacillus clausii*	361	269
ADD64465	63.2	*Bacillus sp. JB99*	361	269
BAD02409	63.0	*Bacillus sp. KSM-LD1*	404	273
YP_003426762	61.9	*Bacillus pseudofirmus OF4*	374	273
BAA06158	61.9	*Bacillus sp.*	374	273
WP_022628745	61.9	*Bacillus marmarensis DSM 21297*	374	273
BAF34115	61.5	*Alkaliphilus transvaalensis*	376	273
ABD33463	61.2	*Bacillus sp. hr08*	355	255
BAD11988	60.8	*Bacillus sp. KSM-LD1*	376	273
YP_003972439	60.7	*Bacillus atrophaeus UCMB-5137*	382	275
WP_010192405	60.6	*Bacillus sp. m3-13*	379	274
WP_021837258	60.6	*Bacillus licheniformis CG-B52*	379	274
AAG31027	60.4	*Bacillus licheniformis*	374	268
AAG31028	60.4	*Bacillus licheniformis*	374	268
WP_017729072	60.4	*Bacillus sp. L1(2012)*	362	273

Table 3. List of sequences with percent identity to BspAL03240 protein identified from the NCBI non-redundant protein database

Accession #	PID	*Organism*	Sequence Length	Alignment Length
AAC43581	60.2	*Bacillus sp.*	379	274
WP_023856571	60.2	*Bacillus sp. CPSM8*	378	274
AAG00494	60.2	*Bacillus licheniformis*	310	274
WP_003 180337	60.2	*Bacillus licheniformis WX-02*	379	274
ABY65723	60.2	*Bacillus subtilis*	354	274
CAJ70731	60.2	*Bacillus licheniformis*	379	274
AAT75303	60.2	*Bacillus mojavensis*	379	274
AAG00493	60.2	*Bacillus licheniformis*	310	274
YP_078307	60.2	*Bacillus licheniformis*	379	274
ADK11044	60.2	*Bacillus licheniformis*	379	274
AAG31026	60.1	*Bacillus licheniformis*	374	268
AEQ38580	60.0	*Bacillus licheniformis*	370	275

Table 4. List of sequences with percent identity to Bps02592 protein identified from the NCBI non-redundant protein database

Accession #	PID	Organism	Sequence Length	Alignment Length
WP_047986748	100	*Bacillus pseudalcaliphilus*	382	269
WP_003321226	90.3	*Bacillus alcalophilus ATCC 27647*	382	269
WP_034632645	90.3	*Bacillus okhensis*	382	269
P27693	82.5	*Bacillus alcalophilus*	380	268
P41362	82.1	*Bacillus alcalophilus*	380	268
WP_038486090	81.0	*Bacillus lehensis*	374	268
AFR78140	82.1	*synthetic construct*	269	268
P29599	80.6	*Bacillus lentus*	269	268
BAA25184	80.2	*Bacillus sp.AprN*	379	268
AFK08970	80.2	*Bacillus lehensis*	378	268
P20724	79.9	*Bacillus subtilis*	378	268
AGS78407	78.4	*Bacillus gibsonii*	375	268
ADK62564	71.3	*Bacillus sp. B001*	375	268
BAA02443	64.7	*Bacillus halodurans*	361	269
BAA05540	63.9	*Bacillus sp.*	361	269
NP_241721	63.9	*Bacillus halodurans C-125*	361	269
ADD64465	63.6	*Bacillus sp. JB99*	361	269
ABI26631	63.2	*Bacillus clausii*	361	269
WP_017729072	61.2	*Bacillus sp. LI (2012)*	362	273
WP_022628745	61.0	*Bacillus marmarensis DSM 21297*	374	272
YP_003426762	60.7	*Bacillus pseudofirmus OF4*	374	272

Table 4. List of sequences with percent identity to Bps02592 protein identified from the NCBI non-redundant protein database				
Accession #	**PID**	**Organism**	**Sequence Length**	**Alignment Length**
BAA06158	60.7	*Bacillus sp.*	374	272

Table 5. List of sequences with percent identity to BspQ01211 protein identified from the NCBI non-redundant protein database				
Accession #	**PID**	**Organism**	**Sequence Length**	**Alignment Length**
WP_034632645	99.2	*Bacillus okhensis*	382	269
WP_003321226	91.4	*Bacillus alcalophilus ATCC 27647*	382	269
WP_047986748	90.3	*Bacillus pseudalcaliphilus*	382	269
P27693	82.8	*Bacillus alcalophilus*	380	268
P41362	82.5	*Bacillus alcalophilus*	380	268
AFR78140	82.1	*synthetic construct*	269	268
P29599	81.7	Bacillus lentus	269	268
AFK08970	80.2	*Bacillus lehensis*	378	268
AGS78407	78.3	*Bacillus gibsonii*	375	268
BAA25184	80.6	*Bacillus sp.AprN*	379	268
P20724	79.8	*Bacillus subtilis*	378	268
ADK62564	71.3	*Bacillus sp. B001*	375	268
BAA02443	65.4	*Bacillus halodurans*	361	269
BAA05540	65.1	*Bacillus sp.*	361	269
NP_241721	65.1	*Bacillus halodurans C-125*	361	269
ABI26631	64.3	*Bacillus clausii*	361	269
ADD64465	64.3	*Bacillus sp. JB99*	361	269
YP_003426762	61.8	*Bacillus pseudofirmus OF4*	374	272
BAA06158	61.8	*Bacillus sp.*	374	272
WP_022628745	61.4	*Bacillus marmarensis DSM 21297*	374	272
WP_017729072	60.8	*Bacillus sp. L1(2012)*	362	273
WP_010192403	60.7	*Bacillus sp. m3-13*	381	272
BAF34115	60.7	*Alkaliphilus transvaalensis*	376	272
BAD11988	60.3	*Bacillus sp. KSM-LD1*	376	272

Table 6. List of sequences with percent identity to Bpan01744 protein identified from the Genome Quest Patent database				
Patent ID #	**PID**	**Organism**	**Sequence Length**	**Alignment Length**
EP1160327	90.3	*Bacillus sp*	269	269
WO9402618	90.3	*Bacillus novalis*	269	269
US20140045268-0050	90.3	*Bacillus clausii*	269	269

Table 6. List of sequences with percent identity to Bpan01744 protein identified from the Genome Quest Patent database

Patent ID #	PID	Organism	Sequence Length	Alignment Length
WO20051 18793-0018	90.3	*Bacillus sp. DSM 14390*	380	269
JP2006296268-0008	90.3	*Bacillus clausii KSM-K16*	380	269
JP2008022828-0033	90.3	*Bacillus clausii KSM-K16*	355	269
US20140045268-0040	90.0	*Bacillus clausii*	353	269
WO2012151480	90.0	*Bacillus lentus*	269	269
WO9402618	90.0	*Bacillus novalis*	269	269
EP1 160327	90.0	*Bacillus sp*	269	269
EP0571049	90.0	*Bacillus sp*	269	269
US6271012	90.0	*Bacillus sp*	269	269
US20130217607-0001	90.0	*Bacillus Alkalophilus PB92*	269	269
WO20 11072099	90.0	*Bacillus lentus*	269	269
CA2829859	90.0	*Bacillus lentus*	269	269
CN1606626-0002	90.0	*spore bacillus plants*	380	269
WO02077289	90.0	*Bacillus alcalophilus*	377	269
US20130323816-0002	90.0	*Bacillus nov. Sp. PB92*	380	269
EP0283075-0003	90.0	*Bacillus sp.*	380	269
EP0328229-0001	90.0	*Bacillus sp*	270	269
US20140045268-0061	90.0	*Artificial Sequence*	382	269
WO201 1140364	90.0	*Bacillus lentus*	380	269
WO03054185	89.9	*Bacillus alkalophilus*	268	268
US20140045268-0038	89.6	*Artificial Sequence*	353	269
WO2012151480	89.6	*Bacillus lentus*	269	269
WO20 11072099	89.6	*Bacillus lentus*	269	269
WO2008153934	89.6	*Bacillus lentus*	269	269
WO9402618	89.6	*Bacillus novalis*	269	269
EP1 160327	89.6	*Bacillus sp*	269	269
WO9402618	89.6	*Bacillus novalis*	269	269
EP2589651-0002	89.6	*Bacillus clausii*	269	269
JP2013153763-0002	89.6	*B. lentus*	273	269
EP2628785-0004	89.6	*Bacillus lentus, Bacillus clausii*	380	269
DE4224125	89.6	*B. alcalophilus HA1 DSM 5466*	380	269
EP2578679-0561	89.6	*Artificial Sequence*	380	269
US20140045268-0059	89.6	*Bacillus clausii*	382	269
WO201 1140364	89.6	*Bacillus lentus*	380	269
JP2013524855-0003	89.6	*Bacillus lentus*	380	269

Table 7. List of sequences with percent identity to BspAL03240 protein identified from the Genome Quest Patent database

Patent ID #	PID	Organism	Sequence Length	Alignment Length

Table 7. List of sequences with percent identity to BspAL03240 protein identified from the Genome Quest Patent database

Patent ID #	PID	Organism	Sequence Length	Alignment Length
EP1 160327	90.0	*Bacillus sp*	269	269
WO9402618	90.0	*Bacillus novalis*	269	269
US20140045268-0050	90.0	*Bacillus clausii*	269	269
WO20051 18793-0018	90.0	*Bacillus sp.*	380	269
JP2006296268-0008	90.0	*Bacillus clausii KSM-K16*	380	269
JP2008022828-0033	90.0	*Bacillus clausii KSM-K16*	355	269
US20140045268-0040	89.6	*Artificial Sequence*	353	269
WO2012151480	89.6	*Bacillus lentus*	269	269
WO9402618	89.6	*Bacillus novalis*	269	269
EP1 160327	89.6	*Bacillus sp*	269	269
EP0571049	89.6	*Bacillus sp*	269	269
US6271012	89.6	*Bacillus sp; PB92*	269	269
US20130217607-0001	89.6	*Bacillus Alkalophilus PB92*	269	269
WO20 11072099	89.6	*Bacillus lentus*	269	269
CA2829859	89.6	*Bacillus lentus*	269	269
CN1606626-0002	89.6	*spore bacillus plants*	380	269
WO02077289	89.6	*Bacillus alcalophilus*	377	269
US20130323816-0002	89.6	*Bacillus nov. Sp. PB92*	380	269
EP0283075-0003	89.6	*Bacillus sp.*	380	269
WO8907642-0001	89.6	*Construct, Bacillus sp.*	270	269
US20140045268-0061	89.6	*Artificial Sequence*	382	269
WO201 1140364	89.6	*Bacillus lentus*	380	269
WO03054185	89.6	*Bacillus alkalophilus*	268	268
US20140045268-0038	89.2	*Artificial Sequence*	353	269
WO2012151480	89.2	*Bacillus lentus*	269	269
WO20 11072099	89.2	*Bacillus lentus*	269	269
WO2012151480	89.2	*Bacillus lentus*	269	269
WO9402618	89.2	*Bacillus novalis*	269	269
EP1 160327	89.2	*Bacillus sp*	269	269
EP2589651-0002	89.2	*Construct*	269	269
JP2013153763-0002	89.2	*B. lentus*	273	269
EP2628785-0004	89.2	*Bacillus lentus*	380	269
DE4224125	89.2	*B. alcalophilus HAl DSM 5466*	380	269
EP2578679-0561	89.2	*Bacillus subtilis*	380	269
US20140045268-0059	89.2	*Bacillus clausii*	382	269
WO201 1140364	89.2	*Bacillus lentus*	380	269
JP2013524855-0003	89.2	*Bacillus lentus*	380	269

Table 8. List of sequences with percent identity to Bps02592 protein identified from the Genome Quest Patent database

Patent ID #	PID	Organism	Sequence Length	Alignment Length
WO2015089441-0056	90.3	Bacillus sp. G-825-6	269	269
US2014001 1259-0034	90.3	Bacillus species.	269	269
WO02077289	90.3	*Bacillus sp*	382	269
US20140045268-0051	90.3	*Bacillus sp*	269	269
US200501 13273-0008	90.3	*Bacillus sp*	267	268
WO03054185	90.3	*Bacillus sp*	268	268
US20050009167-0008	89.9	*Bacillus sp.*	267	268
W09211348	82.8	*Bacillus subtilis*	269	268
US20140045268-0040	82.5	*Artificial Sequence*	353	268
WO2012151480	82.5	*Bacillus lentus*	269	268
WO9402618	82.5	*Bacillus novalis*	269	268
EP1 160327	82.5	*Bacillus sp*	269	268
EP2647692-0003	82.5	*Bacillus alcalophilus*	269	268
WO20 11072099	82.5	*Bacillus lentus*	269	268
US20130123162	82.5	*Bacillus lentus*	269	268
CA2829859	82.5	*Bacillus lentus*	269	268
EP0415296	82.5	*Bacillus alcalophilus*	269	268
WO0218588	82.5	*Hordeum sp B. lentus Chimeric*	368	268
WO2012151534	82.5	*Bacillus lentus*	269	268
WO02077289	82.5	*Bacillus alcalophilus*	377	268
EP1 160327	82.5	*Bacillus sp*	380	268
EP0283075-0003	82.5	*Bacillus sp.*	380	268
EP0328229-0001	82.5	*Bacillus sp*	270	268
WO03054185	82.5	*Bacillus alkalophilus*	268	268
US20140045268-0061	82.5	*Artificial Sequence*	382	268
WO201 1140364	82.5	*Bacillus lentus*	380	268
US20140045268-0038	82.1	*Artificial Sequence*	353	268
WO20 11072099	82.1	*Bacillus lentus*	269	268
WO2008153934	82.1	*Bacillus lentus*	269	268
WO9402618	82.1	*Bacillus novalis*	269	268
EP1 160327	82.1	*Bacillus sp*	269	268
US6271012	82.1	*Bacillus novalis*	269	268
WO2012151534	82.1	*Bacillus lentus*	269	268
EP2650354-0004	82.1	*Bacillus lentus*	269	268
JP2013153763-0002	82.1	*B. lentus (subtilisin 309)*	273	268
EP0415296	82.1	*Bacillus alcalophilus*	269	268
US20130123162	82.1	*Bacillus lentus*	269	268
WO2006136160-0012	82.1	*Bacillus clausii*	380	268

Table 8. List of sequences with percent identity to Bps02592 protein identified from the Genome Quest Patent database				
Patent ID #	**PID**	**Organism**	**Sequence Length**	**Alignment Length**
DE4411223	82.1	*Bacillus sp*	380	268
DE4224125	82.1	*B. alcalophilus; HA1 DSM 5466*	380	268
EP2589651-0006	82.1	*Bacillus subtilis*	380	268
US20140045268-0059	82.1	*Bacillus clausii*	382	268
WO201 1140364	82.1	*Bacillus lentus*	380	268
JP2013527767-0004	82.1	*Bacillus lentus*	380	268
CA2829859	81.8	*Bacillus lentus*	269	269
WO9402618	81.7	*Bacillus novalis*	269	268

Table 9. List of sequences with percent identity to BspQ01211 protein identified from the Genome Quest Patent database				
Patent ID #	**PID**	**Organism**	**Sequence Length**	**Alignment Length**
US20140011259-0034	91.5	*Bacillus sp.*	269	269
WO02077289	91.5	*Bacillus sp.*	382	269
US20140045268-0051	91.5	*Bacillus sp.*	269	269
WO03054185	91.4	*Bacillus sp.*	268	268
US20050113273-0008	91.0	*Bacillus sp.*	267	268
US20050009167-0008	91.0	*Bacillus sp.*	267	268
WO9402618	83.2	*Bacillus novalis*	269	268
EP1160327	83.2	*Bacillus sp.*	269	268
US20140045268-0040	82.8	*Bacillus clausii*	353	268
WO9402618	82.8	*Bacillus novalis*	269	268
EP1160327	82.8	*Bacillus sp*	269	268
EP2589651-0003	82.8	*Artificial Sequence*	269	268
WO2011072099	82.8	*Bacillus lentus*	269	268
WO2007006305	82.8	*Bacillus lentus*	269	268
US20130123162	82.8	*Bacillus lentus*	269	268
WO2012151534	82.8	*Bacillus lentus*	269	268
WO2012151480	82.8	*Bacillus lentus*	269	268
EP0415296	82.8	*Bacillus alcalophilus*	269	268
WO0218588	82.8	*Hordeum sp B. lentus Chimeric*	368	268
WO02077289	82.8	*Bacillus alcalophilus*	377	268
US8623630-0025	82.8	*Bacillus alcalophilus*	380	268
US20100105599-0261	82.8	*Bacillus*	380	268
EP0283075-0003	82.8	*Bacillus sp.*	380	268
EP0328229-0001	82.8	*Bacillus sp.*	270	268
WO03054185	82.8	*Bacillus alkalophilus*	268	268

Table 9. List of sequences with percent identity to BspQ01211 protein identified from the Genome Quest Patent database

Patent ID #	PID	Organism	Sequence Length	Alignment Length
US20140045268-0061	82.8	*Artificial Sequence*	382	268
WO201 1140364	82.8	*Bacillus lentus*	380	268
US20140045268-0038	82.5	*Artificial Sequence*	353	268
WO20 11072099	82.5	*Bacillus lentus*	269	268
WO2012151480	82.5	*Bacillus lentus*	269	268
WO2008153934	82.5	*Bacillus lentus*	269	268
WO9402618	82.5	*Bacillus novalis*	269	268
EP1 160327	82.5	*Bacillus sp*	269	268
WO20 11072099	82.5	*Bacillus lentus*	269	268
US20130225466-0004	82.5	*Bacillus lentus*	269	268
JP2013153763-0002	82.5	*B. lentus*	273	268
WO2007006305	82.5	*Bacillus lentus*	269	268
JP1993361428-0006	82.5	*Bacillus clausii KSM-K16*	269	268
EP0415296	82.5	*Bacillus alcalophilus*	269	268
CA2829859	82.5	*Bacillus lentus*	269	268
WO2012151534	82.5	*Bacillus lentus*	269	268
CA2829859	82.5	*Bacillus lentus*	269	268
WO20051 18793-0018	82.5	*Bacillus sp*	380	268
JP2007074934-0008	82.5	*Bacillus clausii KSM-K16*	380	268
W09855634	82.5	*Bacillus lentus*	380	268
DE4411223	82.5	*Bacillus sp*	380	268
DE4224125	82.5	*B. alcalophilus; HA1 DSM 5466*	380	268
EP2647692-0006	82.5	*Artificial Sequence*	380	268
JP2008022828-0033	82.5	*Bacillus clausii KSM-K16*	355	268
US20140045268-0059	82.5	*Bacillus clausii*	382	268
WO201 1140364	82.5	*Bacillus lentus*	380	268
JP2013526853-0019	82.5	*Bacillus lentus*	380	268
CA2829859	82.2	*Bacillus lentus*	269	269

Alignment of Homologous Sequences

[00203] The amino acid sequences of mature Bpan01744 (SEQ ID NO:3), BspAL03240 (SEQ ID NO:6), Bps02592 (SEQ ID NO:9), and BspQ01211 (SEQID NO: 12) proteases were aligned with multiple subtilisin sequences from Tables 2-9 using the MUSCLE program from Geneious software (Biomatters Ltd.) (Robert C. Edgar. MUSCLE: multiple sequence alignment with high accuracy and high throughput Nucl. Acids Res. (2004) 32 (5): 1792-1797) with the default

parameters. Figures 8.1-8.3 show the alignment of Bpan01744, BspAL03240, Bps02592 and BspQ01211 proteases with these protease sequences over the region of residues 1 to 269 of Bpan01744, BspAL03240, Bps02592 and BspQ01211.

[00204] A phylogenetic tree (Figure 9) for mature sequences of Bpan01744 (SEQ ID NO:3), BspAL03240 (SEQ ID NO:6), Bps02592 (SEQ ID NO:9), and BspQ01211 (SEQ ID NO: 12) was built from Figure 8.1-8.3 using the Geneious Tree builder program.

[00205] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein can be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

We claim:

1. A recombinant polypeptide or an active fragment thereof, comprising an amino acid sequence having at least 93% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:3, 6, 9, and 12.
2. The recombinant polypeptide or active fragment thereof of Claim 1, with the proviso that the polypeptide or active fragment thereof does not comprise WP_034632645 or WP_047986748.
3. The recombinant polypeptide or active fragment thereof of Claim 1 or 2, wherein the polypeptide has protease activity.
4. The recombinant polypeptide or active fragment thereof of Claim 3, wherein the protease activity is subtilisin protease activity.
5. The recombinant polypeptide or active fragment thereof of any one of the above Claims, wherein the polypeptide has protease activity in the presence of a surfactant.
6. The recombinant polypeptide or active fragment thereof of any of the above claims, wherein the polypeptide has cleaning activity in a detergent composition.
7. The recombinant polypeptide or active fragment thereof of Claim 6, wherein the detergent composition is an automatic dishwashing detergent and/or the cleaning activity comprises hydrolysis of an egg yolk substrate.
8. The recombinant polypeptide or active fragment thereof of Claim 6, wherein the detergent composition is a laundry detergent and/or the cleaning activity comprises hydrolysis of a substrate selected from the group consisting of blood, milk, ink and combinations thereof.
9. The recombinant polypeptide or active fragment thereof of Claim 8, wherein the laundry detergent is a liquid laundry detergent or a powder laundry detergent.
10. The recombinant polypeptide or active fragment thereof of any of the above claims, wherein the polypeptide has a thermostability $T_{50\%}$ value of at least 60°C.
11. A composition comprising a surfactant and the recombinant polypeptide or active fragment thereof of the above Claims.
12. The composition of Claim 11, wherein the surfactant is selected from the group consisting of an anionic surfactant, a cationic surfactant, a zwitterionic surfactant, an ampholytic surfactant, a semi-polar non-ionic surfactant, and a combination thereof.

13. The composition of Claim 11 or 12, wherein the composition is a detergent composition.
14. The composition of Claim 13, wherein the detergent composition is selected from the group consisting of a laundry detergent, a fabric softening detergent, a dishwashing detergent, and a hard-surface cleaning detergent.
15. The composition of any one of Claims 11-14, wherein said composition further comprises at least one calcium ion and/or zinc ion; at least one stabilizer; from about 0.001% to about 1.0 weight % of said recombinant polypeptide; at least one bleaching agent; at least one adjunct ingredient; and/or one or more additional enzymes or enzyme derivatives selected from the group consisting of acyl transferases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinosidases, aryl esterases, beta-galactosidases, carrageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1, 4-glucanases, endo-beta-mannanases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipoxygenases, mannanases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases, peroxidases, phenoloxidases, phosphatases, phospholipases, phytases, polygalacturonases, proteases, pullulanases, reductases, rhamnogalacturonases, beta-glucanases, tannases, transglutaminases, xylan acetyl-esterases, xylanases, xyloglucanases, xylosidases, metalloproteases, additional serine proteases, and combinations thereof.
16. The composition of any one of Claims 11-15, wherein said composition contains phosphate or is phosphate-free and/or contains borate or is borate-free.
17. The composition of any one of Claims 11-16, wherein said composition is a granular, powder, solid, bar, liquid, tablet, gel, paste or unit dose composition.
18. The composition of any one of Claims 11-17, wherein said composition is formulated at a pH of from about 8 to about 12.
19. A method of cleaning comprising contacting a surface or an item in need of cleaning with the recombinant polypeptide or active fragment thereof of any one of Claims 1-10, or the composition of any one of Claims 11-18; and optionally further comprising the step of rinsing said surface or item after contacting said surface or item with said recombinant polypeptide or composition.
20. The method of Claim 19, wherein said item is dishware or fabric.
21. A polynucleotide comprising a nucleic acid sequence encoding the recombinant

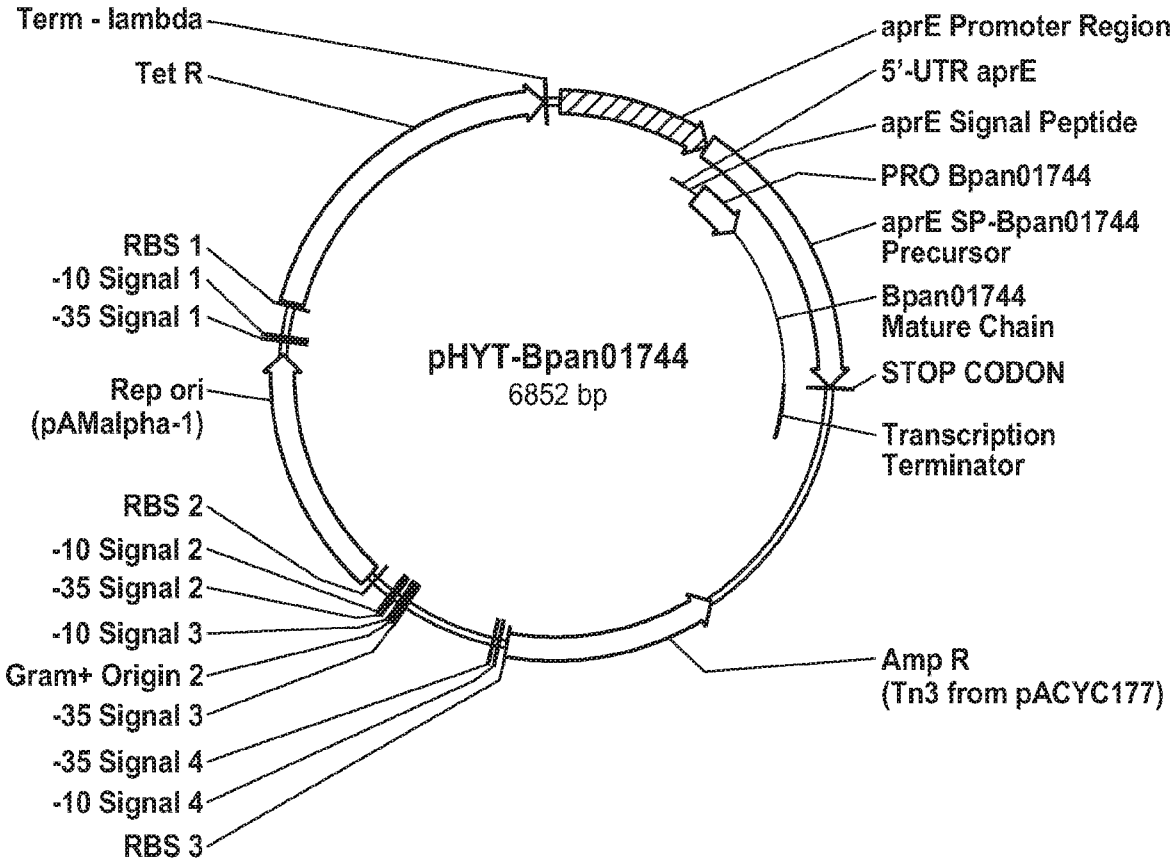
polypeptide or active fragment thereof of any one of Claims 1-10.

22. The polynucleotide of claim 21, comprising a nucleic acid sequence having at least 93% identity to the nucleic acid sequence of SEQ ID NO:l, 4, 7, or 10.
23. An expression vector comprising the polynucleotide of Claim 21 or 22.
24. A host cell comprising the vector of Claim 23.
25. The host cell of Claim 24, wherein the host cell is of a species selected from *Bacillus spp., Streptomyces spp., Escherichia spp., Aspergillus spp., Trichoderma spp., Pseudomonas spp., Corynebacterium spp., Saccharomyces spp.,* and *Pichia spp.*
26. The host cell of Claim 25, wherein said *Bacillus spp.* is *Bacillus subtilis.*
27. A method for producing the recombinant polypeptide, or active fragment thereof of Claims 1-10 comprising:

    (a) stably transforming the host cell of any one of Claims 24-26 with the expression vector of Claim 23;

    (b) cultivating said transformed host cell under conditions suitable for said host cell to produce said polypeptide; and

    (c) recovering said polypeptide.
28. The method of Claim 27, wherein said expression vector comprises a heterologous polynucleotide sequence encoding a heterologous pro-peptide.
29. The method of Claim 27 or 28, wherein said expression vector comprises one or both of a heterologous promoter and a polynucleotide sequence encoding a heterologous signal peptide.
30. A composition comprising the recombinant polypeptide or active fragment thereof of any one of Claims 1-10, wherein said composition is an animal feed, contact lens cleaning, wound cleaning, or textile, leather or feather processing composition.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Rep ori
(pAMalpha-1)
RBS 2
-10 Signal 2
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Gram+ Origin 2
-35 Signal 3
-35 Signal 4
-10 Signal 4
RBS 3
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6852 bp
aprE Promoter Region
5'-UTR aprE
aprE Signal Peptide
PRO Bpan01744
aprE SP-Bpan01744
Precursor
Bpan01744
Mature Chain
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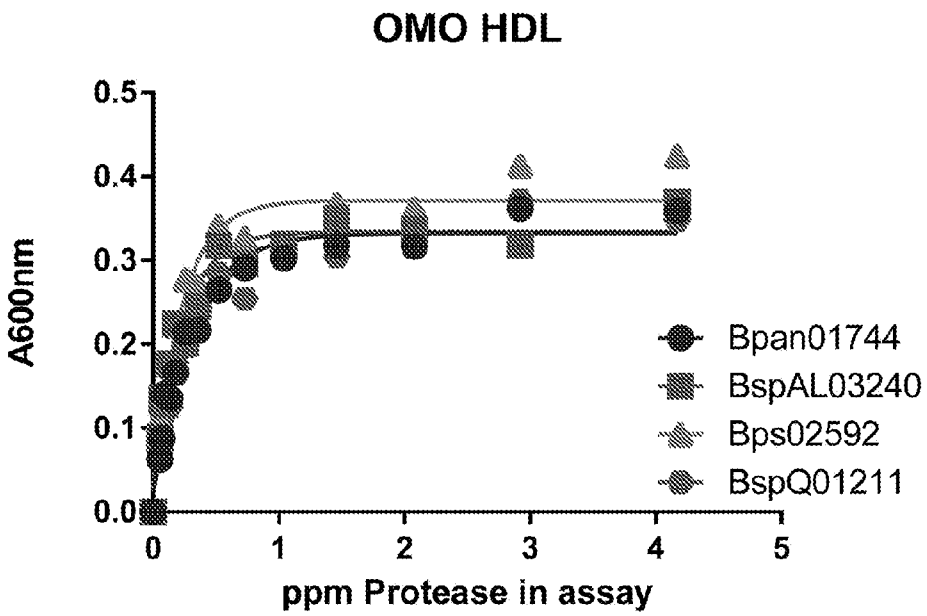
Transcription
Terminator
Amp R
(Tn3 from pACYC177)

FIG. 1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0.3
0.2
0.1
0.0
0
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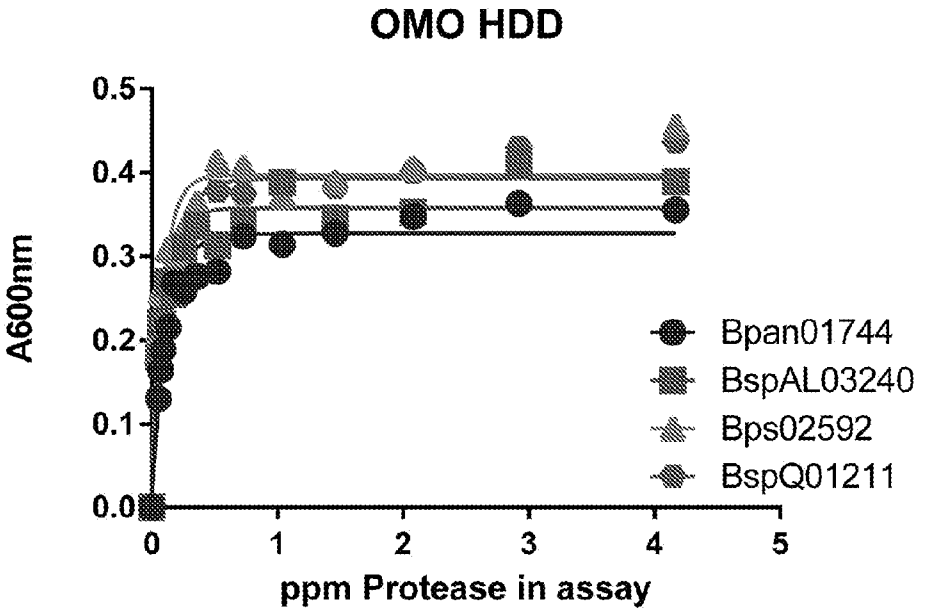
2
3
4
5
ppm Protease in assay
Bpan01744
BspAL03240
Bps02592
BspQ01211

FIG. 2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0.1
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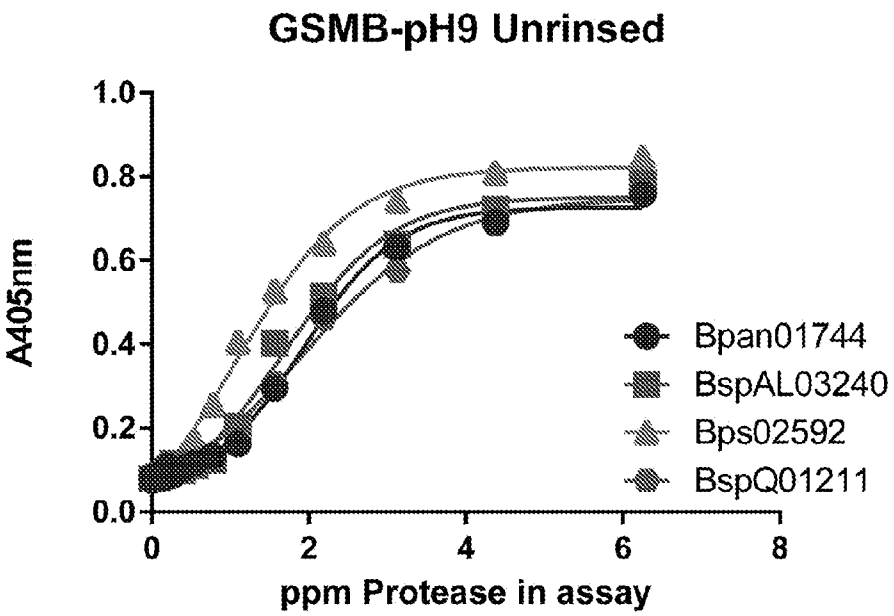
Bpan01744
BspAL03240
Bps02592
BspQ01211

FIG. 3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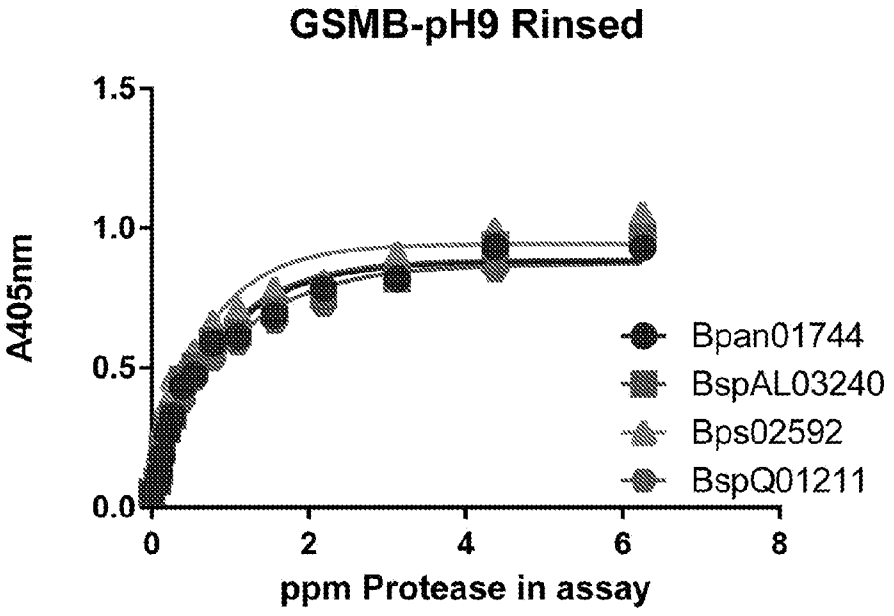
0.4
0.2
0.0
0
2
4
6
8
ppm Protease in assay
Bpan01744
BspAL03240
Bps02592
BspQ01211

FIG. 4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1.0
0.5
0.0
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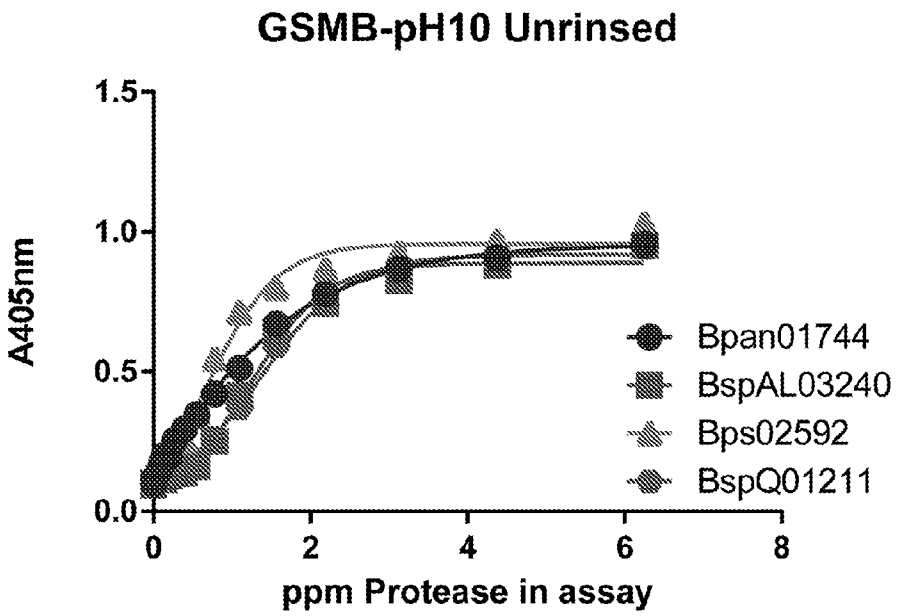
2
4
6
8
ppm Protease in assay
Bpan01744
BspAL03240
Bps02592
BspQ01211

FIG. 5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1.0
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0
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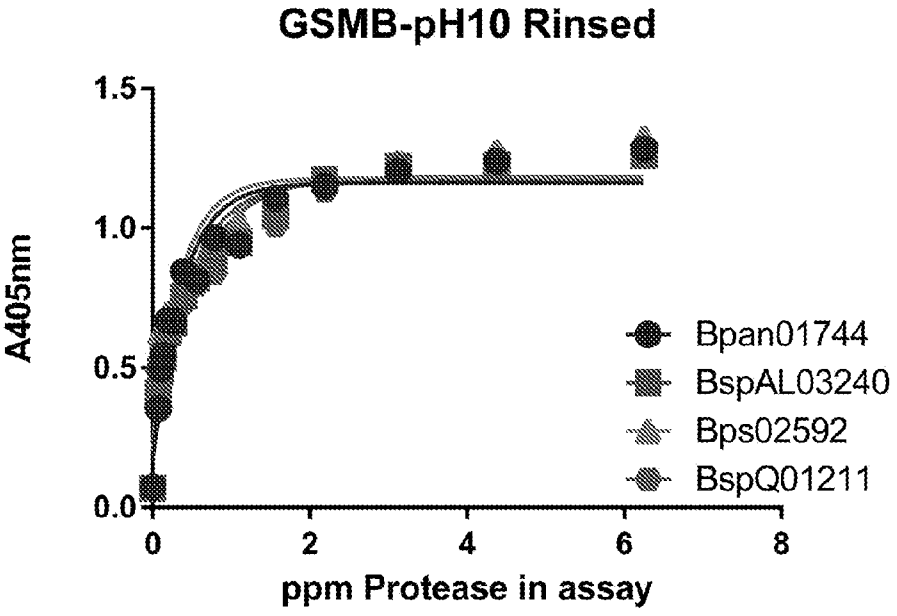
4
6
8
ppm Protease in assay
Bpan01744
BspAL03240
Bps02592
BspQ01211

FIG. 6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1.0
0.5
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0
2
4
6
8
ppm Protease in assay
Bpan01744
BspAL03240
Bps02592
BspQ01211

FIG. 7

```
                                1       10        20        30        40        50        60
                                |       |         |         |         |         |         |
                    Bpan01744   AQSVPWGISRVQAQSAHNRGITGSGVKVAVLDTGI-STHEDLNVRGGASFVAGEPG-YQD
                  BspAL03240    AQSVPWGISRVQAQSAHNRGITGSGVKVAVLDTGI-STHEDLNVRGGASFVAGEPG-YQD
                      Bps02592  NQTTPWGITRVQAPTALNRGFTGSGVRVAVLDTGI-ATHSDLNIRGGVSFVSGEPG-YQD
                    BspQ01211   NQTIPWGITRVQAPAAAINRGFTGAGVRVAVLDTGI-SNHPDLNIRGGVSFVPGEST-YQD
Bacillus_okhensis_WP_034632645.1 NQTIPWGITRVQAPAAAINRGFTGAGVRVAVLDTGI-SNHPDLNIRGGVSFVPGEST-YQD
      B_lehensis_WP_038486090.1 MQTVPWGINRVQAPIAQSRGFTGTGVRVAVLDTGI-SNHADLRIRGGASFVPGEPN-ISD
B_alcalophilus_WP_003321226.1   NQVTPWGITRVQAPTAWTRGYTGTGVRVAVLDTGI-STHPDLNIRGGVSFVPGEPS-YQD
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                B_lentus_P29600 AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLNIRGGASFVPGEPS-TQD
        B_pseudofirmus_ADC49870 AQTVPWGIPYIYSDVVHRQGYFGNGVKVAVLDTGV-APHPDLHIRGGVSFISTENT-YVD
B_amyloliquefaciens_CAA24990    AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQD
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      B_mojavensis_WP010333625  AQSVPYGISQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLNVRGGASFVPSETNPYQD
          Bacillus_sp_sprC_AAC43580 AQTVPWGIPHIKADKAHAAGVTGSGVKVAILDTGIDANHADLNVKGGASFVSGEPNALQD
B_sonorensis_WP_006636716       AQTVPYGIPLIKADKVQAQGYKGANVKVGIIDTGIASSHTDLKVVGGASFVSGESY-NTD
        B_licheniformis_CAJ70731.1 AQTVPYGIPLIKADKVQAQGFKGANVKVAVLDTGIQASHPDLNVVGGASFVAGEAY-NTD
```

FIG. 8A

Bpan01744	GNGHGTHVAGTIAALNNSIGVLGVAPNAELYAVKVLGASGSGSISGIAQGLQWAGNNGMH
BspAL03240	GNGHGTHVAGTIAALNNSLGVLGVAPNAELYAVKVLGASGSGSISGIAQGLQWAGNNGMH
Bps02592	GNGHGTHVAGTIAALNNSIGVIGVAPNAELYAVKVLGANGSGSVSAIAQGLQWSAQNNMH
BspQ01211	GNGHGTHVAGTIAALNNSIGVVGVAPNTELYAVKVLGANGSGSISSIAQGLQWTAQNNIH
Bacillus_okhensis_WP_034632645.1	GNGHGTHVAGTIAALNNSIGVVGVAPNTELYAVKVLGANGSGSISSIAQGLQWTAQNNIH
B_lehensis_WP_038486090.1	GNGHGTHVAGTIAALNNSIGVLGVAPNVDLYGVKVLGASGSGSISGIAQGLQWAANNGMH
B_alcalophilus_WP_003321226.1	GNGHGTHVAGTIAALNNSIGVVGVAPNAELYAVKVLGANGSGSVSSIAQGLQWTAQNNIH
WO2015089441-0056	GNGHGTHVAGTIAALNNSIGVVGVAPNAELYAVKVLGANGSGSVSSIAQGLQWTAQNNIH
US20140011259-0034	GNGHGTHVAGTIAALNNSIGVVGVAPNAELYAVKVLGANGSGSVSSIAQGLQWTAQNNIH
US20140045268-0051	GNGHGTHVAGTIAALNNSIGVVGVAPNAELYAVKVLGANGSGSVSSIAQGLQWTAQNNIH
B_gibsonii_AGS78407.1	LNGHGTHVAGTVAALNNSIGVLGVAPNAELYAVKVLGANGSGSVSGIAQGLEWAATNNMH
B_lentus_P29600	GNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
B_pseudofirmus_ADC49870	YNGHGTHVAGTVAALNNSYGVLGVAPGAELYAVKVLDRNGSGSHASIAQGIEWAMNNGMD
B_amyloliquefaciens_CAA24990	NNSHGTHVAGTVAALNNSIGVLGVAPSASLYAVKVLGADGSGQVSWIINGIEWAIANNMD
B_vallismortis_WP010329279	GSSHGTHVAGTVAALNNSIGVLGVAPNASLYAVKVLDSTGNGQVSWIINGIEWAISNKMD
B_mojavensis_WP010333625	GSSHGTHVAGTVAALNNTIGVLGVAPSASLYAVKVLDSTGSGQVSWIINGIEWAISNNMD
Bacillus_sp_sprC_AAC43580	GNGHGTHVAGTVAALNNTTGVLGVAYNADLYAVKVLSASGSGTLSGIAQGIEWSISNGMN
B_sonorensis_WP_006636716	GNGHGTHVAGTVAALDNTTGVLGVAPNVSLYAIKVLNSSGSGTYSAIVSGIEWATQNGLD
B_licheniformis_CAJ70731.1	GNGHGTHVAGTVAALDNTTGVLGVAPSVSLYAVKVLNSSGSGSYSGIVSGIEWATTNGMD

FIG. 8B

```
Bpan01744                    IANMSLGTSAPSATLEQAVNAATSRGVLVIAASGNSGA----GSVGYPARYANAMAVGAT
BspAL03240                   IANMSLGTSAPSATLEQAVNAATSRGVLVIAASGNSGA----GSVGYPARYANAMAVGAT
Bps02592                     IANLSLGSPTGSQTLELAVNQANSAGVLVVAASGNNGS----GTVSYPARYTNALAVGAT
BspQ01211                    VANLSLGSSTGSQTLELAVNQATSAGVLVVAASGNNGS----GTISYPARYANALAVGAT
Bacillus_okhensis_WP_034632645.1 VANLSLGSPTGSQTLELAVNQATSAGVLVVAASGNNGS----GTISYPARYANALAVGAT
B_lehensis_WP_038486090.1    IANMSLGSSAGSATMEQAVNQATASGVLVVAASGNSGA----GNVGFPARYANAMAVGAT
B_alcalophilus_WP_003321226.1 VANLSLGSPVGSQTLELAVNQATNAGVLVVAATGNNGS----GTVSYPARYANALAVGAT
WO2015089441-0056            VANLSLGSPVGSQTLELAVNQATNAGVLVVAATGNNGS----GTVSYPARYANALAVGAT
US20140011259-0034           VANLSLGSPVGSQTLELAVNQATNAGVLVVAATGNNGS----GTVSYPARYANALAVGAT
US20140045268-0051           VANLSLGSPVGSQTLELAVNQATNAGVLVVAATGNNGS----GTVSYPARYANALAVGAT
B_gibsonii_AGS78407.1        IANMSLGSDFPSSTLERAVNYATSRDVLVIAATGNNGS----GSVGYPARYANAMAVGAT
B_lentus_P29600              VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
B_pseudofirmus_ADC49870      IANMSLGSPSGSTTLQLAADRARNAGVLLIGAAGNSGQQGGSNNMGYPARYASVMAVGAV
B_amyloliquefaciens_CAA24990 VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAV
B_vallismortis_WP010329279   VINMSLGGPSGSTALKSVVDRAVASGIVVVAAAGNEGTSGSSSTIGYPAKYPSTIAVGAV
B_mojavensis_WP010333625     VINMSLGGPTGSTALKTVVDKAVASGIVVVAAAGNEGSSGSTSTVGYPAKYPSTIAVGAV
Bacillus_sp_sprC_AAC43580    VINMSLGGSSGSTALQQACNNAYNRGIVVIAAAGNSGSSGNRNTMGYPARYSSVIAVGAV
B_sonorensis_WP_006636716    VINMSLGGPSGSTALKQAVDKAYASGIVVVAAAGNSGSSGSQNTIGYPAKYDSVIAVGAV
B_licheniformis_CAJ70731.1   VINMSLGGASGSTAMKQAVDNAYARGVVVVAAAGNSGSSGNTNTIGYPAKYDSVIAVGAV
```

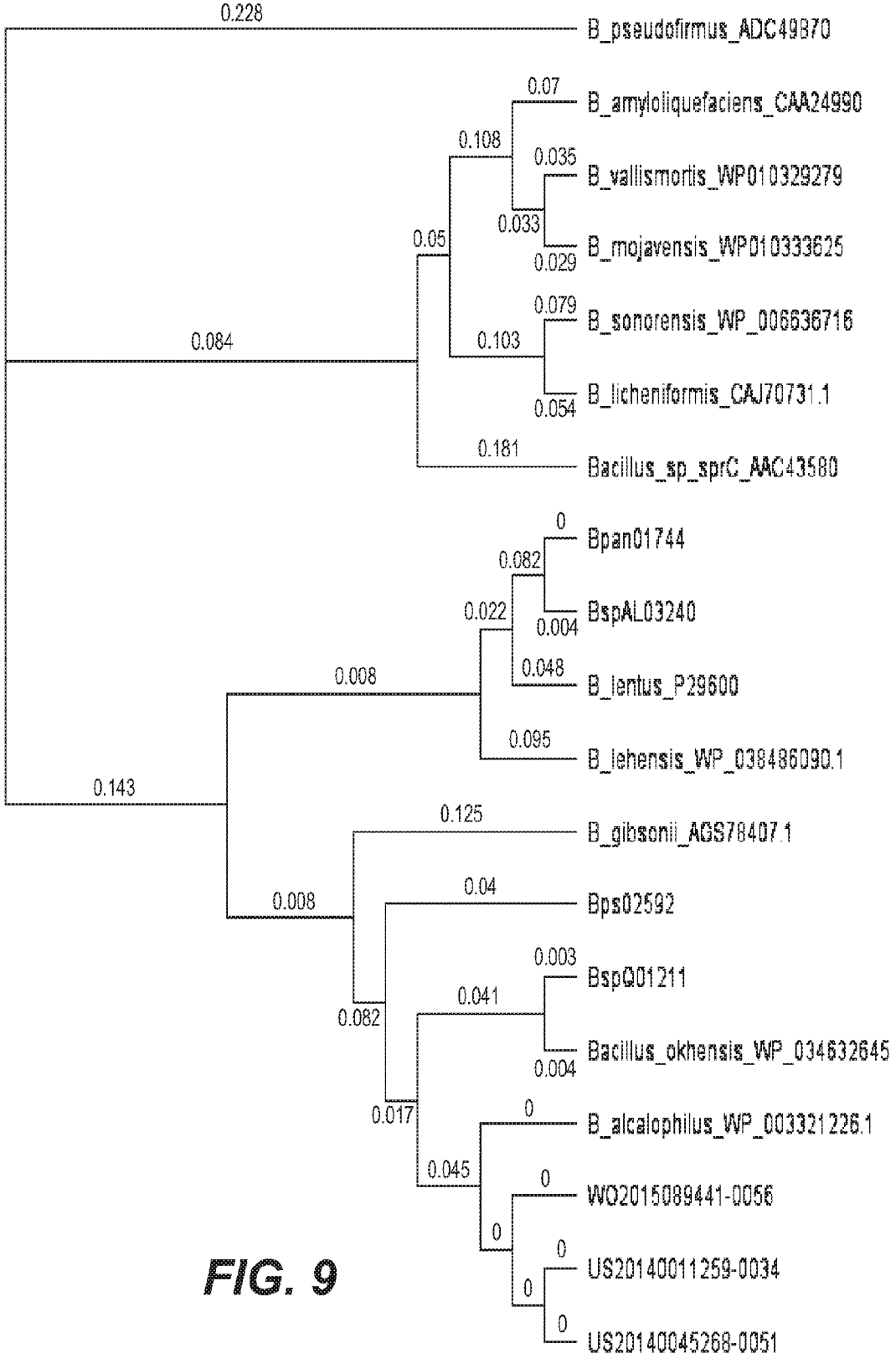
FIG. 8C

```
Bpan01744                    DQNNNRASFSQYGAGLDIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQKNPS
BspAL03240                   DQNNNRASFSQYGAGLDIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQKNPS
Bps02592                     DQNNNRASFSQYGTGLNIVAPGVGVQSTYPGNRYASLNGTSMATPHVAGVAALVKQKNPS
BspQ01211                    DQNNNRASFSQYGTGLNIVAPGVGVQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPS
Bacillus_okhensis_WP_034632645.1 DQNNNRASFSQYGTGLNIVAPGVGVQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPG
B_lehensis_WP_038486090.1    DQNNNRASFSQYGAGLDIVAPGVGVQSTVPGNGYASFNGTSMATPHVAGVAALVKQKNPS
B_alcalophilus_WP_003321226.1 DQNNNRASFSQYGTGLNIVAPGVGIQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPS
WO2015089441-0056            DQNNNRASFSQYGTGLNIVAPGVGIQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPS
US20140011259-0034           DQNNNRASFSQYGTGLNIVAPGVGIQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPS
US20140045268-0051           DQNNNRASFSQYGTGLNIVAPGVGIQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPS
B_gibsonii_AGS78407.1        DQNNRRANFSQYGTGIDIVAPGVNVQSTYPGNRYVSMNGTSMATPHVAGAAALVKQRYPS
B_lentus_P29600              DQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSIYASLNGTSMATPHVAGAAALVKQKNPS
B_pseudofirmus_ADC49870      DQNGNRANFSSYGSELEIMAPGVNINSTYLNNGYRSLNGTSMASPHVAGVAALVKQKHPH
B_amyloliquefaciens_CAA24990 DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
B_vallismortis_WP010329279   NSSNQRGSFSSVGPELDVMAPGVSIQSTLPGGTYGSYNGTSMATPHVAGAAALILSKHPT
B_mojavensis_WP010333625     NSSNQRASFSSAGSELDVMAPGVSIQSTLPGGTYGAYNGTSMATPHVAGAAALILSKHPT
Bacillus_sp_sprC_AAC43580    SSNNTRASFSSVGSELEVMAPGVNILSTTPGNNYASFNGTSMAAPHVAGAAALIKAKYPS
B_sonorensis_WP_006636716    DSNKNRASFSSVGSELEVMAPGVSVYSTYPSNTYTSLNGTSMASPHVAGAAALILSKYPT
B_licheniformis_CAJ70731.1   DSNSNRASFSSVGAELEVMAPGAGVYSTYPTNTYATLNGTSMASPHVAGAAALILSKHPN
```

FIG. 8D

Bpan01744	WSNVQVRNHLKNTATNLGNTNLYGSGLVNAEAATR
BspAL03240	WSNVQVRNHLKNTATNLGNTNLYGSGLVNAEAATR
Bps02592	WSNTQIRNHLLNTATSLGSSTQFGSGLVNAEAATR
BspQ01211	WSNTQIRQHLLNTATPLGSSNQYGSGLVNAEAATR
Bacillus_okhensis_WP_034632645.1	WSNTQIRQHLLNTATPLGSSNQYGSGLVNAEAATR
B_lehensis_WP_038486090.1	WSNVQIRNHLKNTATNLGNTTQFGSGLVNAEAATR
B_alcalophilus_WP_003321226.1	WSNTQIRQHLTSTATSLGNSNQFGSGLVNAEAATR
WO2015089441-0056	WSNTQIRQHLTSTATSLGNSNQFGSGLVNAEAATR
US20140011259-0034	WSNTQIRQHLTSTATSLGNSNQFGSGLVNAEAATR
US20140045268-0051	WSNTQIRQHLTSTATSLGNSNQFGSGLVNAEAATR
B_gibsonii_AGS78407.1	WNATQIRNHLKNTATNLGNSSQFGSGLVNAEAATR
B_lentus_P29600	WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR
B_pseudofirmus_ADC49870	LTAAQIRNRMNQTAIPLGNSTYYGNGLVDAEYAAQ
B_amyloliquefaciens_CAA24990	WTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ
B_vallismortis_WP010329279	WTNTQVRNRLESTITYLGSSFYYGKGLINVQAAAQ
B_mojavensis_WP010333625	WTNAQVRDRLESTATYLGSSFYYGKGLINVQAAAQ
Bacillus_sp_sprC_AAC43580	MTNVQIRERLKNTATNLGDPTFYGKGVINVESALQ
B_sonorensis_WP_006636716	LSASQVRNRLSSTATNLGDSFYYGKGLINVEAAAQ
B_licheniformis_CAJ70731.1	LSASQVRNRLSSTATYLGSSFYYGKGLINVEAAAQ

FIG. 8E


0.228
B_pseudofirmus_ADC49870
0.07
B_amyloliquefaciens_CAA24990
0.108
0.035
B_vallismortis_WP010329279
0.033
0.05
B_mojavensis_WP010333625
0.029
0.079
B_sonorensis_WP_006636716
0.084
0.103
B_licheniformis_CAJ70731.1
0.054
0.181
Bacillus_sp_sprC_AAC43580
0
Bpan01744
0.082
0.022
BspAL03240
0.004
0.048
0.008
B_lentus_P29600
0.095
B_lehensis_WP_038486090.1
0.143
0.125
B_gibsonii_AGS78407.1
0.04
0.008
Bps02592
0.003
BspQ01211
0.041
0.082
Bacillus_okhensis_WP_034632645
0.004
0
0.017
B_alcalophilus_WP_003321226.1
0.045
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WO2015089441-0056
0
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US20140011259-0034
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0
US20140045268-0051

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/057520

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/54 C11D3/386
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C11D

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 100 64 983 Al (HENKEL KGAA [DE]) 18 July 2002 (2002-07-18) examples 5,6; table 3; sequence 2	1-30
X	EP 1 160 327 A2 (GENENC0R INT [US]) 5 December 2001 (2001-12-05) figure 2; example 1	1-30
X	W0 2009/058303 A2 (DANISC0 US INC GENENC0R DIV [US]; ESTELL DAVID A [US]; H0MMES RONALDUS) 7 May 2009 (2009-05-07) cited in the application example 7	1-30

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2016	18/04/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	van Klompenburg, Wim

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/057520

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2012009651 A1	12-01-2012
		US 2014099698 A1	10-04-2014
		US 2016032266 A1	04-02-2016
		WO 2009058303 A2	07-05-2009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-30(partially)

   A recombinant polypeptide or an active fragment thereof comprising an amino acid sequence having at least 93% identity to SEQ ID N0:3. Compositions comprising the polypeptide. A method of cleaning. A polynucleotide comprising a nucleic acid encoding said recombinant polypeptide. A method for producing the recombinant polypeptide.

---

2. claims: 1-30(partially)

   A recombinant polypeptide or an active fragment thereof comprising an amino acid sequence having at least 93% identity to SEQ ID N0: 6. Compositions comprising the polypeptide. A method of cleaning. A polynucleotide comprising a nucleic acid encoding said recombinant polypeptide. A method for producing the recombinant polypeptide.

---

3. claims: 1-30(partially)

   A recombinant polypeptide or an active fragment thereof comprising an amino acid sequence having at least 93% identity to SEQ ID N0:9. Compositions comprising the polypeptide. A method of cleaning. A polynucleotide comprising a nucleic acid encoding said recombinant polypeptide. A method for producing the recombinant polypeptide.

---

4. claims: 1-30(partially)

   A recombinant polypeptide or an active fragment thereof comprising an amino acid sequence having at least 93% identity to SEQ ID NO: 12. Compositions comprising the polypeptide. A method of cleaning. A polynucleotide comprising a nucleic acid encoding said recombinant polypeptide. A method for producing the recombinant polypeptide.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/057520

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-30(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.