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(56) Fremdragne publikationer:

**EP-A1- 2 287 318****WO-A1-2012/149317****WO-A1-2012/149325****WO-A1-2012/149333****WO-A1-2014/100018****WO-A2-2009/108941****CN-A- 1 904 052****DATABASE UniProt [Online] 2 November 2010 (2010-11-02), "SubName: Full=Endoglucanase  
{ECO:0000313|EMBL:ADM68451.1}; EC=3.2.1.78 {ECO:0000313|EMBL:ADM68451.1};", XP002779513, retrieved  
from EBI accession no. UNIPROT:E0RFB6 Database accession no. E0RFB6****DATABASE UniProt [Online] 11 January 2011 (2011-01-11), "SubName: Full=Endoglucanase  
{ECO:0000313|EMBL:ADO54643.1};", XP002779514, retrieved from EBI accession no. UNIPROT:E3EGK4  
Database accession no. E3EGK4**

DATABASE UniProt [Online] 22 September 2009 (2009-09-22), "SubName: Full=Beta-mannanase {ECO:0000313|EMBL:ACU30843.1}; EC=3.2.1.78 {ECO:0000313|EMBL:ACU30843.1};", XP002779515, retrieved from EBI accession no. UNIPROT:C7FFF2 Database accession no. C7FFF2

LI YANAN ET AL: "Gene cloning, expression, and characterization of a novel beta-mannanase from *Bacillus circulans* CGMCC 1416", JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, KOREAN SOCIETY FOR APPLIED MICROBIOLOGY, SEOUL, KR, vol. 18, no. 1, 1 January 2008 (2008-01-01), pages 160-166, XP009186548, ISSN: 1017-7825

PEILONG YANG ET AL: "A Novel [beta]-mannanase with High Specific Activity from *Bacillus circulans* CGMCC1554: Gene Cloning, Expression and Enzymatic Characterization", APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, vol. 159, no. 1, 24 September 2008 (2008-09-24), pages 85-94, XP055219229, United States ISSN: 0273-2289, DOI: 10.1007/s12010-008-8364-3

ZHAO YUEJU ET AL: "Crystallization and preliminary X-ray study of alkaline .beta.-mannanase from the alkaliphilic *Bacillus* sp N16-5", ACTA CRYSTALLOGRAPHICA. SECTION F: STRUCTURAL BIOLOGY AND CRYSTALLIZATION COMMUNICATIONS, WILEY-BLACKWELL PUBLISHING, INC, US, vol. 64, no. Part 10, 1 October 2008 (2008-10-01), pages 957-959, XP008120224, ISSN: 1744-3091, DOI: 10.1107/S1744309108028571 -& DATABASE UniProt [Online] 23 November 2004 (2004-11-23), "SubName: Full=Beta-mannanase {ECO:0000313|EMBL:AAT06599.1}; EC=3.2.1.78 {ECO:0000313|EMBL:AAT06599.1};", XP002747724, retrieved from EBI accession no. UNIPROT:Q5YEX6 Database accession no. Q5YEX6

YOSHIDA S ET AL: "CLONING, SEQUENCE ANALYSIS, AND EXPRESSION IN *ESCHERICHIA COLI* OF A GENE CODING FOR AN ENZYME FROM *BACILLUS CIRCULANS* K-1 THAT DEGRADES GUAR GUM", BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY, JAPAN SOCIETY FOR BIOSCIENCE, BIOTECHNOLOGY, AND AGROCHEMISTRY, TOKYO, JAPAN, vol. 62, no. 3, 1 January 1998 (1998-01-01), pages 514-520, XP000881879, ISSN: 0916-8451, DOI: 10.1271/BBB.62.514 -& DATABASE UniProt [Online] 1 August 1998 (1998-08-01), "SubName: Full=Mannanase {ECO:0000313|EMBL:BAA25878.1};", XP002747725, retrieved from EBI accession no. UNIPROT:O66185 Database accession no. O66185

PRAKRAM SINGH CHAUHAN ET AL: "Mannanases: microbial sources, production, properties and potential biotechnological applications", APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 93, no. 5, 8 February 2012 (2012-02-08), pages 1817-1830, XP035019260, ISSN: 1432-0614, DOI: 10.1007/S00253-012-3887-5

DHAWAN SAMRITIL ET AL: "Microbial mannanases: An overview of production and applications", CRC CRITICAL REVIEWS IN BIOTECHNOLOGY, CRC PRESS, BOCA RATON, FL, US, vol. 27, no. 4, 1 January 2007 (2007-01-01), pages 197-216, XP009161462, ISSN: 0738-8551

## DESCRIPTION

**[0001]** The present disclosure relates to endo- $\beta$ -mannanases from *Paenibacillus* or *Bacillus* spp, polynucleotides encoding such endo- $\beta$ -mannanases, compositions containing such mannanases, and methods of use thereof. Compositions containing such endo- $\beta$ -mannanases are suitable for use as detergents and cleaning fabrics and hard surfaces, as well as a variety of other industrial applications.

**[0002]** Mannanase enzymes, including endo- $\beta$ -mannanases, have been employed in detergent cleaning compositions for the removal of gum stains by hydrolyzing mannans. A variety of mannans are found in nature, such as, for example, linear mannan, glucomannan, galactomannan, and glucogalactomannan. Each such mannan is comprised of polysaccharides that contain a  $\beta$ -1,4-linked backbone of mannose residues that may be substituted up to 33% with glucose residues (Yeoman et al., *Adv Appl Microbiol*, Elsevier). In galactomannans or glucogalactomannans, galactose residues are linked in alpha-1,6-linkages to the mannan backbone (Moreira and Filho, *Appl Microbiol Biotechnol*, 79:165, 2008). Therefore, hydrolysis of mannan to its component sugars requires endo-1,4- $\beta$ -mannanases that hydrolyze the backbone linkages to generate short chain manno-oligosaccharides that are further degraded to monosaccharides by 1,4- $\beta$ -mannosidases. EP 2 287 318 A, WO 2014/100018 A1, WO 2012/149333 A1 and WO 2012/149325 disclose mannanases and their use in cleaning applications. CN 1 904 052 A discloses a  $\beta$ -mannanase designated MANB48, but suggests that SDS has an inhibitory effect on the enzyme.

**[0003]** Although endo- $\beta$ -mannanases have been known in the art of industrial enzymes, there remains a need for further endo- $\beta$ -mannanases that are suitable for particular conditions and uses.

**[0004]** The invention provides a cleaning composition comprising a surfactant and an endo- $\beta$ -mannanase polypeptide comprising an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NO: 8, 10, 12, 42, 43, 44, 46, 47, 48, 50, 51 and 52, or an active fragment thereof, wherein said polypeptide or active fragment thereof has mannanase activity in the presence of a surfactant.

**[0005]** In some embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment thereof contains Asn33-Asp-34-Leu35 (NDL), wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0006]** The endo- $\beta$ -mannanase polypeptide or active fragment thereof may further comprise a WXaKNDLXXAI motif at positions 30-38, wherein X<sub>a</sub> is F or Y and X is any amino acid, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0007]** For example, the endo- $\beta$ -mannanase polypeptide or active fragment thereof may further comprise a  $WX_aKNDLX_bX_cA$  motif at positions 30-38, wherein  $X_a$  is F or Y,  $X_b$  is N, Y or A, and  $X_c$  is A or T, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0008]** The endo- $\beta$ -mannanase polypeptide or active fragment thereof may further comprise a  $L_{262}D_{263}XXXGPXGXL_{272}T_{273}$ , motif at positions 262-273, where X is any amino acid and wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0009]** For example, the endo- $\beta$ -mannanase polypeptide or active fragment thereof may further comprise a  $L_{262}D_{263}M/LV/AT/AGPX_1GX_2L_{272}T_{273}$  motif at positions 262-273, where  $X_1$  is N, A or S and  $X_2$  is S, T or N, and wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0010]** The endo- $\beta$ -mannanase polypeptide may be an NDL-Clade-1 polypeptide further comprising a LDM/LATGPA/NGS/TLT motif at positions 262-273, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0011]** Alternatively, the endo- $\beta$ -mannanase polypeptide may be an NDL-Clade 2 polypeptide further comprising a LDLA/VA/TGPS/NGNLT motif at positions 262-273, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0012]** The endo- $\beta$ -mannanase polypeptide or active fragment thereof may have mannanase activity on locust bean gum galactomannan or konjac glucomannan, or is capable of hydrolyzing a substrate selected from the group consisting of guar gum, locust bean gum, and combinations thereof.

**[0013]** In some embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment thereof retains at least 70% of its maximal mannanase activity at a pH range of 4.5-9.0; retains at least 70% of its maximal mannanase activity at a pH range of 5.5-8.5; retains at least 70% of its maximal mannanase activity at a pH range of 6.0-7.5; retains at least 70% of its maximal mannanase activity at a temperature range of 40°C to 70°C; retains at least 70% of its maximal mannanase activity at a temperature range of 45°C to 65°C; and/or retains at least 70% of its maximal mannanase activity at a temperature range of 50°C to 60°C.

**[0014]** In some embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment thereof

does not comprise a carbohydrate-binding module.

**[0015]** The cleaning composition may further comprise at least one adjunct ingredient; and/or an enzyme selected from the group consisting of acyl transferases, amylases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinases, arabinosidases, aryl esterases, beta-galactosidases, beta-glucanases, carrageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1, 4-glucanases, endo-beta-mannanases, exo-beta-mannanases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipolytic enzymes, lipoxygenases, mannanases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases, perhydrolases, peroxidases, phenoloxidases, phosphatases, phospholipases, phytases, polygalacturonases, proteases, pullulanases, reductases, rhamnogalacturonases, beta-glucanases, tannases, transglutaminases, xylan acetyl-esterases, xylanases, xyloglucanases, xylosidases, metalloproteases, and combinations thereof.

**[0016]** In some embodiments, the cleaning composition:

1. (i) is a detergent composition selected from the group consisting of a laundry detergent, a fabric softening detergent, a dishwashing detergent, and a hard-surface cleaning detergent;
2. (ii) is in a form selected from the group consisting of a liquid, a powder, a granulated solid, a tablet, a sheet, and a unit dose;
3. (iii) is phosphate-free; or
4. (iv) is boron-free.

**[0017]** In some preferred embodiments, the surfactant is an ionic surfactant. In some embodiments, the ionic surfactant is selected from the group consisting of an anionic surfactant, a cationic surfactant, a zwitterionic surfactant, and a combination thereof.

**[0018]** The present disclosure further provides methods for hydrolyzing a mannan substrate present in a soil or stain on a surface, comprising: contacting the surface with the detergent composition to produce a clean surface. Also provided are methods of textile cleaning comprising: contacting a soiled textile with the detergent composition to produce a clean textile.

**[0019]** These and other aspects of compositions and methods of the present invention will be apparent from the following description.

#### **DESCRIPTION OF THE DRAWINGS**

**[0020]**

Figure 1 provides a plasmid map of p2JM-PspMan4.

Figures 2A-B show the cleaning performance of *Paenibacillus* and *Bacillus spp.* mannanases on Locust bean gum (CS-73) at pH 8, 20 minutes.

Figures 3A-C show the CLUSTAL W (1.83) multiple sequence alignment of mannanases including BciMan1, BciMan3, BciMan4, PamMan2, PpaMan2, PpoMan1, PpoMan2, PspMan4, PspMan5, PspMan9, and PtuMan2.

Figure 4 shows a phylogenetic tree of mannanases including BciMan1, BciMan3, BciMan4, PamMan2, PpaMan2, PpoMan1, PpoMan2, PspMan4, PspMan5, PspMan9, and PtuMan2 showing the branching of the NDL-Clade mannanases from other mannanases and the differentiation of NDL-Clade 1 and NDL-Clade 2.

Figure 5 shows the motif of the NDL-Clade mannanases at positions 30-38, using the conserved linear sequence numbering.

Figure 6 shows the motif of the NDL-Clade mannanases, including the NDL-Clade 1 and NDL-Clade 2 mannanases, that is between the conserved Leu262-Asp263 (LD) and conserved Leu272-Thr273 (LT) residues, using the conserved linear sequence numbering.

Figure 7 shows the potential structural consequences of motif changes found in the NDL-Clade mannanases. The closest known mannanase structure from *Bacillus* sp. JAMB-602 (1WKY) is shown in black while modelled structures of PspMan4, PspMan9 and PpaMan2 are shown in gray. The location of the deletion motif is highlighted by an arrow. The deletion motif is postulated to impact the structure of the loop in which it is located.

**[0021]** Described herein are endo- $\beta$ -mannanases from *Paenibacillus* or *Bacillus spp.*, polynucleotides encoding such endo- $\beta$ -mannanases, compositions containing such mannanases, and methods of use thereof. In one embodiment, the *Paenibacillus* and *Bacillus spp.* endo- $\beta$ -mannanases described herein have glycosyl hydrolase activity in the presence of detergent compositions. This feature of the endo- $\beta$ -mannanases described herein makes them well suited for use in a variety of cleaning and other industrial applications, for example, where the enzyme can hydrolyze mannans in the presence of surfactants and other components found in detergent compositions.

**[0022]** The following terms are defined for clarity. Terms and abbreviations not defined should be accorded their ordinary meaning as used in the art:

**[0023]** As used herein, a "mannan endo-1,4- $\beta$ -mannosidase," "endo-1,4- $\beta$ -mannanase," "endo- $\beta$ -1,4-mannase," " $\beta$ -mannanase B," " $\beta$ -1, 4-mannan 4-mannanohydrolase," "endo- $\beta$ -mannanase," " $\beta$ -D-mannanase," "1,4- $\beta$ -D-mannan mannanohydrolase," or "endo- $\beta$ -mannanase" (EC 3.2.1.78) refers to an enzyme capable of the random hydrolysis of 1,4- $\beta$ -D-mannosidic linkages in mannans, galactomannans and glucomannans. Endo-1,4- $\beta$ -mannanases are members of several families of glycosyl hydrolases, including GH26 and

GH5. In particular, endo- $\beta$ -mannanases constitute a group of polysaccharases that degrade mannans and denote enzymes that are capable of cleaving polyose chains containing mannose units (*i.e.*, are capable of cleaving glycosidic bonds in mannans, glucomannans, galactomannans and galactoglucomannans). The "endo- $\beta$ -mannanases" of the present disclosure may possess additional enzymatic activities (e.g., endo-1,4- $\beta$ -glucanase, 1,4- $\beta$ -mannosidase, celldextrinase activities, etc.).

**[0024]** As used herein, a "mannanase," "mannosidic enzyme," "mannolytic enzyme," "mannanase enzyme," "mannanase polypeptides," or "mannanase proteins" refers to an enzyme, polypeptide, or protein exhibiting a mannan degrading capability. The mannanase enzyme may be, for example, an endo- $\beta$ -mannanase, an exo- $\beta$ -mannanase, or a glycosyl hydrolase. As used herein, mannanase activity may be determined according to any procedure known in the art (See, e.g., Lever, Anal. Biochem, 47:248, 1972; U.S. Pat. No. 6, 602, 842; and International Publication No. WO 95/35362A1).

**[0025]** As used herein, "mannans" are polysaccharides having a backbone composed of  $\beta$ -1,4-linked mannose; "glucomannans" are polysaccharides having a backbone of more or less regularly alternating  $\beta$ -1,4 linked mannose and glucose; "galactomannans" and "galactoglucomannans" are mannans and glucomannans with alpha-1,6 linked galactose sidebranches. These compounds may be acetylated. The degradation of galactomannans and galactoglucomannans is facilitated by full or partial removal of the galactose sidebranches. Further the degradation of the acetylated mannans, glucomannans, galactomannans and galactoglucomannans is facilitated by full or partial deacetylation. Acetyl groups can be removed by alkali or by mannan acylesterases. The oligomers that are released from the mannanases or by a combination of mannanases and alpha-galactosidase and/or mannan acetyl esterases can be further degraded to release free maltose by  $\beta$ -mannosidase and/or  $\beta$ -glucosidase

**[0026]** As used herein, "catalytic activity" or "activity" describes quantitatively the conversion of a given substrate under defined reaction conditions. The term "residual activity" is defined as the ratio of the catalytic activity of the enzyme under a certain set of conditions to the catalytic activity under a different set of conditions. The term "specific activity" describes quantitatively the catalytic activity per amount of enzyme under defined reaction conditions.

**[0027]** As used herein, "pH-stability" describes the property of a protein to withstand a limited exposure to pH-values significantly deviating from the pH where its stability is optimal (e.g., more than one pH-unit above or below the pH-optimum, without losing its activity under conditions where its activity is measurable).

**[0028]** As used herein, the phrase "detergent stability" refers to the stability of a specified detergent composition component (such as a hydrolytic enzyme) in a detergent composition mixture.

**[0029]** As used herein, a "perhydrolase" is an enzyme capable of catalyzing a reaction that

results in the formation of a peracid suitable for applications such as cleaning, bleaching, and disinfecting.

**[0030]** As used herein, the term "aqueous," as used in the phrases "aqueous composition" and "aqueous environment," refers to a composition that is made up of at least 50% water. An aqueous composition may contain at least 50% water, at least 60% water, at least 70% water, at least 80% water, at least 90% water, at least 95% water, at least 97% water, at least 99% water, or even at least 99% water.

**[0031]** As used herein, the term "surfactant" refers to any compound generally recognized in the art as having surface active qualities. Surfactants generally include anionic, cationic, nonionic, and zwitterionic compounds, which are further described, herein.

**[0032]** As used herein, "surface property" is used in reference to electrostatic charge, as well as properties such as the hydrophobicity and hydrophilicity exhibited by the surface of a protein.

**[0033]** The term "oxidation stability" refers to endo- $\beta$ -mannanases of the present disclosure that retain a specified amount of enzymatic activity over a given period of time under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein, for example while exposed to or contacted with bleaching agents or oxidizing agents. In some embodiments, the endo- $\beta$ -mannanases retain at least about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 92%, about 95%, about 96%, about 97%, about 98%, or about 99% endo- $\beta$ -mannanase activity after contact with a bleaching or oxidizing agent over a given time period, for example, at least about 1 minute, about 3 minutes, about 5 minutes, about 8 minutes, about 12 minutes, about 16 minutes, about 20 minutes, etc.

**[0034]** The term "chelator stability" refers to endo- $\beta$ -mannanases of the present disclosure that retain a specified amount of enzymatic activity over a given period of time under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein, for example while exposed to or contacted with chelating agents. In some embodiments, the endo- $\beta$ -mannanases retain at least about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 92%, about 95%, about 96%, about 97%, about 98%, or about 99% endo- $\beta$ -mannanase activity after contact with a chelating agent over a given time period, for example, at least about 10 minutes, about 20 minutes, about 40 minutes, about 60 minutes, about 100 minutes, etc.

**[0035]** The terms "thermal stability" and "thermostable" refer to endo- $\beta$ -mannanases of the present disclosure that retain a specified amount of enzymatic activity after exposure to identified temperatures over a given period of time under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein, for example, while exposed to altered temperatures. Altered temperatures include increased or decreased temperatures. In some embodiments, the endo- $\beta$ -mannanases retain at least about 50%,

about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 92%, about 95%, about 96%, about 97%, about 98%, or about 99% endo- $\beta$ -mannanase activity after exposure to altered temperatures over a given time period, for example, at least about 60 minutes, about 120 minutes, about 180 minutes, about 240 minutes, about 300 minutes, etc.

**[0036]** The term "cleaning activity" refers to the cleaning performance achieved by the endo- $\beta$ -mannanase under conditions prevailing during the mannosidic, hydrolyzing, cleaning, or other process disclosed herein. In some embodiments, cleaning performance is determined by the application of various cleaning assays concerning enzyme sensitive stains arising from food products, household agents or personal care products. Some of these stains include, for example, ice cream, ketchup, BBQ sauce, mayonnaise, soups, chocolate milk, chocolate pudding, frozen desserts, shampoo, body lotion, sun protection products, toothpaste, locust bean gum, or guar gum as determined by various chromatographic, spectrophotometric or other quantitative methodologies after subjection of the stains to standard wash conditions. Exemplary assays include, but are not limited to those described in WO 99/34011, U.S. Pat. No. 6,605,458, and U.S. Pat. No. 6,566,114, as well as those methods included in the Examples.

**[0037]** As used herein, the terms "clean surface" and "clean textile" refer to a surface or textile respectively that has a percent stain removal of at least 10%, preferably at least 15%, 20%, 25%, 30%, 35%, or 40% of a soiled surface or textile.

**[0038]** The term "cleaning effective amount" of an endo- $\beta$ -mannanase refers to the quantity of endo- $\beta$ -mannanase described herein 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 endo- $\beta$ -mannanase used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar, powder, solid, liquid, tablet, gel, paste, foam, sheet, or unit dose) composition is required, etc.

**[0039]** The term "cleaning adjunct materials", as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, granule, powder, bar, paste, spray, tablet, gel, unit dose, sheet, or foam composition), which materials are also preferably compatible with the endo- $\beta$ -mannanase enzyme used in the composition. In some embodiments, granular compositions are in "compact" form, while in other embodiments, the liquid compositions are in a "concentrated" form.

**[0040]** As used herein, "cleaning compositions" and "cleaning formulations" refer to admixtures of chemical ingredients that find use in the removal of undesired compounds (e.g., soil or stains) from items to be cleaned, such as fabric, dishes, contact lenses, other solid surfaces, hair, skin, teeth, and the like. The compositions or formulations may be in the form of a liquid, gel, granule, powder, bar, paste, spray tablet, gel, unit dose, sheet, or foam, depending on the surface, item or fabric to be cleaned and the desired form of the composition or formulation.

**[0041]** As used herein, the terms "detergent composition" and "detergent formulation" refer to mixtures of chemical ingredients intended for use in a wash medium for the cleaning of soiled objects. Detergent compositions/formulations generally include at least one surfactant, and may optionally include hydrolytic enzymes, oxido-reductases, builders, bleaching agents, bleach activators, bluing agents and fluorescent dyes, caking inhibitors, masking agents, enzyme activators, antioxidants, and solubilizers.

**[0042]** As used herein, "dishwashing composition" refers to all forms of compositions for cleaning dishware, including cutlery, including but not limited to granular and liquid forms. In some embodiments, the dishwashing composition is an "automatic dishwashing" composition that finds use in automatic dish washing machines. It is not intended that the present disclosure be limited to any particular type or dishware composition. Indeed, the present disclosure finds use in cleaning dishware (e.g., dishes including, but not limited to plates, cups, glasses, bowls, etc.) and cutlery (e.g., utensils including, but not limited to spoons, knives, forks, serving utensils, etc.) of any material, including but not limited to ceramics, plastics, metals, china, glass, acrylics, etc. The term "dishware" is used herein in reference to both dishes and cutlery.

**[0043]** 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 under appropriate pH and 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 ClO<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, peracids, NO<sub>2</sub>, etc.

**[0044]** As used herein, "wash performance" of a variant endo- $\beta$ -mannanase refers to the contribution of a variant endo- $\beta$ -mannanase to washing that provides additional cleaning performance to the detergent composition. Wash performance is compared under relevant washing conditions.

**[0045]** 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 dish or laundry detergent market segment.

**[0046]** 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.

**[0047]** 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, a preferred filler salt is sodium sulfate.

**[0048]** The terms "textile" or "textile material" refer to woven fabrics, as well as staple fibers and filaments suitable for conversion to or use as yarns, woven, knit, and non-woven fabrics. The term encompasses yarns made from natural, as well as synthetic (e.g., manufactured) fibers.

**[0049]** 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.

**[0050]** 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.

**[0051]** As used herein, a "polypeptide" refers to a molecule comprising a plurality of amino acids linked through peptide bonds. The terms "polypeptide," "peptide," and "protein" are used interchangeably. Proteins may optionally be modified (e.g., glycosylated, phosphorylated, acylated, farnesylated, prenylated, sulfonated, and the like) to add functionality. Where such amino acid sequences exhibit activity, they may be referred to as an "enzyme." The conventional one-letter or three-letter codes for amino acid residues are used, with amino acid sequences being presented in the standard amino-to-carboxy terminal orientation (*i.e.*, N→C).

**[0052]** The terms "polynucleotide" encompasses DNA, RNA, heteroduplexes, and synthetic molecules capable of encoding a polypeptide. Nucleic acids may be single-stranded or doublestranded, and may have chemical modifications. The terms "nucleic acid" and "polynucleotide" are used interchangeably. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and the present compositions and methods encompass nucleotide sequences which encode a particular amino acid sequence. Unless otherwise indicated, nucleic acid sequences are presented in a 5'-to-3' orientation.

**[0053]** As used herein, the terms "wild-type" and "native" refer to polypeptides or polynucleotides that are found in nature.

**[0054]** The terms, "wild-type," "parental," or "reference," with respect to a polypeptide, refer to a naturally-occurring polypeptide that does not include a man-made substitution, insertion, or deletion at one or more amino acid positions. Similarly, the terms "wild-type," "parental," or "reference," with respect to a polynucleotide, refer to a naturally-occurring polynucleotide that does not include a man-made nucleoside change. However, note that a polynucleotide encoding a wild-type, parental, or reference polypeptide is not limited to a naturally-occurring polynucleotide, and encompasses any polynucleotide encoding the wild-type, parental, or reference polypeptide.

**[0055]** As used herein, a "variant polypeptide" refers to a polypeptide that is derived from a parent (or reference) polypeptide by the substitution, addition, or deletion, of one or more amino acids, typically by recombinant DNA techniques. Variant polypeptides may differ from a parent polypeptide by a small number of amino acid residues and may be defined by their level of primary amino acid sequence homology/identity with a parent polypeptide. Preferably, variant polypeptides have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or even at least 99% amino acid sequence identity with a parent polypeptide.

**[0056]** Sequence identity may be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (See, *e.g.*, Altschul et al. [1990] *J. Mol. Biol.* 215:403-410; Henikoff et al. [1989] *Proc. Natl. Acad. Sci. USA* 89:10915; Karin et al. [1993] *Proc. Natl. Acad. Sci USA* 90:5873; and Higgins et al. [1988] *Gene* 73:237-244). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Databases may also be searched using FASTA (Pearson et al. [1988] *Proc. Natl.*

Acad. Sci. USA 85:2444-2448). One indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-reactive with the second polypeptide. Typically, polypeptides that differ by conservative amino acid substitutions are immunologically cross-reactive. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution..

**[0057]** As used herein, a "variant polynucleotide" encodes a variant polypeptide, has a specified degree of homology/identity with a parent polynucleotide, or hybridizes under stringent conditions to a parent polynucleotide or the complement, thereof. Preferably, a variant polynucleotide has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or even at least 99% nucleotide sequence identity with a parent polynucleotide. Methods for determining percent identity are known in the art and described immediately above.

**[0058]** The term "derived from" encompasses the terms "originated from," "obtained from," "obtainable from," "isolated from," and "created from," and generally indicates that one specified material finds its origin in another specified material or has features that can be described with reference to the another specified material.

**[0059]** As used herein, the term "hybridization" refers to the process by which a strand of nucleic acid joins with a complementary strand through base pairing, as known in the art.

**[0060]** As used herein, the phrase "hybridization conditions" refers to the conditions under which hybridization reactions are conducted. These conditions are typically classified by degree of "stringency" of the conditions under which hybridization is measured. The degree of stringency can be based, for example, on the melting temperature (T<sub>m</sub>) of the nucleic acid binding complex or probe. For example, "maximum stringency" typically occurs at about T<sub>m</sub>-5 °C (5° below the T<sub>m</sub> of the probe); "high stringency" at about 5-10° below the T<sub>m</sub>; "intermediate stringency" at about 10-20° below the T<sub>m</sub> of the probe; and "low stringency" at about 20-25° below the T<sub>m</sub>. Alternatively, or in addition, hybridization conditions can be based upon the salt or ionic strength conditions of hybridization and/or one or more stringency washes, e.g.,: 6X SSC = very low stringency; 3X SSC = low to medium stringency; 1X SSC = medium stringency; and 0.5X SSC = high stringency. Functionally, maximum stringency conditions may be used to identify nucleic acid sequences having strict identity or near-strict identity with the hybridization probe; while high stringency conditions are used to identify nucleic acid sequences having about 80% or more sequence identity with the probe. For applications requiring high selectivity, it is typically desirable to use relatively stringent conditions to form the hybrids (e.g., relatively low salt and/or high temperature conditions are used). As used herein, stringent conditions are defined as 50°C and 0.2X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0).

**[0061]** The phrases "substantially similar" and "substantially identical" in the context of at least two nucleic acids or polypeptides means that a polynucleotide or polypeptide comprises a

sequence that has at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identical to a parent or reference sequence, or does not include amino acid substitutions, insertions, deletions, or modifications made only to circumvent the present description without adding functionality.

**[0062]** As used herein, an "expression vector" refers to a DNA construct containing a DNA sequence that encodes a specified polypeptide and is operably linked to a suitable control sequence capable of effecting the expression of the polypeptides in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself.

**[0063]** The term "recombinant," refers to genetic material (*i.e.*, nucleic acids, the polypeptides they encode, and vectors and cells comprising such polynucleotides) that has been modified to alter its sequence or expression characteristics, such as by mutating the coding sequence to produce an altered polypeptide, fusing the coding sequence to that of another gene, placing a gene under the control of a different promoter, expressing a gene in a heterologous organism, expressing a gene at a decreased or elevated levels, expressing a gene conditionally or constitutively in manner different from its natural expression profile, and the like. Generally recombinant nucleic acids, polypeptides, and cells based thereon, have been manipulated by man such that they are not identical to related nucleic acids, polypeptides, and cells found in nature.

**[0064]** A "signal sequence" refers to a sequence of amino acids bound to the N-terminal portion of a polypeptide, and which facilitates the secretion of the mature form of the protein from the cell. The mature form of the extracellular protein lacks the signal sequence which is cleaved off during the secretion process.

**[0065]** The term "selective marker" or "selectable marker" refers to a gene capable of expression in a host cell that allows for ease of selection of those hosts containing an introduced nucleic acid or vector. Examples of selectable markers include but are not limited to antimicrobial substances (*e.g.*, hygromycin, bleomycin, or chloramphenicol) and/or genes that confer a metabolic advantage, such as a nutritional advantage, on the host cell. The terms "selectable marker" or "selectable gene product" as used herein refer to the use of a gene, which encodes an enzymatic activity that confers resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed.

**[0066]** The term "regulatory element" as used herein refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding

region. Additional regulatory elements include splicing signals, polyadenylation signals and termination signals.

**[0067]** As used herein, "host cells" are generally prokaryotic or eukaryotic hosts which are transformed or transfected with vectors constructed using recombinant DNA techniques known in the art. Transformed host cells are capable of either replicating vectors encoding the protein variants or expressing the desired protein variant. In the case of vectors which encode the pre- or pro-form of the protein variant, such variants, when expressed, are typically secreted from the host cell into the host cell medium.

**[0068]** The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means transformation, transduction or transfection. Means of transformation include protoplast transformation, calcium chloride precipitation, electroporation, naked DNA, and the like as known in the art. (See, Chang and Cohen [1979] Mol. Gen. Genet. 168:111-115; Smith et al. [1986] Appl. Env. Microbiol. 51:634; and the review article by Ferrari et al., in Harwood, *Bacillus*, Plenum Publishing Corporation, pp. 57-72, 1989).

**[0069]** Other technical and scientific terms have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains (See, e.g., Singleton and Sainsbury, *Dictionary of Microbiology and Molecular Biology*, 2d Ed., John Wiley and Sons, NY 1994; and Hale and Marham, *The Harper Collins Dictionary of Biology*, Harper Perennial, NY 1991).

**[0070]** The singular terms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise.

**[0071]** As used herein in connection with a numerical value, the term "about" refers to a range of -10% to +10% of the numerical value. For instance, the phrase a "pH value of about 6" refers to pH values of from 5.4 to 6.6.

**[0072]** Headings are provided for convenience and should not be construed as limitations. The description included under one heading may apply to the specification as a whole.

#### ***Paenibacillus and Bacillus spp. Polypeptides***

**[0073]** The compositions and methods of the invention employ an endo- $\beta$ -mannanase polypeptide comprising an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NO: 8, 10, 12, 42, 43, 44, 46, 47, 48, 50, 51 and 52, or an active fragment thereof, wherein said polypeptide or active fragment thereof has mannanase activity in the presence of a surfactant. In some embodiments, the polypeptide or recombinant polypeptide or fragment, active fragment, or variant thereof, described herein contains Asn33-Asp-34-Leu35 (NDL), wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on

conserved linear sequence numbering.

**[0074]** In some embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment thereof comprises an Asn33-Asp-34-Leu35, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering. In another embodiment, the endo- $\beta$ -mannanase polypeptide or active fragment thereof comprises a WX<sub>a</sub>KNDLXXAI motif at positions 30-38, wherein X<sub>a</sub> is F or Y and X is any amino acid, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering. In some embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment thereof comprises a WX<sub>a</sub>KNDLX<sub>b</sub>X<sub>c</sub>AI motif at positions 30-38, wherein X<sub>a</sub> is F or Y, X<sub>b</sub> is N, Y or A, and X<sub>c</sub> is A or T, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0075]** In a further embodiment, the endo- $\beta$ -mannanase polypeptide or active fragment thereof comprises a L<sub>262</sub>D<sub>263</sub>XXXGPXGXL<sub>272</sub>T<sub>273</sub>, motif at positions 262-273, where X is any amino acid and wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering. In yet a still further embodiment, the endo- $\beta$ -mannanase polypeptide or active fragment thereof comprises a L<sub>262</sub>D<sub>263</sub>M/LV/AT/AGPX<sub>1</sub>GX<sub>2</sub>L<sub>272</sub>T<sub>273</sub> motif at positions 262-273, where X<sub>1</sub> is N, A or S and X<sub>2</sub> is S, T or N, and wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering. In some embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment thereof is an NDL-Clade 1 polypeptide which comprises a LDM/LATGPN/AGS/TLT motif at positions 262-273, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering. In some embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment thereof is an NDL-Clade 2 polypeptide which comprises an LDLA/VA/TGPS/NGNLT motif at positions 262-273, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32 and are based on the conserved linear sequence numbering.

**[0076]** Exemplary *Paenibacillus* or *Bacillus* spp. polypeptides which may be used in the compositions and methods described include PpoMan1 (SEQ ID NO:8) isolated from *Paenibacillus polymyxa* E681, PpoMan2 (SEQ ID NO:10) isolated from *Paenibacillus polymyxa* SC2, and PspMan4 (SEQ ID NO:12) isolated from *Paenibacillus* sp. A1. These and other isolated endo- $\beta$ -mannanase polypeptides are described in more detail below.

**[0077]** In other embodiments, the endo- $\beta$ -mannanase polypeptide or active fragment comprises an amino acid sequence which has at least 95% identity to the amino acid sequence

selected from the group consisting of SEQ ID NO: 8, 10, 12, 42, 43, 44, 46, 47, 48, 50, 51 or 52.

**[0078]** In some embodiments, the endo- $\beta$ -mannanase polypeptide polypeptide or active fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 10, 12, 42, 43, 44, 46, 47, 48, 50, 51 or 52.

**[0079]** Sequence identity can be determined by amino acid sequence alignment, e.g., using a program such as BLAST, ALIGN, or CLUSTAL, as described herein. In some embodiments, the polypeptides of the present invention are isolated polypeptides.

**[0080]** The polypeptide or active fragment has mannanase activity in the presence of a surfactant. In some embodiments, the mannanase activity is activity on mannan gum. In some embodiments, the mannanase activity is activity on locust bean gum galactomannan. In some embodiments, the mannanase activity is activity on konjac glucomannan.

**[0081]** In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a pH range of 4.5-9.0. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a pH range of 5.5-8.5. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a pH range of 6.0-7.5. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a pH above 3.0, 3.5, 4.0 or 4.5. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a pH below 10.0, 9.5, or 9.0.

**[0082]** In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a temperature range of 40°C to 70°C. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a temperature range of 45°C to 65°C. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a temperature range of 50°C to 60°C. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a temperature above 20°C, 25°C, 30°C, 35°C, or 40°C. In some embodiments, the polypeptide or active fragment retains at least 70% of its maximal protease activity at a temperature below 90°C, 85°C, 80°C, 75°C, or 70°C.

**[0083]** In some embodiments, the polypeptide or active fragment has cleaning activity in a detergent composition.

**[0084]** In some embodiments, the polypeptide or active fragment has mannanase activity in the presence of a protease. In some embodiments, the polypeptide or active fragment is capable of hydrolyzing a substrate selected from the group consisting of guar gum, locust bean gum, and combinations thereof.

**[0085]** In some embodiments, the polypeptide or active fragment does not further comprise a

carbohydrate-binding module.

**[0086]** The polypeptides and active fragments employed in the present invention may be produced recombinantly, produced synthetically, or may be purified from a native source.

**[0087]** In certain embodiments, the polypeptide or active fragment may includ substitutions that do not substantially affect the structure and/or function of the polypeptide. Exemplary substitutions are conservative mutations, as summarized in Table I.

**Table I. Amino Acid Substitutions**

Original Residue	Code	Acceptable Substitutions
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, beta-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

**[0088]** Substitutions involving naturally occurring amino acids are generally made by mutating a nucleic acid encoding a recombinant a polypeptide of the present invention, and then expressing the variant polypeptide in an organism. Substitutions involving non-naturally

occurring amino acids or chemical modifications to amino acids are generally made by chemically modifying a recombinant a polypeptide of the present invention after it has been synthesized by an organism.

**[0089]** In some embodiments, the polypeptides are substantially identical to SEQ ID NO: 8, 10, 12, 42, 43, 44, 46, 47, 48, 50, 51 or 52, meaning that they do not include amino acid substitutions, insertions, or deletions that do not significantly affect the structure, function, or expression of the polypeptide.

**[0090]** In some embodiments, the polypeptide has 1,4- $\beta$ -D-mannosidic hydrolase activity, which includes mannanase, endo-1,4- $\beta$ -D-mannanase, exo-1,4- $\beta$ -D-mannanase/galactomannanase, and/or glucosidase activity. 1,4- $\beta$ -D-mannosidic hydrolase activity can be determined and measured using the assays described herein, or by other assays known in the art. In some embodiments, the polypeptide has activity in the presence of a detergent composition.

**[0091]** It will be clear that fragments of "full-length" polypeptides that retain 1,4- $\beta$ -D-mannosidic hydrolase activity may also be employed. Such fragments preferably retain the active site of the full-length polypeptides but may have deletions of non-critical amino acid residues. The activity of fragments can readily be determined using the assays described, herein, or by other assays known in the art. In some embodiments, the fragments retain 1,4- $\beta$ -D-mannosidic hydrolase activity in the presence of a detergent composition.

**[0092]** In some embodiments, the polypeptide or active fragment is produced as a N- and/or C-terminal fusion protein, for example to aid in extraction, detection and/or purification and/or to add functional properties to a polypeptide of the present invention. Examples of fusion protein partners include, but are not limited to, glutathione-S-transferase (GST), 6XHis, GAL4 (DNA binding and/or transcriptional activation domains), FLAG, MYC, BCE103 (WO 2010/044786), or other tags well known to anyone skilled in the art. In some embodiments, a proteolytic cleavage site is provided between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably, the fusion protein does not hinder the activity of the polypeptide.

**[0093]** In some embodiments, a polypeptide or active fragment is fused to a functional domain including a leader peptide, propeptide, one or more binding domain (modules) and/or catalytic domain. Suitable binding domains include, but are not limited to, carbohydrate-binding modules (e.g., CBM) of various specificities, providing increased affinity to carbohydrate components present during the application of a polypeptide. As described herein, the CBM and catalytic domain of a polypeptide are operably linked.

**[0094]** A carbohydrate-binding module (CBM) is defined as a contiguous amino acid sequence within a carbohydrate-active enzyme with a discreet fold having carbohydrate-binding activity. A few exceptions are CBMs in cellulosomal scaffold in proteins and rare instances of independent putative CBMs. The requirement of CBMs existing as modules within larger

enzymes sets this class of carbohydrate-binding protein apart from other non-catalytic sugar binding proteins such as lectins and sugar transport proteins. CBMs were previously classified as cellulose-binding domains (CBDs) based on the initial discovery of several modules that bound cellulose (Tomme et al., Eur J Biochem, 170:575-581, 1988; and Gilkes et al., J Biol Chem, 263:10401-10407, 1988). However, additional modules in carbohydrate-active enzymes are continually being found that bind carbohydrates other than cellulose yet otherwise meet the CBM criteria, hence the need to reclassify these polypeptides using more inclusive terminology. Previous classification of cellulose-binding domains was based on amino acid similarity. Groupings of CBDs were called "Types" and numbered with roman numerals (e.g. Type I or Type II CBDs). In keeping with the glycoside hydrolase classification, these groupings are now called families and numbered with Arabic numerals. Families 1 to 13 are the same as Types I to XIII (Tomme et al., in Enzymatic Degradation of Insoluble Polysaccharides (Saddler, J.N. & Penner, M., eds.), Cellulose-binding domains: classification and properties. pp. 142-163, American Chemical Society, Washington, 1995). A detailed review on the structure and binding modes of CBMs can be found in (Boraston et al., Biochem J, 382:769-81, 2004). The family classification of CBMs is expected to: aid in the identification of CBMs, in some cases, predict binding specificity, aid in identifying functional residues, reveal evolutionary relationships and possibly be predictive of polypeptide folds. Because the fold of proteins is better conserved than their sequences, some of the CBM families can be grouped into superfamilies or clans. The current CBM families are 1-63. CBMs/CBDs have also been found in algae, e.g., the red alga *Porphyra purpurea* as a non-hydrolytic polysaccharide-binding protein. However, most of the CBDs are from cellulases and xylanases. CBDs are found at the N-and C-termini of proteins or are internal. Enzyme hybrids are known in the art (See e.g., WO 90/00609 and WO 95/16782) and may be prepared by transforming into a host cell a DNA construct comprising at least a fragment of DNA encoding the cellulose-binding domain ligated, with or without a linker, to a DNA sequence encoding a disclosed polypeptide and growing the host cell to express the fused gene. Enzyme hybrids may be described by the following formula: CBM-MR-X or X-MR-CBM

**[0095]** In the above formula, the CBM is the N-terminal or the C-terminal region of an amino acid sequence corresponding to at least the carbohydrate-binding module; MR is the middle region (the linker), and may be a bond, or a short linking group preferably of from about 2 to about 100 carbon atoms, more preferably of from 2 to 40 carbon atoms; or is preferably from about 2 to about 100 amino acids, more preferably from 2 to 40 amino acids; and X is an N-terminal or C-terminal region of a polypeptide or active fragment having mannanase catalytic activity. In addition, a mannanase may contain more than one CBM or other module(s)/domain(s) of non-glycolytic function. The terms "module" and "domain" are used interchangeably in the present disclosure.

**[0096]** Suitable enzymatically active domains possess an activity that supports the action of a polypeptide in producing a desired product. Non-limiting examples of catalytic domains include: cellulases, hemicellulases such as xylanase, exo-mannanases, glucanases, arabinases, galactosidases, pectinases, and/or other activities such as proteases, lipases, acid phosphatases and/or others or functional fragments thereof. Fusion proteins are optionally

linked to a polypeptide through a linker sequence that simply joins a polypeptide and the fusion domain without significantly affecting the properties of either component, or the linker optionally has a functional importance for the intended application.

**[0097]** Alternatively, polypeptides described herein are used in conjunction with one or more additional proteins of interest. Non-limiting examples of proteins of interest include: acyl transferases, amylases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinases, arabinosidases, aryl esterases, beta-galactosidases, beta-glucanases, carrageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1, 4-glucanases, endo-beta-mannanases, exo-beta-mannanases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipolytic enzymes, 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 and/or other enzymes.

**[0098]** In other embodiments, a polypeptide as described is fused to a signal peptide for directing the extracellular secretion of the polypeptide. For example, in certain embodiments, the signal peptide is the native signal peptide of the polypeptide. In other embodiments, the signal peptide is a non-native signal peptide such as the *B. subtilis* AprE signal peptide. In some embodiments, a polypeptide has an N-terminal extension of Ala-Gly-Lys between the mature form and the signal peptide.

**[0099]** In some embodiments, a polypeptide or active fragment as described is expressed in a heterologous organism, *i.e.*, an organism other than *Paenibacillus* and *Bacillus* spp.. Exemplary heterologous organisms are Gram(+) bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus latus*, *Bacillus brevis*, *Geobacillus* (formerly *Bacillus*) *stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus latus*, *Bacillus megaterium*, *Bacillus thuringiensis*, *Streptomyces lividans*, or *Streptomyces murinus*; Gram(-) bacteria such as *Escherichia coli*; yeast such as *Saccharomyces* spp. or *Schizosaccharomyces* spp., *e.g.* *Saccharomyces cerevisiae*; and filamentous fungi such as *Aspergillus* spp., *e.g.*, *Aspergillus oryzae* or *Aspergillus niger*, and *Trichoderma reesei*. Methods from transforming nucleic acids into these organisms are well known in the art. A suitable procedure for transformation of *Aspergillus* host cells is described in EP 238 023.

**[0100]** In particular embodiments, a polypeptide or active fragment is expressed in a heterologous organism as a secreted polypeptide.

## Polynucleotides

**[0101]** Also described herein is a polynucleotide that encodes a polypeptide for use in the present invention (including active fragments thereof). In one aspect, the polynucleotide is provided in the context of an expression vector for directing the expression of a polypeptide in a heterologous organism, such as those identified, herein. The polynucleotide that encodes a polypeptide may be operably-linked to regulatory elements (e.g., a promoter, terminator, enhancer, and the like) to assist in expressing the encoded polypeptides.

**[0102]** Exemplary polynucleotide sequences encoding a polypeptide for use in the present invention has the nucleotide sequence of SEQ ID NO: 7, 9, 11, 41, 45 or 49. Similar, including substantially identical, polynucleotides encoding suitable polypeptides may occur in nature, e.g., in other strains or isolates of *B. agaradhaerens*. In view of the degeneracy of the genetic code, it will be appreciated that polynucleotides having different nucleotide sequences may encode the same polypeptide.

**[0103]** In some embodiments, polynucleotides can have a specified degree of nucleotide sequence identity to the polynucleotides described herein, e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to the nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 9, 11, 41, 45 or 49. Homology can be determined by amino acid sequence alignment, e.g., using a program such as BLAST, ALIGN, or CLUSTAL, as described herein.

**[0104]** In some embodiments, the polynucleotide that encodes a polypeptide for use in the present invention is fused in frame behind (*i.e.*, downstream of) a coding sequence for a signal peptide for directing the extracellular secretion of the polypeptide. Heterologous signal sequences include those from bacterial cellulase genes. Expression vectors may be provided in a heterologous host cell suitable for expressing a polypeptide, or suitable for propagating the expression vector prior to introducing it into a suitable host cell.

**[0105]** A polynucleotide may be naturally occurring or synthetic (*i.e.*, man-made), and may be codon-optimized for expression in a different host, mutated to introduce cloning sites, or otherwise altered to add functionality.

#### **Vectors and Host Cells**

**[0106]** In order to produce a disclosed polypeptide, the DNA encoding the polypeptide can be chemically synthesized from published sequences or obtained directly from host cells harboring the gene (e.g., by cDNA library screening or PCR amplification). In some embodiments, a polynucleotide is included in an expression cassette and/or cloned into a suitable expression vector by standard molecular cloning techniques. Such expression cassettes or vectors contain sequences that assist initiation and termination of transcription (e.g., promoters and terminators), and generally contain a selectable marker.

**[0107]** The expression cassette or vector is introduced in a suitable expression host cell, which

then expresses the corresponding polynucleotide. Particularly suitable expression hosts are bacterial expression host genera including *Escherichia* (e.g., *Escherichia coli*), *Pseudomonas* (e.g., *P. fluorescens* or *P. stutzeri*), *Proteus* (e.g., *Proteus mirabilis*), *Ralstonia* (e.g., *Ralstonia eutropha*), *Streptomyces*, *Staphylococcus* (e.g., *S. carnosus*), *Lactococcus* (e.g., *L. lactis*), or *Bacillus* (*subtilis*, *megaterium*, *licheniformis*, etc.). Also particularly suitable are yeast expression hosts such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Hansenula polymorpha*, *Kluyveromyces lactis* or *Pichia pastoris*. Especially suited are fungal expression hosts such as *Aspergillus niger*, *Chrysosporium lucknowense*, *Aspergillus* (e.g., *A. oryzae*, *A. niger*, *A. nidulans*, etc.) or *Trichoderma reesei*. Also suited are mammalian expression hosts such as mouse (e.g., NS0), Chinese Hamster Ovary (CHO) or Baby Hamster Kidney (BHK) cell lines. Other eukaryotic hosts such as insect cells or viral expression systems (e.g., bacteriophages such as M13, T7 phage or Lambda, or viruses such as Baculovirus) are also suitable for producing a polypeptide.

**[0108]** Promoters and/or signal sequences associated with secreted proteins in a particular host of interest are candidates for use in the heterologous production and secretion of endo- $\beta$ -mannanases in that host or in other hosts. As an example, in filamentous fungal systems, the promoters that drive the genes for cellobiohydrolase I (cbh1), glucoamylase A (glaA), TAKA-amylase (amyA), xylanase (exlA), the gpd-promoter cbh1, cbhII, endoglucanase genes EGI-EGV, Cel61B, Cel74A, egl1-egl5, gpd promoter, Pgk1, pki1, EF-1alpha, tef1, cDNA1 and hex1 are particularly suitable and can be derived from a number of different organisms (e.g., *A. niger*, *T. reesei*, *A. oryzae*, *A. awamori* and *A. nidulans*). A polynucleotide encoding a polypeptide for use in the present invention may be recombinantly associated with a polynucleotide encoding a suitable homologous or heterologous signal sequence that leads to secretion of the polypeptide into the extracellular (or periplasmic) space, thereby allowing direct detection of enzyme activity in the cell supernatant (or periplasmic space or lysate). Particularly suitable signal sequences for *Escherichia coli*, other Gram negative bacteria and other organisms known in the art include those that drive expression of the HlyA, DsbA, Pbp, PhoA, PelB, OmpA, OmpT or M13 phage Gill genes. For *Bacillus subtilis*, Gram-positive organisms and other organisms known in the art, particularly suitable signal sequences further include those that drive expression of the AprE, NprB, Mpr, AmyA, AmyE, Blac, SacB, and for *S. cerevisiae* or other yeast, include the killer toxin, Bar1, Suc2, Mating factor alpha, Inu1 or Ggpl signal sequence. Signal sequences can be cleaved by a number of signal peptidases, thus removing them from the rest of the expressed protein. In some embodiments, the rest of the polypeptide is expressed alone or as a fusion with other peptides, tags or proteins located at the N- or C-terminus (e.g., 6XHis, HA or FLAG tags). Suitable fusions include tags, peptides or proteins that facilitate affinity purification or detection (e.g., BCE103, 6XHis, HA, chitin binding protein, thioredoxin or FLAG tags), as well as those that facilitate expression, secretion or processing of the target endo- $\beta$ -mannanase. Suitable processing sites include enterokinase, STE13, Kex2 or other protease cleavage sites for cleavage in vivo or in vitro.

**[0109]** Polynucleotides can be introduced into expression host cells by a number of transformation methods including, but not limited to, electroporation, lipid-assisted transformation or transfection ("lipofection"), chemically mediated transfection (e.g., CaCl

and/or CaP), lithium acetate-mediated transformation (e.g., of host-cell protoplasts), biolistic "gene gun" transformation, PEG-mediated transformation (e.g., of host-cell protoplasts), protoplast fusion (e.g., using bacterial or eukaryotic protoplasts), liposome-mediated transformation, *Agrobacterium tumefaciens*, adenovirus or other viral or phage transformation or transduction.

**[0110]** Alternatively, a polypeptide can be expressed intracellularly. Optionally, after intracellular expression of the enzyme variants, or secretion into the periplasmic space using signal sequences such as those mentioned above, a permeabilisation or lysis step can be used to release the polypeptide into the supernatant. The disruption of the membrane barrier is effected by the use of mechanical means such as ultrasonic waves, pressure treatment (French press), cavitation or the use of membrane-digesting enzymes such as lysozyme or enzyme mixtures. As a further alternative, the polynucleotides encoding the polypeptide can be expressed by use of a suitable cell-free expression system. In cell-free systems, the polynucleotide of interest is typically transcribed with the assistance of a promoter, but ligation to form a circular expression vector is optional. In other embodiments, RNA is exogenously added or generated without transcription and translated in cell free systems.

**[0111]** The polypeptides disclosed herein may have enzymatic activity over a broad range of pH conditions. In certain embodiments the disclosed polypeptides of the present invention have enzymatic activity from about pH 4.0 to about pH 11.0, or from about pH 4.5 to about pH 11.0. In preferred embodiments, the polypeptides have substantial enzymatic activity, for example, at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity from about pH 4.0 to 11.0, pH 4.5 to 11.0, pH 4.5 to 9.0, pH 5.5 to 8.5, or pH 6.0 to 7.5. It should be noted that the pH values described herein may vary by  $\pm$  0.2. For example a pH value of about 8.0 could vary from pH 7.8 to pH 8.2.

**[0112]** The polypeptides disclosed herein may have enzymatic activity over a wide range of temperatures, e.g., from about 20°C or lower to 90°C, 30°C to 80°C, 40°C to 70°C, 45°C to 65°C, or 50°C to 60°C. In certain embodiments, the polypeptides have substantial enzymatic activity, for example, at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity at a temperature range of about 20°C or lower to 90°C, 30°C to 80°C, 40°C to 70°C, 45°C to 65°C, or 50°C to 60°C. It should be noted that the temperature values described herein may vary by  $\pm$  0.2°C. For example a temperature of about 50°C could vary from 49.8°C to 50.2°C.

### **Detergent Compositions**

**[0113]** An aspect of the compositions and methods disclosed herein is a detergent composition comprising an isolated polypeptide as described (including active fragments thereof) and methods for using such compositions in cleaning applications. Cleaning applications include, but are not limited to, laundry or textile cleaning, laundry or textile softening, dishwashing (manual and automatic), stain pre-treatment, and the like. Particular applications are those where mannans (e.g., locust bean gum, guar gum, etc.) are a component of the soils or stains

to be removed. Detergent compositions typically include an effective amount of any of the polypeptides described herein, e.g., at least 0.0001 weight percent, from about 0.0001 to about 1, from about 0.001 to about 0.5, from about 0.01 to about 0.1 weight percent, or even from about 0.1 to about 1 weight percent, or more. An effective amount of a polypeptide in the detergent composition results in the polypeptide having enzymatic activity sufficient to hydrolyze a mannan-containing substrate, such as locust bean gum, guar gum, or combinations thereof.

**[0114]** Additionally, detergent compositions having a concentration from about 0.4 g/L to about 2.2 g/L, from about 0.4 g/L to about 2.0 g/L, from about 0.4 g/L to about 1.7 g/L, from about 0.4 g/L to about 1.5 g/L, from about 0.4 g/L to about 1 g/L, from about 0.4 g/L to about 0.8 g/L, or from about 0.4 g/L to about 0.5 g/L may be mixed with an effective amount of an isolated polypeptide as described. The detergent composition may also be present at a concentration of about 0.4 ml/L to about 2.6 ml/L, from about 0.4 ml/L to about 2.0 ml/L, from about 0.4 ml/L to about 1.5 ml/L, from about 0.4 ml/L to about 1 ml/L, from about 0.4 ml/L to about 0.8 ml/L, or from about 0.4 ml/L to about 0.5 ml/L.

**[0115]** 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. 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.

**[0116]** In some embodiments, the detergent composition comprises one or more surfactants, which may be non-ionic, semi-polar, anionic, cationic, zwitterionic, or combinations and mixtures thereof. The surfactants are typically present at a level of from about 0.1% to 60% by weight. Exemplary surfactants include but are not limited to sodium dodecylbenzene sulfonate, C12-14 pareth-7, C12-15 pareth-7, sodium C12-15 pareth sulfate, C14-15 pareth-4, sodium laureth sulfate (e.g., Steol CS-370), sodium hydrogenated cocoate, C12 ethoxylates (Alfonic 1012-6, Hetoxol LA7, Hetoxol LA4), sodium alkyl benzene sulfonates (e.g., Nacconol 90G), and combinations and mixtures thereof.

**[0117]** Anionic surfactants that may be used with the detergent compositions described herein include but are not limited to linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. It may also contain 0-40% of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamine oxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide (e.g., as described in WO 92/06154),

and combinations and mixtures thereof.

**[0118]** Nonionic surfactants that may be used with the detergent compositions described herein include but are not limited to polyoxyethylene esters of fatty acids, polyoxyethylene sorbitan esters (e.g., TWEENS), polyoxyethylene alcohols, polyoxyethylene isoalcohols, polyoxyethylene ethers (e.g., TRITONs and BRIJ), polyoxyethylene esters, polyoxyethylene-*p*-tert-octylphenols or octylphenyl-ethylene oxide condensates (e.g., NONIDET P40), ethylene oxide condensates with fatty alcohols (e.g., LUBROL), polyoxyethylene nonylphenols, polyalkylene glycols (SYNPERONIC F108), sugar-based surfactants (e.g., glycopyranosides, thioglycopyranosides), and combinations and mixtures thereof.

**[0119]** The detergent compositions disclosed herein may have mixtures that include, but are not limited to 5-15% anionic surfactants, < 5% nonionic surfactants, cationic surfactants, phosphonates, soap, enzymes, perfume, butylphenyl methylptopionate, geraniol, zeolite, polycarboxylates, hexyl cinnamal, limonene, cationic surfactants, citronellol, and benzisothiazolinone.

**[0120]** Detergent compositions may additionally include one or more detergent builders or builder systems, a complexing agent, a polymer, a bleaching system, a stabilizer, a foam booster, a suds suppressor, an anti-corrosion agent, a soil-suspending agent, an anti-soil redeposition agent, a dye, a bactericide, a hydrotope, a tarnish inhibitor, an optical brightener, a fabric conditioner, and a perfume. The detergent compositions may also include enzymes, including but not limited to proteases, amylases, cellulases, lipases, pectin degrading enzymes, xyloglucanases, or additional carboxylic ester hydrolases. The pH of the detergent compositions should be neutral to basic, as described herein.

**[0121]** In some embodiments incorporating at least one builder, the detergent 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 may include, but are not limited to, the alkali metals, 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 metals, 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 disclosure.

**[0122]** 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 tripolyphosphate hexahydrate, potassium tripolyphosphate, and mixed sodium and potassium tripolyphosphate, etc.). It is contemplated that any suitable builder will find use in the

present disclosure, including those known in the art (See, e.g., EP 2 100 949).

**[0123]** As indicated herein, in some embodiments, the cleaning compositions described herein further comprise adjunct materials including, but not limited to surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dispersants, suds suppressors, dyes, perfumes, colorants, filler salts, hydrotropes, 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 (See, e.g., U.S. Pat. Nos. 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). Embodiments of specific cleaning composition materials are exemplified in detail below. In embodiments in which the cleaning adjunct materials are not compatible with the polypeptides of the present invention in the cleaning compositions, suitable methods of keeping the cleaning adjunct materials and the endo- $\beta$ -mannanase(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.).

**[0124]** The cleaning compositions described herein are advantageously employed for example, in laundry applications, hard surface cleaning, dishwashing applications, as well as cosmetic applications such as dentures, teeth, hair, and skin. In addition, due to the unique advantages of increased effectiveness in lower temperature solutions, the polypeptides described herein are ideally suited for laundry and fabric softening applications. Furthermore, the polypeptides of the present invention may find use in granular and liquid compositions.

**[0125]** A polypeptide or isolated polypeptide described herein may also find use cleaning in additive products. In some embodiments, low temperature solution cleaning applications find use. In some embodiments, the present disclosure provides cleaning additive products including at least one disclosed a polypeptide 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 endo- $\beta$ -mannanases. 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 disclosure, 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 described more fully below.

**[0126]** In one embodiment, the present cleaning compositions or cleaning additives contain an effective amount of at least one polypeptide described herein, optionally in combination with other endo- $\beta$ -mannanases and/or additional enzymes. In certain embodiments, the additional enzymes include, but are not limited to, at least one enzyme selected from acyl transferases, amylases, alpha-amylases, beta-amylases, alpha-galactosidases, arabinases, arabinosidases, aryl esterases, beta-galactosidases, beta-glucanases, carageenases, catalases, cellobiohydrolases, cellulases, chondroitinases, cutinases, endo-beta-1, 4-glucanases, endo-beta-mannanases, exo-beta-mannanases, esterases, exo-mannanases, galactanases, glucoamylases, hemicellulases, hyaluronidases, keratinases, laccases, lactases, ligninases, lipases, lipolytic enzymes, lipoxygenases, mannanases, metalloproteases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases, perhydrolases, peroxidases, phenoloxidases, phosphatases, phospholipases, phytases, polygalacturonases, proteases, pullulanases, reductases, rhamnogalacturonases, beta-glucanases, tannases, transglutaminases, xylan acetyl-esterases, xylanases, xyloglucanases, xylosidases, and mixtures thereof.

**[0127]** The required level of enzyme is achieved by the addition of one or more polypeptide or active fragment as described. Typically the present cleaning compositions will comprise at least about 0.0001 weight percent, from about 0.0001 to about 10, from about 0.001 to about 1, or even from about 0.01 to about 0.1 weight percent of at least one of the disclosed polypeptides.

**[0128]** 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 3.0 to about 11. Liquid product formulations are typically formulated to have a neat pH from about 5.0 to about 9.0. Granular laundry products are typically formulated to have a pH from about 8.0 to about 11.0. 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.

**[0129]** Suitable low pH cleaning compositions typically have a neat pH of from about 3.0 to about 5.0 or even from about 3.5 to about 4.5. Low pH cleaning compositions are typically free of surfactants that hydrolyze in such a pH environment. Such surfactants include sodium alkyl sulfate surfactants that comprise at least one ethylene oxide moiety or even from about 1 to about 16 moles of ethylene oxide. Such cleaning compositions typically comprise a sufficient amount of a pH modifier, such as sodium hydroxide, monoethanolamine, or hydrochloric acid, to provide such cleaning composition with a neat pH of from about 3.0 to about 5.0. Such compositions typically comprise at least one acid stable enzyme. In some embodiments, the compositions are liquids, while in other embodiments, they are solids. The pH of such liquid compositions is typically measured as a neat pH. The pH of such solid compositions is measured as a 10% solids solution of the composition wherein the solvent is distilled water. In these embodiments, all pH measurements are taken at 20°C, unless otherwise indicated.

**[0130]** Suitable high pH cleaning compositions typically have a neat pH of from about 9.0 to about 11.0, or even a net pH of from 9.5 to 10.5. Such cleaning compositions typically comprise a sufficient amount of a pH modifier, such as sodium hydroxide, monoethanolamine, or hydrochloric acid, to provide such cleaning composition with a neat pH of from about 9.0 to about 11.0. Such compositions typically comprise at least one base-stable enzyme. In some embodiments, the compositions are liquids, while in other embodiments, they are solids. The pH of such liquid compositions is typically measured as a neat pH. The pH of such solid compositions is measured as a 10% solids solution of said composition wherein the solvent is distilled water. In these embodiments, all pH measurements are taken at 20°C, unless otherwise indicated.

**[0131]** In some embodiments, when the polypeptide is employed in a granular composition or liquid, it is desirable for the polypeptide to be in the form of an encapsulated particle to protect the polypeptide from other components of the granular composition during storage. In addition, encapsulation is also a means of controlling the availability of the polypeptide during the cleaning process. In some embodiments, encapsulation enhances the performance of the polypeptide and/or additional enzymes. In this regard, the polypeptide is encapsulated with any suitable encapsulating material known in the art. In some embodiments, the encapsulating material typically encapsulates at least part of the catalyst for the polypeptide described herein. 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 the PCT application WO 97/11151. 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., EP 0 922 499; U.S. 4,977,252; U.S. 5,354,559; and U.S. 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 PM 6545, PM 6550, PM 7220, PM 7228, EXTENDOSPHERES®, LUXSIL®, Q-CEL®, and SPHERICEL® (PQ Corp., Valley Forge, PA).

**[0132]** The term "granular composition" refers to a conglomeration of discrete solid, macroscopic particles. Powders are a special class of granular material due to their small particle size, which makes them more cohesive and more easily suspended.

**[0133]** In using detergent compositions that include a polypeptide of the present invention in cleaning applications, the fabrics, textiles, dishes, or other surfaces to be cleaned are incubated in the presence of a detergent composition for a time sufficient to allow the active polypeptide to hydrolyze mannan substrates including, but not limited to, locust bean gum,

guar gum, and combinations thereof present in soil or stains, and then typically rinsed with water or another aqueous solvent to remove the detergent composition along with hydrolyzed mannans.

**[0134]** As described herein, the compositions of the present invention find particular use in the cleaning industry, including, but not limited to laundry and dish detergents. These applications place enzymes under various environmental stresses. A polypeptide as described may provide advantages over many currently used enzymes, due to their stability under various conditions.

**[0135]** Indeed, there are a variety of wash conditions including varying detergent formulations, wash water volumes, wash water temperatures, and lengths of wash time, to which endo- $\beta$ -mannanases involved in washing are exposed. In addition, detergent formulations used in different geographical areas have different concentrations of their relevant components present in the wash water. For example, European detergents typically have about 4500-5000 ppm of detergent components in the wash water, while Japanese detergents typically have approximately 667 ppm of detergent components in the wash water. In North America, particularly the United States, detergents typically have about 975 ppm of detergent components present in the wash water.

**[0136]** A low detergent concentration system includes detergents where less than about 800 ppm of the detergent components are present in the wash water. Japanese detergents are typically considered low detergent concentration system as they have approximately 667 ppm of detergent components present in the wash water.

**[0137]** A medium detergent concentration includes detergents where between about 800 ppm and about 2000 ppm of the detergent components are present in the wash water. North American detergents are generally considered to be medium detergent concentration systems as they have approximately 975 ppm of detergent components present in the wash water. Brazil typically has approximately 1500 ppm of detergent components present in the wash water.

**[0138]** A high detergent concentration system includes detergents where greater than about 2000 ppm of the detergent components are present in the wash water. European detergents are generally considered to be high detergent concentration systems as they have approximately 4500-5000 ppm of detergent components in the wash water.

**[0139]** Latin American detergents are generally high suds phosphate builder detergents and the range of detergents used in Latin America can fall in both the medium and high detergent concentrations as they range from 1500 ppm to 6000 ppm of detergent components in the wash water. As mentioned above, Brazil typically has approximately 1500 ppm of detergent components present in the wash water. However, other high suds phosphate builder detergent geographies, not limited to other Latin American countries, may have high detergent concentration systems up to about 6000 ppm of detergent components present in the wash water.

**[0140]** In light of the foregoing, it is evident that concentrations of detergent compositions in typical wash solutions throughout the world varies from less than about 800 ppm of detergent composition ("low detergent concentration geographies"), for example about 667 ppm in Japan, to between about 800 ppm to about 2000 ppm ("medium detergent concentration geographies"), for example about 975 ppm in U.S. and about 1500 ppm in Brazil, to greater than about 2000 ppm ("high detergent concentration geographies"), for example about 4500 ppm to about 5000 ppm in Europe and about 6000 ppm in high suds phosphate builder geographies.

**[0141]** The concentrations of the typical wash solutions are determined empirically. For example, in the U.S., a typical washing machine holds a volume of about 64.4 L of wash solution. Accordingly, in order to obtain a concentration of about 975 ppm of detergent within the wash solution about 62.79 g of detergent composition must be added to the 64.4 L of wash solution. This amount is the typical amount measured into the wash water by the consumer using the measuring cup provided with the detergent.

**[0142]** As a further example, different geographies use different wash temperatures. The temperature of the wash water in Japan is typically less than that used in Europe. For example, the temperature of the wash water in North America and Japan is typically between about 10 and about 30°C (e.g., about 20°C), whereas the temperature of wash water in Europe is typically between about 30 and about 60°C (e.g., about 40°C). Accordingly, in certain embodiments, the detergent compositions described herein may be utilized at temperature from about 10°C to about 60°C, or from about 20°C to about 60°C, or from about 30°C to about 60°C, or from about 40°C to about 60°C, as well as all other combinations within the range of about 40°C to about 55°C, and all ranges within 10°C to 60°C. However, in the interest of saving energy, many consumers are switching to using cold water washing. In addition, in some further regions, cold water is typically used for laundry, as well as dish washing applications. In some embodiments, the "cold water washing" of the present disclosure utilizes 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.

**[0143]** As a further example, different geographies typically have different water hardness. Water hardness is usually described in terms of the grains per gallon mixed  $\text{Ca}^{2+}/\text{Mg}^{2+}$ . Hardness is a measure of the amount of calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ) 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 (parts per million converted to grains per U.S. gallon is ppm # divided by 17.1 equals grains per gallon) of hardness minerals.

**Table II. Water Hardness Levels**

Water	Grains per gallon	Parts per million
Soft	less than 1.0	less than 17

Water	Grains per gallon	Parts per million
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

**[0144]** European water hardness is typically greater than about 10.5 (for example about 10.5 to about 20.0) grains per gallon mixed  $\text{Ca}^{2+}/\text{Mg}^{2+}$  (e.g., about 15 grains per gallon mixed  $\text{Ca}^{2+}/\text{Mg}^{2+}$ ). North American water hardness is typically greater than Japanese water hardness, but less than European water hardness. For example, North American water hardness can be between about 3 to about 10 grains, about 3 to about 8 grains or about 6 grains. Japanese water hardness is typically lower than North American water hardness, usually less than about 4, for example about 3 grains per gallon mixed  $\text{Ca}^{2+}/\text{Mg}^{2+}$ .

**[0145]** Accordingly, polypeptides and fragments as described may 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 polypeptide is comparable in wash performance to other endo- $\beta$ -mannanases. In some embodiments, the polypeptide exhibits enhanced wash performance as compared to endo- $\beta$ -mannanases currently commercially available. Thus, in some preferred embodiments, the polypeptide exhibits enhanced oxidative stability, enhanced thermal stability, enhanced cleaning capabilities under various conditions, and/or enhanced chelator stability. In addition, the polypeptide may find use in cleaning compositions that do not include detergents, again either alone or in combination with builders and stabilizers.

**[0146]** In some embodiments of the present disclosure, the cleaning compositions comprise at least one a polypeptide of the present invention of the present disclosure 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 other aspects of the present disclosure, the cleaning compositions comprises at least one a polypeptide of the present invention 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.

**[0147]** In addition to the polypeptides provided herein, any other suitable endo- $\beta$ -mannanases find use in the compositions described herein. Suitable endo- $\beta$ -mannanases include, but are not limited to, endo- $\beta$ -mannanases of the GH26 family of glycosyl hydrolases, endo- $\beta$ -mannanases of the GH5 family of glycosyl hydrolases, acidic endo- $\beta$ -mannanases, neutral endo- $\beta$ -mannanases, and alkaline endo- $\beta$ -mannanases. Examples of alkaline endo- $\beta$ -mannanases include those described in U.S. Pat. Nos. 6,060,299, 6,566,114, and 6,602,842;

WO 9535362A1, WO 9964573A1, WO9964619A1, and WO2015022428. Additionally, suitable endo- $\beta$ -mannanases include, but are not limited to those of animal, plant, fungal, or bacterial origin. Chemically or genetically modified mutants are encompassed by the present disclosure.

**[0148]** Examples of useful endo- $\beta$ -mannanases include *Bacillus* endo- $\beta$ -mannanases such as *B. subtilis* endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,060,299, and WO 9964573A1), *B. sp.* 1633 endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *Bacillus* sp. AAI12 endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. sp.* AA349 endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. agaradhaerens* NCIMB 40482 endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. halodurans* endo- $\beta$ -mannanase, *B. clausii* endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *B. licheniformis* endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), *Humicola* endo- $\beta$ -mannanases such as *H. insolens* endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1), and *Caldocellulosiruptor* endo- $\beta$ -mannanases such as *C. sp.* endo- $\beta$ -mannanase (See, e.g., U.S. Pat. No. 6,566,114 and WO9964619A1).

**[0149]** Furthermore, a number of identified mannanases (i.e., endo- $\beta$ -mannanases and exo- $\beta$ -mannanases) find use in some embodiments of the present disclosure, including but not limited to *Agaricus bisporus* mannanase (See, Tang et al., [2001] Appl. Environ. Microbiol. 67: 2298-2303), *Aspergillus tamarii* mannanase (See, Civas et al., [1984] Biochem. J. 219: 857-863), *Aspergillus aculeatus* mannanase (See, Christgau et al., [1994] Biochem. Mol. Biol. Int. 33: 917-925), *Aspergillus awamori* mannanase (See, Setati et al., [2001] Protein Express Purif. 21: 105-114), *Aspergillus fumigatus* mannanase (See, Puchart et al., [2004] Biochimica et biophysica Acta. 1674: 239-250), *Aspergillus niger* mannanase (See, Ademark et al., [1998] J. Biotechnol. 63: 199-210), *Aspergillus oryzae* NRRL mannanase (See, Regalado et al., [2000] J. Sci. Food Agric. 80: 1343-1350), *Aspergillus sulphureus* mannanase (See, Chen et al., [2007] J. Biotechnol. 128(3): 452-461), *Aspergillus terrus* mannanase (See, Huang et al., [2007] Wei Sheng Wu Xue Bao. 47(2): 280-284), *Paenibacillus* and *Bacillus spp.* mannanase (See, U.S. Pat No. 6,376,445.), *Bacillus AM001* mannanase (See, Akino et al., [1989] Arch. Microbiol. 152: 10-15), *Bacillus brevis* mannanase (See, Araujo and Ward, [1990] J. Appl. Bacteriol. 68: 253-261), *Bacillus circulans* K-1 mannanase (See, Yoshida et al., [1998] Biosci. Biotechnol. Biochem. 62(3): 514-520), *Bacillus polymyxa* mannanase (See, Araujo and Ward, [1990] J. Appl. Bacteriol. 68: 253-261), *Bacillus sp* JAMB-750 mannanase (See, Hatada et al., [2005] Extremophiles. 9: 497-500), *Bacillus sp.* M50 mannanase (See, Chen et al., [2000] Wei Sheng Wu Xue Bao. 40: 62-68), *Bacillus sp.* N 16-5 mannanase (See, Yanhe et al., [2004] Extremophiles 8: 447-454), *Bacillus stearothermophilu* mannanase (See, Talbot and Sygusch, [1990] Appl. Environ. Microbiol. 56: 3505-3510), *Bacillus subtilis* mannanase (See, Mendoza et al., [1994] World J. Microbiol. Biotechnol. 10: 51-54), *Bacillus subtilis* B36 mannanase (Li et al., [2006] Z. Naturforsch (C). 61: 840-846), *Bacillus subtilis* BM9602 mannanase (See, Cui et al., [1999] Wei Sheng Wu Xue Bao. 39(1): 60-63), *Bacillus subtilis* SA-22 mannanase (See, Sun et al., [2003] Sheng Wu Gong Cheng Xue Bao. 19(3): 327-330), *Bacillus subtilis* 168 mannanase (See, Helow and Khattab, [1996] Acta Microbiol. Immunol. Hung. 43: 289-299), *Bacteroides ovatus* mannanase (See, Gherardini et al., [1987] J. Bacteriol. 169: 2038-2043), *Bacteroides*

*ruminicola* mannanase (See, Matsushita et al., [1991] *J. Bacteriol.* 173: 6919-6926), *Caldibacillus cellulovorans* mannanase (See, Sunna et al., [2000] *Appl. Environ. Microbiol.* 66: 664-670), *Caldocellulosiruptor saccharolyticus* mannanase (See, Morris et al., [1995] *Appl. Environ. Microbiol.* 61: 2262-2269), *Caldocellum saccharolyticum* mannanase (See, Bicho et al., [1991] *Appl. Microbiol. Biotechnol.* 36: 337-343), *Cellulomonas fimi* mannanase (See, Stoll et al., [1999] *Appl. Environ. Microbiol.* 65(6):2598-2605), *Clostridium butyricum/ beijerinckii* mannanase (See, Nakajima and Matsuura, [1997] *Biosci. Biotechnol. Biochem.* 61: 1739-1742), *Clostridium cellulolyticum* mannanase (See, Perret et al., [2004] *Biotechnol. Appl. Biochem.* 40: 255-259), *Clostridium tertium* mannanase (See, Kataoka and Tokiwa, [1998] *J. Appl. Microbiol.* 84: 357-367), *Clostridium thermocellum* mannanase (See, Halstead et al., [1999] *Microbiol.* 145: 3101-3108), *Dictyoglomus thermophilum* mannanase (See, Gibbs et al., [1999] *Curr. Microbiol.* 39(6): 351-357), *Flavobacterium* sp mannanase (See, Zakaria et al., [1998] *Biosci. Biotechnol. Biochem.* 62: 655-660), *Gastropoda pulmonata* mannanase (See, Charrier and Rouland, [2001] *J. Expt. Zool.* 290: 125-135), *Littorina brevicula* mannanase (See, Yamamura et al., [1996] *Biosci. Biotechnol. Biochem.* 60: 674-676), *Lycopersicon esculentum* mannanase (See, Filichkin et al., [2000] *Plant Physiol.* 134:1080-1087), *Paenibacillus curdlanolyticus* mannanase (See, Pason and Ratanakhanokchai, [2006] *Appl. Environ. Microbiol.* 72: 2483-2490), *Paenibacillus polymyxa* mannanase (See, Han et al., [2006] *Appl. Microbiol Biotechnol.* 73(3): 618-630), *Phanerochaete chrysosporium* mannanase (See, Wymelenberg et al., [2005] *J. Biotechnol.* 118: 17-34), *Piromyces* sp. mannanase (See, Fanutti et al., [1995] *J. Biol. Chem.* 270(49): 29314-29322), *Pomacea insularis* mannanase (See, Yamamura et al., [1993] *Biosci. Biotechnol. Biochem.* 7: 1316-1319), *Pseudomonas fluorescens* subsp. *Cellulose* mannanase (See, Braithwaite et al., [1995] *Biochem J.* 305: 1005-1010), *Rhodothermus marinus* mannanase (See, Politz et al., [2000] *Appl. Microbiol. Biotechnol.* 53 (6): 715-721), *Sclerotium rolfsii* mannanase (See, Sachslehner et al., [2000] *J. Biotechnol.* 80:127-134), *Streptomyces galbus* mannanase (See, Kansoh and Nagieb, [2004] *Anton. van. Leeuwenhoek.* 85: 103-114), *Streptomyces lividans* mannanase (See, Arcand et al., [1993] *J.Biochem.* 290: 857-863), *Thermoanaerobacterium Polysaccharolyticum* mannanase (See, Cann et al., [1999] *J. Bacteriol.* 181: 1643-1651), *Thermomonospora fusca* mannanase (See, Hilge et al., [1998] *Structure* 6: 1433-1444), *Thermotoga maritima* mannanase (See, Parker et al., [2001] *Biotechnol. Bioeng.* 75(3): 322-333), *Thermotoga neapolitana* mannanase (See, Duffaud et al., [1997] *Appl. Environ. Microbiol.* 63: 169-177), *Trichoderma harzanium* strain T4 mannanase (See, Franco et al., [2004] *Biotechnol Appl. Biochem.* 40: 255-259), *Trichoderma reesei* mannanase (See, Stalbrand et al., [1993] *J. Biotechnol.* 29: 229-242), and *Vibrio* sp. mannanase (See, Tamaru et al., [1997] *J. Ferment. Bioeng.* 83: 201-205).

**[0150]** Additional suitable endo- $\beta$ -mannanases include commercially available endo- $\beta$ -mannanases such as HEMICELL® (Chemgen); GAMANASE® and MANNAWAY®, (Novozymes A/S, Denmark); PURABRITE™ and MANNASTAR™ (Genencor, A Danisco Division, Palo Alto, CA); and PYROLASE® 160 and PYROLASE® 200 (Diversa).

**[0151]** In some embodiments of the present disclosure, the cleaning compositions of the present disclosure further comprise endo- $\beta$ -mannanases at a level from about 0.00001% to

about 10% of additional endo- $\beta$ -mannanase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In other aspects of the present disclosure, the cleaning compositions of the present disclosure also comprise endo- $\beta$ -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% endo- $\beta$ -mannanase by weight of the composition.

**[0152]** In some embodiments of the present disclosure, any suitable protease may be used. Suitable proteases include those of animal, vegetable or microbial origin. 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. Various proteases are described in PCT applications WO 95/23221 and WO 92/21760; U.S. Pat. Publication No. 2008/0090747; and U.S. Pat. Nos. 5,801,039; 5,340,735; 5,500,364; 5,855,625; U.S. RE 34,606; 5,955,340; 5,700,676; 6,312,936; 6,482,628; and various other patents. In some further embodiments, metalloproteases find use in the present disclosure, including but not limited to the neutral metalloprotease described in PCT application WO 07/044993. 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, P110, P280), EFFECTENZ™ proteases (e.g. P1000, P1050, P2000), EXCELLENZ™ proteases (e.g. P1000), ULTIMASE®, and PURAFAST™ (DuPont); ALCALASE®, SAVINASE®, PRIMASE®, DURAZYME™, POLARZYME®, OVOZYME®, KANNASE®, LIQUANASE®, NEUTRASE®, RELEASE® and ESPERASE® (Novozymes); BLAP™ and BLAP™ variants (Henkel Kommanditgesellschaft auf Aktien, Duesseldorf, Germany), and KAP (*B. alkalophilus* subtilisin; Kao Corp., Tokyo, Japan).

**[0153]** In some embodiments of the present disclosure, any suitable amylase may be used. 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 disclosure include, but are not limited to  $\alpha$ -amylases obtained from *B. licheniformis* (See, e.g., GB 1,296,839). Commercially available amylases that find use in the present disclosure include, but are not limited to DURAMYL®, TERMAMYL®, FUNGAMYL®, STAINZYME®, STAINZYME PLUS®, STAINZYME ULTRA®, and BAN™ (Novozymes A/S, Denmark), as well as PURASTAR®, POWERASE™, RAPIDASE®, and MAXAMYL® P (Genencor, A Danisco Division, Palo Alto, CA).

**[0154]** In some embodiments of the present disclosure, the disclosed cleaning compositions 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 other aspects of the present disclosure, the cleaning compositions 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.

**[0155]** In some embodiments of the present disclosure, any suitable pectin degrading enzyme

may be used. As used herein, "pectin degrading enzyme(s)" encompass arabinanase (EC 3.2.1.99), galactanases (EC 3.2.1.89), polygalacturonase (EC 3.2.1.15) exo-polygalacturonase (EC 3.2.1.67), exo-poly-alpha-galacturonidase (EC 3.2.1.82), pectin lyase (EC 4.2.2.10), pectin esterase (EC 3.2.1.11), pectate lyase (EC 4.2.2.2), exo-polygalacturonate lyase (EC 4.2.2.9) and hemicellulases such as endo-1,3- $\beta$ -xylosidase (EC 3.2.1.32), xylan-1,4- $\beta$ -xylosidase (EC 3.2.1.37) and  $\alpha$ -L-arabinofuranosidase (EC 3.2.1.55). Pectin degrading enzymes are natural mixtures of the above mentioned enzymatic activities. Pectin enzymes therefore include the pectin methylesterases which hydrolyse the pectin methyl ester linkages, polygalacturonases which cleave the glycosidic bonds between galacturonic acid molecules, and the pectin transeliminases or lyases which act on the pectic acids to bring about non-hydrolytic cleavage of  $\alpha$ -1,4 glycosidic linkages to form unsaturated derivatives of galacturonic acid.

**[0156]** Suitable pectin degrading enzymes include those of plant, fungal, or microbial origin. In some embodiments, chemically or genetically modified mutants are included. In some embodiments, the pectin degrading enzymes are alkaline pectin degrading enzymes, i.e., enzymes having an enzymatic activity of at least 10%, preferably at least 25%, more preferably at least 40% of their maximum activity at a pH of from about 7.0 to about 12. In certain other embodiments, the pectin degrading enzymes are enzymes having their maximum activity at a pH of from about 7.0 to about 12. Alkaline pectin degrading enzymes are produced by alkalophilic microorganisms e.g., bacterial, fungal, and yeast microorganisms such as *Bacillus* species. In some embodiments, the microorganisms are *Bacillus firmus*, *Bacillus circulans*, and *Bacillus subtilis* as described in JP 56131376 and JP 56068393. Alkaline pectin decomposing enzymes may include but are not limited to galacturn-1,4- $\alpha$ -galacturonase (EC 3.2.1.67), polygalacturonase activities (EC 3.2.1.15, pectin esterase (EC 3.1.1.11), pectate lyase (EC 4.2.2.2) and their iso enzymes. Alkaline pectin decomposing enzymes can be produced by the *Erwinia* species. In some embodiments, the alkaline pectin decomposing enzymes are produced by *E. chrysanthemi*, *E. carotovora*, *E. amylovora*, *E. herbicola*, and *E. dissolvens* as described in JP 59066588, JP 63042988, and in World J. Microbiol. Microbiotechnol. (8, 2, 115-120) 1992. In certain other embodiments, the alkaline pectin enzymes are produced by *Bacillus* species as disclosed in JP 73006557 and Agr. Biol. Chem. (1972), 36 (2) 285-93.

**[0157]** In some embodiments of the present disclosure, the disclosed cleaning compositions further comprise pectin degrading enzymes at a level from about 0.00001% to about 10% of additional pectin degrading enzyme by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In other aspects of the present disclosure, the cleaning compositions also comprise pectin degrading 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% pectin degrading enzyme by weight of the composition.

**[0158]** In some other embodiments, any suitable xyloglucanase finds used in the cleaning compositions of the present disclosure. Suitable xyloglucanases include, but are not limited to those of plant, fungal, or bacterial origin. Chemically or genetically modified mutants are included in some embodiments. As used herein, "xyloglucanase(s)" encompass the family of enzymes described by Vincken and Voragen at Wageningen University [Vincken et al (1994)

Plant Physiol., 104, 99-107] and are able to degrade xyloglucans as described in Hayashi et al (1989) Plant. Physiol. Plant Mol. Biol., 40, 139-168. Vincken et al demonstrated the removal of xyloglucan coating from cellulose of the isolated apple cell wall by a xyloglucanase purified from *Trichoderma viride* (endo-IV-glucanase). This enzyme enhances the enzymatic degradation of cell wall-embedded cellulose and work in synergy with pectic enzymes. Rapidase LIQ+ from Gist-Brocades contains a xyloglucanase activity.

**[0159]** In some embodiments of the present disclosure, the disclosed cleaning compositions further comprise xyloglucanases at a level from about 0.00001% to about 10% of additional xyloglucanase by weight of the composition and the balance of cleaning adjunct materials by weight of composition. In other aspects of the present disclosure, the cleaning compositions also comprise xyloglucanases 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% xyloglucanase by weight of the composition. In certain other embodiments, xyloglucanases for specific applications are alkaline xyloglucanases, *i.e.*, enzymes having an enzymatic activity of at least 10%, preferably at least 25%, more preferably at least 40% of their maximum activity at a pH ranging from 7 to 12. In certain other embodiments, the xyloglucanases are enzymes having their maximum activity at a pH of from about 7.0 to about 12.

**[0160]** In some further embodiments, any suitable cellulase finds used in the cleaning compositions of the present disclosure. 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 *Humicola insolens* cellulases (See, *e.g.*, U.S. Pat. No. 4,435,307). Especially suitable cellulases are the cellulases having color care benefits (See, *e.g.*, EP 0 495 257). Commercially available cellulases that find use in the present disclosure include, but are not limited to ENDOLASE®, CELLUCLEAN®, CELLUZYME®, CAREZYME® (Novozymes A/S, Denmark). Additional commercially available cellulases include PURADEX® (Genencor, A Danisco Division, Palo Alto, CA) 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.*, U.S. Pat. No. 5,874,276). In some embodiments, the cleaning compositions of the present disclosure 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 other aspects of the present disclosure, the cleaning compositions 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.

**[0161]** In still further embodiments, any lipase suitable for use in detergent compositions also finds use in the present disclosure. Suitable lipases include, but are not limited to those of bacterial or fungal origin. Chemically or genetically modified mutants are included in some embodiments. Examples of useful lipases include *Humicola lanuginosa* lipase (See, *e.g.*, EP 258 068, and EP 305 216), *Rhizomucor miehei* lipase (See, *e.g.*, EP 238 023), *Candida* lipase, such as *C. antarctica* lipase (*e.g.*, the *C. antarctica* lipase A or B; see, *e.g.*, EP 214 761),

*Pseudomonas* lipases such as *P. alcaligenes* lipase and *P. pseudoalcaligenes* lipase (See, e.g., EP 218 272), *P. cepacia* lipase (See, e.g., EP 331 376), *P. stutzeri* lipase (See, e.g., GB 1,372,034), *P. fluorescens* lipase, *Bacillus* lipase (e.g., *B. subtilis* lipase [Dartois et al., (1993) Biochem. Biophys. Acta 1131:253-260]; *B. stearothermophilus* lipase [See, e.g., JP 64/744992]; and *B. pumilus* lipase [See, e.g., WO 91/16422]). Furthermore, a number of cloned lipases find use in some embodiments of the present disclosure, including but not limited to *Penicillium camembertii* lipase (See, Yamaguchi et al., [1991] Gene 103:61-67), *Geotrichum candidum* lipase (See, Schimada et al., [1989] J. Biochem. 106:383-388), and various *Rhizopus* lipases such as *R. delemar* lipase (See, Hass et al., [1991] Gene 109:117-113), *R. niveus* lipase (Kugimiya et al., [1992] Biosci. Biotech. Biochem. 56:716-719), and *R. oryzae* lipase. Other types of lipolytic enzymes such as cutinases also find use in some embodiments of the present disclosure, including but not limited to the cutinase derived from *Pseudomonas mendocina* (See, WO 88/09367), and the cutinase derived from *Fusarium solani pisi* (See, WO 90/09446). Additional suitable lipases include commercially available lipases such as M1 LIPASE™, LUMA FAST™, and LIPOMAX™ (Genencor, A Danisco Division, Palo Alto, CA); LIPEX®, LIPOCLEAN®, LIPOLASE® and LIPOLASE® ULTRA (Novozymes A/S, Denmark); and LIPASE P™ "Amano" (Amano Pharmaceutical Co. Ltd., Japan).

**[0162]** In some embodiments, the disclosed cleaning compositions 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 other aspects of the present disclosure, the cleaning compositions 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.

**[0163]** 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 disclosure. 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., WO 94/12621 and WO 95/01426). 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 disclosure 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 other aspects of the present disclosure, the cleaning compositions 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.

**[0164]** In some embodiments, additional enzymes find use, including but not limited to perhydrolases (See, e.g., WO 05/056782). In addition, in some particularly preferred 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 disclosure. It is also contemplated that the varying levels of a polypeptide of the present invention(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).

**[0165]** Examples of suitable cleaning adjunct materials include, but are not limited to, surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dye transfer inhibiting agents, catalytic materials, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal agents, structure elasticizing agents, dispersants, suds suppressors, dyes, perfumes, colorants, filler salts, hydrotropes, photoactivators, fluorescers, fabric conditioners, fabric softeners, carriers, hydrotropes, processing aids, solvents, pigments, 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 (See, e.g., U.S. Pat. Nos. 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). Embodiments of specific cleaning composition materials are exemplified in detail below. In embodiments in which the cleaning adjunct materials are not compatible with the disclosed a polypeptide of the present inventions in the cleaning compositions, then suitable methods of keeping the cleaning adjunct materials and the endo- $\beta$ -mannanase(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.).

**[0166]** In some preferred embodiments, an effective amount of one or more polypeptide of the present invention(s) provided herein are included in compositions useful for cleaning a variety of surfaces in need of stain removal. Such cleaning compositions include cleaning compositions for such applications as cleaning hard surfaces, fabrics, and dishes. Indeed, in some embodiments, the present disclosure provides fabric cleaning compositions, while in other embodiments, the present disclosure provides non-fabric cleaning compositions. Notably, the present disclosure 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. Additionally, in still other embodiments, the present disclosure provides fabric softening compositions. It is intended that the present disclosure encompass detergent compositions in any form (i.e., liquid, granular, bar, solid, semisolid, gel, paste, emulsion, tablet, capsule, unit dose, sheet, foam etc.).

**[0167]** By way of example, several cleaning compositions wherein the disclosed a polypeptide of the present inventions find use are described in greater detail below. In some embodiments

in which the disclosed cleaning compositions are formulated as compositions suitable for use in laundry machine washing method(s), the compositions of the present disclosure 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 disclosure also find use 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.

**[0168]** In embodiments formulated as compositions for use in manual dishwashing methods, the compositions of the disclosure 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.

**[0169]** In some embodiments, various cleaning compositions such as those provided in U.S. Pat. No. 6,605,458 find use with a polypeptide or active fragment as described. Thus, in some embodiments, the composition 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 are fabric cleaning compositions such as those described in U.S. Pat. Nos. 6,610,642 and 6,376,450. In addition, a polypeptide as described may find use in granular laundry detergent compositions of particular utility under European or Japanese washing conditions (See, *e.g.*, U.S. Pat. No. 6,610,642).

**[0170]** In some alternative embodiments, the present disclosure provides hard surface cleaning compositions. Thus, in some embodiments, the composition is a hard surface cleaning composition such as those described in U.S. Pat. Nos. 6,610,642; 6,376,450; and 6,376,450.

**[0171]** In yet further embodiments, the present disclosure provides dishwashing compositions. Thus, in some embodiments, the composition is a hard surface cleaning composition such as those in U.S. Pat. Nos. 6,610,642 and 6,376,450. In some still further embodiments, the present disclosure provides dishwashing compositions. In some further embodiments, the compositions comprise oral care compositions such as those in U.S. Pat. Nos. 6,376,450 and 6,605,458. The formulations and descriptions of the compounds and cleaning adjunct materials contained in the aforementioned U.S. Pat. Nos. 6,376,450; 6,605,458; and 6,610,642 may be of use.

**[0172]** In still further embodiments, the compositions comprise fabric softening compositions such as those in GB-A1 400898, GB-A1 514 276, EP 0 011 340, EP 0 026 528, EP 0 242 919, EP 0 299 575, EP 0 313 146, and U.S. Pat. No. 5,019,292. The formulations and descriptions of the compounds and softening agents contained in the aforementioned GB-A1 400898, GB-A1 514 276, EP 0 011 340, EP 0 026 528, EP 0 242 919, EP 0 299 575, EP 0 313 146, and U.S. Pat. No. 5,019,292 may be of use.

**[0173]** The cleaning compositions of the present disclosure are formulated into any suitable form and prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. Pat. Nos. 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. 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 HCl.

**[0174]** In some embodiments, the cleaning compositions of the present invention are provided in unit dose form, including tablets, capsules, sachets, pouches, sheets, and multi-compartment pouches. In some embodiments, the unit dose format is designed to provide controlled release of the ingredients within a multi-compartment pouch (or other unit dose format). Suitable unit dose and controlled release formats are known in the art (See e.g., EP 2 100 949, WO 02/102955, US Pat. Nos. 4,765,916 and 4,972,017, and WO 04/111178 for materials suitable for use in unit dose and controlled release formats). In some embodiments, the unit dose form is provided by tablets wrapped with a water-soluble film or water-soluble pouches. Various unit dose formats are provided in EP 2 100 947 and WO2013/165725, and are known in the art.

**[0175]** While not essential for the purposes of the present disclosure, 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. 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, 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 U.S. Pat. Nos. 5,576,282; 6,306,812; and 6,326,348. The aforementioned adjunct ingredients may constitute the balance of the cleaning compositions of the present disclosure.

**[0176]** In some embodiments, the cleaning compositions according to the present disclosure 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 low pH cleaning composition embodiments (e.g., compositions having a neat pH of from about 3 to about 5), the composition typically does not contain alkyl ethoxylated sulfate, as it is believed that such surfactant may be hydrolyzed by such compositions' acidic contents. 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.

**[0177]** In some embodiments, the cleaning compositions of the present disclosure contain at least one chelating agent. Suitable chelating agents may 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 disclosure 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.

**[0178]** 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.

**[0179]** As indicated herein, in some embodiments, anti-redeposition agents find use in some embodiments of the present disclosure. In some preferred 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 preferred embodiments, the anti-redeposition agent is a non-ionic surfactant as known in the art (See, e.g., EP 2 100 949).

**[0180]** In some embodiments, the cleaning compositions of the present disclosure 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 disclosure 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.

**[0181]** In some embodiments, silicates are included within the compositions of the present disclosure. 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 preferred embodiments, silicates are present at a level of from about 5% to about 15% by weight of the composition.

**[0182]** In some still additional embodiments, the cleaning compositions of the present disclosure 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.

**[0183]** 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. It is contemplated that various techniques for enzyme stabilization will find use in the present disclosure. 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 disclosure. Examples of suitable oligosaccharides and polysaccharides (e.g., dextrins) are known in the art (See, e.g., WO 07/145964). 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.

**[0184]** In some embodiments, bleaches, bleach activators, and/or bleach catalysts are present in the compositions of the present disclosure. In some embodiments, the cleaning compositions of the present disclosure comprise inorganic and/or organic bleaching compound(s). Inorganic bleaches may 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 disclosure (See, e.g., EP 2 100 949).

**[0185]** In some embodiments, bleach activators are used in the compositions of the present disclosure. 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 peroxydicarboxylic 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 disclosure (See, e.g., EP 2 100 949).

**[0186]** In addition, in some embodiments and as further described herein, the cleaning compositions of the present disclosure 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 disclosure (See, e.g., U.S. Pat. No. 4,246,612; U.S. Pat. No. 5,227,084; U.S. Pat. No. 4,810,410; WO 99/06521; and EP 2 100 949).

**[0187]** In some embodiments, the cleaning compositions of the present disclosure contain one or more catalytic metal complexes. In some embodiments, a metal-containing bleach catalyst finds use. In some preferred 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., U.S. Pat. No. 4,430,243). In some embodiments, the cleaning compositions of the present disclosure are catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art (See, e.g., U.S. Pat. No. 5,576,282). In additional embodiments, cobalt bleach catalysts find use in the cleaning compositions of the present disclosure. Various cobalt bleach catalysts are known in the art (See, e.g., U.S. Pat. Nos. 5,597,936 and 5,595,967) and are readily prepared by known procedures.

**[0188]** In some additional embodiments, the cleaning compositions of the present disclosure 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 disclosure 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 preferred embodiments, provide from about 0.005 ppm to about 25 ppm, more preferably from about 0.05 ppm to about 10 ppm, and most preferably from about 0.1 ppm to about 5 ppm, of the MRL in the wash liquor.

**[0189]** In some embodiments, preferred transition-metals in the instant transition-metal bleach catalyst include, but are not limited to manganese, iron, and chromium. Preferred MRLs also include, but are not limited to special ultra-rigid ligands that are cross-bridged (e.g., 5,12-diethyl-1,5,8,12-tetraazabicyclo[6.6.2] hexadecane). Suitable transition metal MRLs are readily prepared by known procedures (See, e.g., WO 2000/32601 and U.S. Pat. No. 6,225,464).

**[0190]** In some embodiments, the cleaning compositions of the present disclosure 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 EP 2 100 949, WO 94/26860, and WO 94/26859). In some embodiments, the metal care agent is a

zinc salt. In some further embodiments, the cleaning compositions of the present disclosure comprise from about 0.1% to about 5% by weight of one or more metal care agent.

**[0191]** As indicated above, the cleaning compositions of the present disclosure are formulated into any suitable form and prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. Pat. Nos. 5,879,584; 5,691,297; 5,574,005; 5,569,645; 5,516,448; 5,489,392; and 5,486,303. In some embodiments in which a low pH cleaning composition is desired, the pH of such composition is adjusted via the addition of an acidic material such as HCl.

**[0192]** The cleaning compositions disclosed herein of find use in cleaning a *situs* (e.g., a surface, dishware, or fabric). Typically, at least a portion of the *situs* is contacted with an embodiment of the present cleaning composition, in neat form or diluted in wash liquor, and then the *situs* is optionally washed and/or rinsed. For purposes of the present disclosure, "washing" includes but is not limited to, scrubbing and mechanical agitation. In some embodiments, the cleaning compositions are typically employed at concentrations of from about 500 ppm to about 15,000 ppm in solution. When the wash solvent is water, the water temperature typically ranges from about 5°C to about 90°C and, when the *situs* comprises a fabric, the water to fabric mass ratio is typically from about 1:1 to about 30:1.

**[0193]** Other aspects and embodiments of the present compositions and methods will be apparent from the foregoing description and following examples.

## EXAMPLES

**[0194]** Certain of the following examples illustrate the invention. Others provide useful context.

### EXAMPLE 1

#### Identification of *Bacillus* and *Paenibacillus* mannanases

**[0195]** The following nucleotide and amino acid sequences for mannanases encoded by *Bacillus* and *Paenibacillus* species were extracted from the NCBI Database.

**[0196]** The nucleotide sequence of the *BciMan1* gene (NCBI Reference Sequence AB007123.1) isolated from *B. circulans* K-1 is set forth as SEQ ID NO:1 (the sequence encoding the predicted native signal peptide is shown in bold):

ATGGGGTGGTTTTAGTGATTACGCAAGTGGTTGATTGCTTTGTCGCATTT  
TTACTGATGTTCTCGTGGACTGGACAACCTACGAACAAAGCACATGCTGCAAGC  
GGATTTATGTAAGCGGTACCAAATTATTGGATGCTACAGGACAACCATTGTGATG

CGAGGAGTCAATCATGCGCACACATGGTATAAAGATCAACTATCCACCGCAATACC  
 AGCCATTGCTAAAACAGGTGCCAACACGATACTGATTGTACTGGCGAATGGACACA  
 AATGGACGCTTGATGATGTAACACCCGTCAACAATATTCTCACCCCTGTGAACAA  
 AACAAACTAATTGCCGTTGGAAAGTACATGACGCTACAGGAAGCGATAGTCTTC  
 CGATTAGACAACGCCGTTAATTACTGGATTGGTATAAAAGCGCGTTGATCGGCA  
 AGGAAGACCGTGTAAATCATTAATATAGCTAACGAGTGGTACGGAACATGGGATGGA  
 GTCGCCTGGCTAATGGTTAAGCAAGCCATACCCAACTGCGTAATGCTGGTCT  
 AACTCATACGCTGATTGTTGACTCCGCTGGATGGGACAATATCCAGATTGGTCAA  
 AAATTATGGGACAGAAGTACTGAATGCAGACCCGTTAAAAACACAGTATTCTCTA  
 TCCATATGTATGAATATGCTGGGGCAATGCAAGTACCGTCAAATCCAATATTGAC  
 GGTGTGCTGAACAAGAATCTTGCAGTGATTATCGCGAATTGGTGGACAACATAC  
 AAACGGTGATGTGGATGAAGCCACCATTATGAGTTATTCCAAGAGAAGGGAGTCG  
 GCTGGTTGGCTTGGCCTGGAAAGGGAAATAGCAGTGATTGGCTTATCTCGATATGA  
 CAAATGATTGGCTGGTAACCTCCTCACCTCGTTCGTAATACCGTAGTGAATGGCA  
 GTAACGGCATTAAAGCAACTTCTGTGTTATCCGGCATTTGGAGGTGTTACGCCA  
 CCTCAAGCCCTACTTCTACACCTACATCTACGCCAACCTCAACTCCTACTCCTACGC  
 CAAGTCCGACCCCGAGTCCAGGTAAATAACGGGACGATCTTATATGATTTCGAAACA  
 GGAACACTCAAGGCTGGTCGGAAACAATATTCGGAGGCCATGGTCACCAATGA  
 ATGGAAAGCAACGGGAGCGCAAACCTCTCAAAGCCGATGTCCTTACAATCCAATT  
 CCACGCATAGTCTATATATAACCTCTAATCAAATCTGTCTGGAAAAAGCAGTCTGA  
 AAGCAACGGTTAACGATGCGAACTGGGCAATATCGCAACGGGATTATGCAAAA  
 CTATACGTAAGACCGGGTCCGGTGGACATGGTACGATTCCGGAGAGAATCTGAT  
 TCAGTCAAACGACGGTACCATTTGACACTATCCCTCAGCGGCATTCAATTTGTC  
 CTCAGTCAAAGAAATTGGGGTAGAATTCCCGCCTCCTCAAACAGTAGTGGCCAAT  
 CAGCTATTATGTAGATAGTGTAGTCTGCAATGA.

**[0197]** The amino acid sequence of the precursor protein encoded by the *BciMan1* gene, BciMan1 (NCBI Accession No. BAA25878.1) is set forth as SEQ ID NO:2 (the predicted native signal peptide is shown in bold):

**MGWFLVILRKWLIAFVAFLMFSWTGQLTNKAHAASGFYVSGTKLLDATGQPFVM**  
 RGVNHAHTWYKDQLSTAIPAIAKTGANTIRIVLANGHKWTLDDVNTVNNILTLCEQNK  
 LIAVLEVHDATGSDSLSDLNAVNYYWIGIKSALIGKEDRVIINIANEWYGTWDGVAWA  
 NGYKQAIPLRNAGLTHTLIVDSAGWGQYPDSVKNYGTEVLNADPLKNTVFSIHMYEY  
 AGGNASTVKSNIDGVLNKNLALIIGEFGGQHTNGDVDEATIMSYSQEKGVGWLAWSW  
 KGNSSDLAYLDMTNDWAGNSLTSFGNTVVNGSNGIKATSVLSGIFGGVPTSSPTSTPT  
 STPTSTPTPSPTPSPGNNGTILYDFETGTQGWSGNNISGGPWVTNEWKATGAQTLKA  
 DVSLQSNSTHSLYITSQNLSGKSSLKATVKHANWGNIGNGIYAKLYVKTGSGWTWYD  
 SGENLIQSNDGTILTLSLSGISNLSSVKEIGVEFRASSNSSGQSAIYVDSVSLQ.

**[0198]** The nucleic acid sequence for the *BciMan3* gene (NCBI Reference Sequence AY907668.1, from 430 to 1413, complement) isolated from *B. circulans* 196 is set forth as SEQ ID NO:3 (the sequence encoding the predicted native signal peptide is shown in bold):

**ATGATGTTGATATGGATGCAGGGATGGAAGTCTATTCTAGTCGCATCTTGGC**  
**GTGTGTGTCAGTAGGCGGTGGCCTCTAGTCCAGAAGCAGGCCACAGGATTTA**  
**TGTAAACGGTACCAAGCTGTATGATTCAACGGCAAGGCCTTGTGATGAGGGGTG**  
**TAAATCATCCCCACACCTGGTACAAGAATGATCTGAACGCGGCTATTCCGGCTATCG**  
**CGCAAACGGGAGCCAATACCGTACGAGTCGTCTTGTGACGGGTCGCAATGGACC**  
**AAGGATGACCTGAACCTCGTCAACAGTATCATCTCGCTGGTGTGCGAGCATCAAAT**  
**GATAGCCGTTCTGGAGGTGCATGATGCGACAGGCAAAGATGAGTATGCTCCCTG**  
**AAGCGGCCGTCGACTATTGGATCAGCATCAAAGGGCATTGATCGGAAAAGAAGA**  
**CCGCGTCATCGTCAATATTGCTAATGAATGGTATGAAATTGGAACAGCAGCGGAT**  
**GGGCCGATGGTTATAAGCAGGCCATTCCCAAATTAAGAAACGCGGGCATTAAGAAT**  
**ACGTTGATCGTTGATGCAGCGGATGGGGCAATACCGCAATCCATCGTGGATGA**  
**GGGGGCCGCGGTATTTGCTTCCGATCAACTGAAGAATACGGTATTCTCCATCCATAT**  
**GTATGAGTATGCCGTAAGGATGCCCTACGGTAAAACGAATATGGACGATGTTT**  
**TAAACAAAGGATTGCCCTTAATCATGGGAGTCGGCGCTATCATCAAGGTGCC**  
**GATGTCGATGAGATTGCTATTATGAAGTACGGACAGCAGAAGGAAGTGGCTGGCT**  
**GGCTTGGCCTGGTACGGAAACAGCCGGAGCTGAACGATTGGATCTGGCTGCAG**  
**GGCCAAGCGGAAACCTGACCGGCTGGGAAACACGGTGGTCATGGAACCGACGG**  
**GATTCAAGCAAACCTCCAAGAAAGCGGGCATTATTAA.**

**[0199]** The amino acid sequence of the precursor protein encoded by the *BciMan3* gene, BciMan3 (NCBI Accession No. AAX87002.1) is set forth as SEQ ID NO:4 (the predicted native signal peptide is shown in bold):

**MMLIWMQGWKSILVAILACSVVGGLPSPEATGFYVNGTKLYDSTGKAFVMRGV**  
**NHPHTWYKNDLNAIIPAIAQTGANTVRVVLNSNGSQWTKDDLNSVNSIISLVSQHQ**  
**MIAVLEVHDATGKDEYASLEAAVDYWISIKGALIGKEDRIVVNIANEWYGNWNSSGWADG**  
**YKQAIPLRNAGIKNTLIVDAAGWGQYPQSIVDEGAAVFASDQLKNTVFSIHM**  
**YEYAGKDAATVKTNMDDVLNKGPLIIGEFGGYHQGADVDEIAIMKYGQQKEVGWLAW**  
**SWYGN SPELNDLDAAGPSGNLTGWGNTVHGTGDIQQTSKKAGIY.**

**[0200]** The nucleic acid sequence for the *BciMan4* gene (NCBI Reference Sequence AY913796.1, from 785 to 1765) isolated from *Bacillus circulans* CGMCC1554 is set forth as SEQ ID NO:5 (the sequence encoding the predicted native signal peptide is shown in bold):

**ATGGCCAAGTTGCAAAAGGGTACAATCTAACAGTCATTGCAGCACTGATGTT**  
**TGTCATTTGGGGAGCGCGGCCAAAGCCGCAGCAGCTACAGGTTTACGT**  
**GAATGGAGGCAAATTGTACGATTCTACGGTAAACCATTACATGAGGGGTATCA**  
**ATCGATCCCCACTGCGCTTAAAGAATTCGAAACGGCTATCGCTCCGATCGCAAA**

ATCATGGGCACTCTGGTTAAAAAATGATTGAAACACGGTATCCCTGGATCCCAA  
 AAACGGGTCCAATACGGTACGAATTGTTTATCAAACGGTACACAATACACCAAG  
 GATGATCTGAATTCCGTAAAAAACATCATTAATGTCGTAATGCAAACAAGATGAT  
 TGCTGTGCTGAAGTACACGATGCCACTGGAAAGATGACTTCAACTCGTGGATG  
 CAGCGTCAACTACTGGATAAGCATCAAAGAACGACTGATCGGAAGGAAGATCG  
 GGTTATTGAAACATTGCAAACAGAGTGGTACGGAACATGGAACGGAAAGCGCGTGG  
 CTGACGGTACAAAAAGCTATTCCGAAATTAGAGATGCGGGTATTAAAAATACC  
 TTGATTGTAGATGCAGCAGGCTGGGTCACTACCCCTCAATCGATCGTCAATTACGG  
 ACAAAGCGTATTGCCCGGATTACAGAAAAATACGGCTTCCATTACATGT  
 ATGAGTATGCAGGCAAGGATGCGGCCACCGTCAAATCCAATATGGAAAATGTGCTG  
 AATAAGGGCTGGCTTAATCATTGGTAGTCGGAGGATATCACACCAATGGAGA  
 TGTCGATGAATATGCAATCATGAAATATGGTCTGGAAAAAGGGTAGGATGGCTTG  
 CATGGTCTGGTACGGTAATAGCTCTGGATTAAACTATCTTGATTGGCAACAGGAC  
 CTAACGGCAGTTGACGAGCTATGGTAATACGGTTGTCAATGATACTTACGGAATTA  
 AAAATACGTCCCAGGGAAATCTTTAA.

**[0201]** The amino acid sequence of the precursor protein encoded by the *BciMan4* gene, BciMan4 (NCBI Accession No. AAX87003.1) is set forth as SEQ ID NO:6 (the predicted native signal peptide is shown in bold):

**MAKLQKG**TILT**VIAALMF**VILGSAAPKAAAATGFYVNGKLYDSTGKPFYMRGINH  
 GHSWFKNLNTAIPAIK**TGANTV**RIVLSNGTQYTKDDNSVKNIINVNANKMIAVLE  
 VHDATGKDDFNSLDAAVNYWISIKEALIGKEDRVIVNIA NEWYGTWNGSAWADGYKK  
 AIPKLRDAGIKNTLIVDAAGWGQYPQSIVDYQSVFAADSKNTAFSIHMYEYAGKDA  
 ATVKSNMENVLNKGLALIIGEFGGYHTNGDVDEYAIMKYGLEKGVGWLAWSWYGN  
 SGLNYLDLATGPNGSLTSYGN  
 TTVNDTYGIKNTSQKAGIF.

**[0202]** The nucleic acid sequence for the *PpoMan1* gene (NCBI Reference Sequence NC\_014483.1, from 649134 to 650117, complement) isolated from *Paenibacillus polymyxa* E681 is set forth as SEQ ID NO:7 (the sequence encoding the predicted native signal peptide is shown in bold):

**ATGAAGGTATTGTTAAGAAAAGCATTATTG**TCTGGACTGGTCGGCTTGCTCATC  
**ATGATTGGTTAGGAGGAGTTCTCC**AAGGTAGAAGCTGCTTCAGGATTATG  
 TAAGCGGTACCAATTGTATGACTCTACAGGCAAGCCATTGTTATGAGAGGCGTC  
 AATCATGCTCACACTGGTACAAAACGATCTTATACAGCTATCCCGCAATTGCC  
 CAGACAGGTGCTAATACCGTCCGAATTGCTCTTAACGGAAACCACTACACCAA  
 GGATGACATTAATTCCGTAAAAATATTATCTCTCTGTCTCCA  
 ACTATAAAATGAT  
 TGCTGTACTGAAGTTCATGCTACAGGCAAAGACGACTACCGCGTCTTGGATGC  
 AGCTGTGAACTACTGGATTAGCATAAAAGATGCTCTGATCGGAAGGAAGACCGGG  
 TTATCGTAAACATTGCGAACGAATGGTATGGTCTTGGAAATGGAAGTGGTGGGCT

GATGGATACAAGCAAGCGATTCCAAGTTGAGAAACGCAGGTATCAAAAATACGCT  
 CATCGTCGATTGTGCCGGATGGGGACAGTATCCTCAGTCTATCAATGACTTGGTAA  
 ATCTGTATTGCAGCTGATTCTTGAAGAATACGGTATTCTCTATTCATATGTATGAG  
 TTCGCTGGTAAAGATGCTCAAACCGTTCGAACCAATATTGATAACGTTCTGAATCAA  
 GGAATTCCCTCTGATTATTGGTGAATTGGAGGTTACCACCAGGGAGCAGACGTCGA  
 CGAGACAGAAATCATGAGATATGCCAATCCAAAGGAGTAGGCTGGTTAGCCTGGT  
 CCTGGTATGGTAATAGTCCAACCTTCCTACCTGATCTGTAACAGGACCTAATG  
 GCAATCTGACGGATTGGGGAAAAACTGTAGTTAACGGAAGCAACGGATCAAAGA  
 AACATCGAAAAAAGCTGGTATCTACTAA.

**[0203]** The amino acid sequence of the protein encoded by the *PpoMan1* gene, PpoMan1 (NCBI Accession No. YP\_003868989.1) is set forth as SEQ ID NO:8 (the predicted native signal peptide is shown in bold):

**MKVLLRKALLSGLVGLIMIGLGGVFSKVEAASGFYVSGTKLYDSTGKPVMRGVN**  
 HAHTWYKNDLYTAIPAIATQGANTRIVLSNGNQYTKDDINSVKNIISLVSNYKMIAVL  
 EVHDATGKDDYASLDAAVNYWISIKDALIGKEDRVIVNIA NEWYGSWNGSGWADGYK  
 QAIPKLRNAGIKNTLIVDCAGWGQYPQSINDFGKSVFAADSLKNTVFSIHMYEFAGKDA  
 QTVRTNIDNVLNQGIPLIIGEFGGYHQGADVDETEIMRYGQSKGVGWLAWSWYGNSSN  
 LSYLDLVTGPNGNLTDWGKTVVNGSNGIKETSKKAGIY.

**[0204]** The nucleic acid sequence for the *PpoMan2* gene (NCBI Reference Sequence NC\_014622.1, from 746871 to 747854, complement) isolated from *Paenibacillus polymyxa* SC2 is set forth as SEQ ID NO:9 (the sequence encoding the predicted native signal peptide is shown in bold):

**GTGAACGCATTGTTAAGAAAAGCATTATTGTCCTGGACTCGCTGGTCTGCTTATC**  
 ATGATTGGTTGGGGGATTCTTCTCAAGGCAGCTGCTTCAGGATTAT  
 GTAAGCGGTACCAATCTGTATGACTCTACAGGCAAACCGTTCTGTTATGAGAGGCGT  
 CAATCATGCTCACACTGGTACAAAACGATCTTATACTGCTATCCCAGCAATTGC  
 TAAAACAGGTGCTAATACAGTCGAATTGTCCTTCTAACGGAAACCAGTACACCA  
 AGGATGACATTAATTCCGTAAAAATATTATCTCTCTCGTCTCCAACCATAAAATGA  
 TTGCTGTACTTGAAGTTCATGACGCTACAGGTAAGACGACTATGCGTCTTGGATG  
 CAGCAGTGAATTACTGGATTAGTATAAAAGATGCTCTGATCGGCAAGGAAGATCGG  
 GTTATCGTGAACATTGCGAACGAATGGTATGGCTCTGGAATGGAGGCAGGTTGGC  
 AGATGGGTATAAGCAAGCGATTCCAAGCTGAGAAACGCAGGCATAAAAATACG  
 CTCATCGTCGATTGTGCTGGATGGGACAATACCTCAGTCTATCAATGACTTGGT  
 AAATCTGTGTTGCAGCTGATTCTTGAAAAATACCGTTCTCCATTATGTATG  
 AATTGCTGGCAAAGATGTTCAAACGGTTCGAACCAATATTGATAACGTTCTGTATC  
 AAGGGCTCCCTTGATTATTGGTGAATTGGCGGTTACCATCAGGGAGCAGACGTCG  
 ACGAGACAGAAATCATGAGATACGCCAATCTAAAGCGTAGGCTGGTTAGCCTGG

TCCTGGTATGGCAATAGCTCCAACCTTAATTATCTTGATCTTGTGACAGGACCTAAC  
GGCAATCTGACCGATTGGGGTCGCACCGTGGTAGAGGGAGCCAACGGGATCAAAG  
AACATCGAAAAAGCGGGTATCTTCTAA.

**[0205]** The amino acid sequence of the hypothetical protein encoded by the *PpoMan2* gene, PpoMan2 (NCBI Accession No. YP\_003944884.1) is set forth as SEQ ID NO:10 (the predicted native signal peptide is shown in bold):

**MNALLRKALLSGLLAGLIMIGLGGFFSKAQAASGFYVSGTNLYDSTGKPFVMRGVN**  
HAHTWYKNDLYTAIPAIAKTGANTVRIVLSNGNQYTKDDINSVKNIISLVSNHKMIAVL  
EVHDATGKDDYASLDAAVNYWISIKDALIGKEDRVIVNIA NEWYGSWNGGGWADGY  
KQAIPKLRNAGIKNTLIVDCAGWGQYPQSINDFGKSVFAADSLKNTVFSIHMYEFAGKD  
VQTVRTNIDNVLYQGLPLIIGEFGGYHQGADVDETEIMRYGQSKSVGWLAWSWYGNSS  
NLNYLDLVTGPNGNLTDWGRTVVEGANGIKETSKKAGIF.

**[0206]** The nucleic acid sequence for the *PspMan4* gene (NCBI Reference Sequence GQ358926.1) isolated from *Paenibacillus* sp. A1 is set forth as SEQ ID NO:11 (the sequence encoding the predicted native signal peptide is shown in bold):

**ATGAAATACCTGCTGCCGACCGCTGCTGGTCTGCTGCCCTCGCTGCCCA**  
**GCCGGCGATGCCATGGCTACAGGTTTTATGTAAGCGGTAAACAAGTTACGATT**  
CCACTGGCAAGCCTTTGTTATGAGAGGTGTTAATCACGGACATTCCCTGGTTCAAAA  
ATGATTGAATACCGCTATCCCTGCCATGCCAAAACAGGTGCCAATACGGTACGC  
ATTGTTCTTCGAATGGTAGCCTGTACACCAAAGATGATCTGAACGCTGTTAAAAAT  
ATTATTAATGTGGTTAACCAGAATAAAATGATAGCTGTACTCGAACGTACATGACGC  
CACAGGGAAAGATGACTATAATTGGTGGATGCCGGTGAACACTACTGGATTAGTA  
TTAAGGAAGCTTGATTGGAAAAGAAGATCGGTAATTGTCAACATGCCAATGAA  
TGGTATGGAACGTGGAATGGAAGTGCCTGGATGGCTGATGGTTACAAAAAAGCCATTCC  
GAAACTCCGAAATGCAGGAATTAAAAATACGCTAATTGGATGCAGCCGGATGGG  
GACAGTTCCCTCAATCCATCGTGGATTATGGACAAAGTGTATTGCAGCCGATTAC  
AGAAAAATACCGTCTCTCATTCATATGTATGAGTATGCTGGCAAAGATGCTGCAA  
CGGTCAAAGCCAATATGGAGAATGTGCTGAACAAAGGATTGGCTCTGATCATTGGT  
GAATTGGGGGATATCACACAAACGGTGATGTGGATGAGTATGCCATCATGAGATA  
TGGTCAGGAAAAAGGGTAGGCTGGCTGGCTGGTCTGGTACGGAAACAGCTCCG  
GTTTGAACTATCTGGACATGCCACAGGTCCGAACGGAAGCTTAACGAGTTTGGC  
AACACTGTTGTTAATGATAACCTATGGTATTAAAACACTTCCAAAAAGCGGGGAT  
TTTCTAA.

**[0207]** The amino acid sequence of the protein encoded by the *PspMan4* gene, PspMan4 (NCBI Accession No. ACU30843.1) is set forth as SEQ ID NO:12 (the predicted native signal peptide is shown in bold):

**MKYLLPTAAAGLLLLAAQPAMAMATGFYVSGNLYDSTGKPFVMRGVNHGHSWFK**  
 NDLNTAIPAIAKTGANTVRIVLSNGSLYT KDDLNAVKNIINVVNQNKMIAVLEVHDATG  
 KDDYNSLDAAVNYWISIKEALIGKEDRVIVNIANEWYGTWNGSAWADGYKKAIPKLR  
 NAGIKNTLIVDAAGWGQFPQSIVDYGQSVFAADSKNTVFSIHMYEYAGKDAATVKA  
 NMENVLNKGLALIIGEFGGYHTNGDVDEYAIMRYGQEKGVGWLAWSWYGNSSGLNY  
 LDMATGPNGSLTSFGNTVVNDTYGIKNTSQKAGIF.

**[0208]** The nucleic acid sequence for the *PspMan5* gene (NCBI Reference Sequence JN603735.1, from 536 to 1519) isolated from *Paenibacillus* sp. CH-3 is set forth as SEQ ID NO:13 (the sequence encoding the predicted native signal peptide is shown in bold):

**ATGAGACAACCTTTAGCAAAAGGTATTTAGCTGCCTGGTCATGATGTTAGCG**  
**ATGTATGGATTGGGAATCTCTCTAAAGCTTCGGCTGCAACAGGTTTATG**  
 TAAGCGGTACCACTCTATGATTCTACTGGTAAACCTTTGTAATGCGCGGTGTCA  
 ATCATTGCATACCTGGTCAAAAATGATCTAAATGCAGCCATCCCTGCTATTGCCA  
 AAACAGGTGCAAATACAGTACGTATCGTTTATCTAATGGTGTTCAGTACTAGAG  
 ATGATGTAACACTCAGTAAAAATTATTCCCTGGTAACCAAAACAAAATGATTG  
 CTGTTCTGAGGTGCATGATGCTACCGGTAAAGACGATTACGCTCTTGATGCCG  
 CTGTAACACTGGATCAGCATCAAAGATGCCTGATTGGCAAGGAAGATCGAGTC  
 ATTGTTAATATTGCCAATGAATGGTACGGTACATGGAATGGCAGTGCTGGCAGA  
 TGGTTATAAGCAGGCTATTCCAAACTAAGAAATGCAGGCATAAAAACACTTTAA  
 TCGTTGATGCCGCCGGCTGGGACAATGCTCAATCGATCGTTGATTACGGCAA  
 AGTGTATTGCAGCAGATTGCTAAAAATACAATTCTCTATTCACATGTATGAA  
 TATGCAGGCGGTACAGATGCGATCGTAAAAGCAATATGGAAAATGTACTGAACAA  
 AGGACTCCTTGATCATCGGTGAATTGGCGGGCAGCATAACACGGCGATGTAG  
 ATGAACATGCAATTGCGTTATGGTCAGAAAAAGGTGTAGGTTGGCTGGCATGG  
 TCGTGGTATGGCAACAATAGTGAACTCAGTTATCTGGATTTGGCTACAGGTCCC  
 GGTAGTCTGACAAGTATCGGAATACGATTGAAATGATCCATATGGTATCAAAGC  
 TACCTCGAAAAAAGCGGGTATCTTCTAA.

**[0209]** The amino acid sequence of the protein encoded by the *PspMan5* gene, PspMan5 (NCBI Accession No. AEX60762.1) is set forth as SEQ ID NO:14 (the predicted native signal peptide is shown in bold):

**MRQLLAKGILAALVMMIAMYGLGNLSSKASAATGFYVSGTLYDSTGKPFVMRGV**  
 NHSHTWFKNLNAIPAIAKTGANTVRIVLSNGVQYTRDDVNSVKNIISL VNQNKMIAV  
 LEVHDATGKDDYASLDAAVNYWISIKDALIGKEDRVIVNIANEWYGTWNGSAWADGY  
 KQAIKLRNAGIKNTLIVDAAGWGQCPQSIVDYGQSVFAADSLKNTIFSIHMYEYAGGT  
 DAIVKSNMENVLNKGLPLIIGEFGGQHTNGDVDEHAIMRYGQQKGVGWLAWSWYGN  
 NSELSYLDLATGPAGSLTSIGNTIVNDPYGIKATSKKAGIF.

**[0210]** In addition, mannanases were identified by sequencing the genomes of *Paenibacillus amylolyticus* DSM11730, DSM15211, and DSM11747, *Paenibacillus pabuli* DSM3036, *Paenibacillus* sp. FeL05 (renamed as *Paenibacillus hunanensis* DSM22170), and *Paenibacillus tundrae* (Culture Collection DuPont). The entire genomes of these organisms were sequenced by BaseClear (Leiden, The Netherlands) using the Illumina's next generation sequencing technology and subsequently assembled by BaseClear. Contigs were annotated by BioXpr (Namur, Belgium).

**[0211]** The nucleotide sequence of the *PamMan2* gene isolated from *Paenibacillus amylolyticus* is set forth as SEQ ID NO:15 (the identical sequence was found in DSM11730, DSM15211, and DSM11747; the sequence encoding the predicted native signal peptide is shown in bold):

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ATGGTTAATCTGAAAAAGTGTACAATCTCACGGTTATTGCTACACTCATGTTCA
ATGGTATTAGGGAGTGCAGCACCCAAAGCATCTGCTGCTACAGGATTTATGTA
AGCGGTAACAAGTTATACGATTCCACAGGCAAGGCTTGTATGAGAGGTGTTAA
TCACGGACATTCCCTGGTCAAAATGATTGAATAACCGCTATCCCTGCAATGCCAA
AACAGGTGCCAATACGGTACGCATTGTTCTCGAATGGTAGCCTGTACACCAAAG
ATGATCTGAACGCTGTTAAAATATTATTAAATGTGGTTACCAAAATAAAATGATA
GCTGTACTCGAGGTGCATGACGCCACAGGGAAAGATGACTATAATTGTTGGATGC
GGCAGTGAACTACTGGATTAGCATTAGGAAGCTTGTGGCAAAGAAGATCGGG
TCATCGTCAATATGCCAATGAATGGTATGGAACGTGGAATGGAAGTGCCTGGCT
GATGGTTACAAAAAAGCCATTCCGAAACTCCGAAATGCCGGATTAAAAATACGCT
AATTGTGGATGCAGCCGGATGGGGACAGTCCCTCAATCCATCGTGGATTATGGAC
AAAGTGTATTGCAACCGATTCTCAGAAAAATACGGTCTTCTCATTCAATGTATG
AGTATGCTGGCAAAGATGCTGCAACCGTCAAAGCCAATATGGAAAATGTGCTGAAC
AAAGGATTGGCTCTGATCATGGTAGTTGGGGATACCACACAAACGGTATGTT
GGACGAGTATGCCATCATGAGATATGGTCAGGAAAAGGGTGGCTGGCTGGCCT
GGCCTGGTATGAAACAGTCTGGTCTGAACCTGGACATGGCTACAGGTCCG
AACGGAAAGTTGACGAGCTCGGAAACACCGTAGTGAATGATAACCTATGGAATTAA
AAAAACTCTCAAAAGCGGGGATTTC.

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**[0212]** The amino acid sequence of the *PamMan2* precursor protein is set forth as SEQ ID NO:16 (the predicted native signal peptide is shown in bold):

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MVNLKKCTIFTVIATLMFMVLGSAAPKASAATGFYVSGNKLYDSTGKAFVMRGVN
GHSWFKNLNTAIPAIAKTGANTVRIVLSNGSLYTKDDLNAVKNIINVVNQNKMIAV
VHDATGKDDYNSLDAAVNYWISIKEALIGKEDRVIVNIANEWYGTWNGSAWADGYKK
AIPKLRNAGIKNTLIVDAAGWGQFPQSIVDYGQSVFATDSQKNTVFSIHMYEYAGKDA
ATVKANMENVLNKGLALIIGFGGYHTNGDVDEYAIMRYGQEKGVGWLAWSWYGN
SGLNYLDMATGPNGSLTSFGNTVVNDTYGIKKSQKAGIF.

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**[0213]** The sequence of the fully processed mature PamMan2 protein (297 amino acids) is set forth as SEQ ID NO:17:

ATGFYVSGNKL<sup>Y</sup>DSTGKAFVMRGVNHGHSWFKNDLNTAIPAIAKTGANTVRIVLSNGS  
 LYTKDDLNAVKNIINVVNQNKMIAVLEVHDATGKDDYNSLDAAVNYWISIKEALIGKE  
 DRVIVNIANEWYGTWNGSAWADGYKKAIPKLRNAGIKNTLIVDAAGWGQFPQSIVDY  
 GQSVFATDSQKNTVFSIHMYEYAGKDAATVKANMENVLNKG<sup>L</sup>ALIIGEFGGYHTNGD  
 VDEYAIMRYGQEKGVGWLAWSWYGNSSGLNYLDMATGPNGSLTSFGNTVVNDTYGI  
 KKTSQKAGIF.

**[0214]** The nucleotide sequence of the *PpaMan2* gene isolated from *Paenibacillus pabuli* DSM3036 is set forth as SEQ ID NO:18 (the sequence encoding the predicted native signal peptide is shown in bold):

**ATGGTCAAGTTGCAAAAGGGTACCGATCATCACCGTCATTGCTGCGCTCATT**  
**GGTTATGTTGGAAAGTGCTGCACCCAAAGCTTCTGCTGCTGGTTTTATGTA**  
 AGCGGTAAACAAGTTGACTCTACGGTAAAGCTTTGTCATGCGGGCGTCAA  
 CCACAGTCATACCTGGTTCAAGAACGATCTAACACACAGCGATAACCGCCATTGCAA  
 AACACAGGTGCGAACACGGTACGTATTGTGCTCTCCAATGGGACGCAATATACAAA  
 GATGATTGAACGCCGTTAAAACATAATCAACCTGGTAGTCAGAACAAAATGAT  
 CGCAGTGCTCGAAGTACATGATGCAACTGGTAAAGATGACTACAATTGTTGGATG  
 CAGCAGTCAACTACTGGATTAGCATCAAGGAAGCTCTGATTGCAAGGAAGACCGC  
 GTTATCGTCAATATTGCCAATGAATGGTACGGGACCTGGAACGGCAGTGCCTGGC  
 TGACGGGTACAAAAAGCAATTCCGAAACTGAGAAATGCCGGATTAAAATACAT  
 TAATTGTAGATGCAGCTGGCTGGGCCAATATCGCAATCTATTGACTATGGTC  
 AAAGTGTGTTGCGAGATGCCGAGAAAAATACGGTTCTCCATTACATGTATG  
 AATATGCAGGTAAAGATGCCAACCGTCAAAGCCAACATGGAAAACGTGCTGAA  
 CAAAGGTTGGCCCTGATCATCGGTGAGTTGGTGGATACCACACCAATGGGACG  
 TCGATGAATATGCAATCATGAAATACGGTCAGGAAAAAGGAGTAGGCTGGCTCGCA  
 TGGCCTGGTATGGGAAACAACCTCGATCTCAATTCTGGATTGGCTACAGGTCCA  
 AACGGAACTTTAACAGCTTGGCAACACGGTGGTTATGACACGTATGGAATTAA  
 AACACACTCGGTAAAAGCAGGGATCTAT.

**[0215]** The amino acid sequence of the *PpaMan2* precursor protein is set forth as SEQ ID NO:19 (the predicted native signal peptide is shown in *italics* and **bold**):

**MVKLQKG<sup>I</sup>ITVIAALILVMLGSAAPKASAAAGFYVSGNKL<sup>Y</sup>DSTGKAFVMRGVNHSHT**  
 WFKNDLNTAIPAIAKTGANTVRIVLSNGTQYT<sup>K</sup>DDLNAVKNIINLVSQNKMIAVLEVHD  
 ATGKDDYNSLDAAVNYWISIKEALIGKEDRVIVNIANEWYGTWNGSAWADGYKKAIP  
 KLRNAGIKNTLIVDAAGWGQYPQSIVDYGQSVFAADAQKNTVFSIHMYEYAGKDAAT  
 VKANMENVLNKG<sup>L</sup>ALIIGEFGGYHTNGDVDEYAIMKYGQEKGVGWLAWSWYGNNSD

LNYLDLATGPNGTLTSFGNTVYDTYGIKNTSVKAGIY.

**[0216]** The nucleotide sequence of the *PspMan9* gene isolated from *Paenibacillus* sp. *FeL05* is set forth as SEQ ID NO:20 (the sequence encoding the predicted native signal peptide is shown in bold):

**GTGTTATGTTAGCGATGTATGGATGGCTGGACTGACTGGTCAAGCTTCAGC**  
**TGCTACAGGTTTATGTAAGCGGTACCAAATTATACGACTCTACAGGCAAGCCATT**  
**TGTGATGCGTGGTGTGAATCATTCCCACACCTGGTCAAAATGACCTGAATGCAGC**  
**GATCCCTGCAATTGCCAAAACAGGCGCCAACACGGTACGTATCGTATTATCGAATG**  
**GCCTGCAGTACACCAGAGATGATGTAACACTCCGTCAAAATATCATCTCTCGTCA**  
**ACCAGAACAAAATGATCGCAGTACTGGAGGTTATGATGCAACAGGCAAGGACGA**  
**TTACGCTCGCTCGATGCCAATCAACTACTGGATCAGCATCAAGGATGCGCTGAT**  
**CGGTAAAGAGGATCGCGTTATCGTCAATATTGCCAACGAATGGTATGGCACATGGA**  
**ATGGAAGCGCATGGCAGATGGCTACAAACAGGCGATTCCAAAGCTCCGTAATGCG**  
**GGTATAAAAATACGCTGATTGGACAAAGTGTATTGCAGCGGATTCTGTTAAAAAATACGGTTT**  
**CTCGATCCATATGTATGAGTATGCAGGTGGAACCGATGCGATGGTCAAAGCCAACA**  
**TGGAGGGCGTACTCAATAAAGGTCTGCCACTGATCATGGTGAATTGGCGGACAG**  
**CACACAAATGGAGACGTGGATGAGCTGGCATGCGTTACGGACAACAAAAAG**  
**GAGTAGGCTGGCTCGCCTGGTACGGCAACAATAGTGTAGTTATCTCG**  
**ATCTAGCGACAGGTCAAATGGTAGCCTGACCACGTTGGTAATACGGTGGTAAAT**  
**GACACCAACGGTATCAAAGCCACCTCCAAAAAAGCAGGTATTTCCAG.**

**[0217]** The amino acid sequence of the *PspMan9* precursor protein is set forth as SEQ ID NO:21 (the predicted native signal peptide is shown in *italics* and **bold**):

***MFMLAMYGWAGLTGQ*ASAATGFYVSGTKLYDSTGKPFVMRGVNHSHTWFKNLNA**  
**IPAIAKTGANTVIRIVLSNGVQYTRDDVNSVKNIISLVNQNKMIAVLEVHDATGKDDYAS**  
**LDAAINYWISIKDALIGKEDRVIVNIA NEWYGTWNGSAWADGYKQAIPLRNAGIKNT**  
**LIVDAAGWGQYPQSIVDYGQSVFAADSLKNTVFSIHMYEYAGGTDAMVKANMEGVLN**  
**KGLPLIIGEFGGQHTNGDVDELAIMRYGQQKGVGWLAWSWYGNNSDLSYLDLATGPN**  
**GSLTTFGNTVVNDTNGIKATSKKAGIFQ.**

**[0218]** The nucleotide sequence of the *PtuMan2* gene isolated from *Paenibacillus tundrae* is set forth as SEQ ID NO:22 (the sequence encoding the predicted native signal peptide is shown in bold):

**ATGGTCAAGTTGCAAAAGTGTACAGTCTTACCGTAATTGCTGCACTTATGTTG**  
**GTGATTCTGGCGAGTGCTGCACCCAAAGCGTCTGCTGCTACAGGATTGTTATGTA**  
**AGCGGAGGCAAATTGTACGATTCTACTGGCAAGGCATTGTTATGAGAGGTGTCAA**

TCATGGACATTATGGTTAAGAACGACTTGAACACGGCTATTCTCGATAGCCAA  
 AACAGGTGCCAACACCGTACGGATTGTGCTCTCAATGGCGTACAGTACACCAAAG  
 ACGATCTGAACCTGTTAAAAACATCATTAATGTTAAGCGTAAACAAAATGATT  
 GCGGTGCTCGAAGTACATGATGCAACAGGTAAAGGATGACTATAATTGTTGGATGC  
 AGCGGTGAACTACTGGATTAGCATCAAGGAAGCACTCATTGGCAAAGAACAGA  
 GTTATCGTAAATATCGCGAACGAATGGTATGGAACATGGAACGGCAGTGCCTGGC  
 TGACGGATACAAAAAAGCAATTCCGAAGCTGAGAAATGCCGGTATTAAAAATACAT  
 TGATCGTGGATGCAGCGGCTGGGGCAGTACCCGCAATCCATCGTGGATTATGGA  
 CAAAGTGTATTGCAGCGGATTCACAGAAAAACACCGTATTCTGATTACATGTAT  
 GAATATGCCGGTAAAGACGCAGCAACCGTAAAGCCAACATGGAAGCGTATTAA  
 ACAAAAGGTCTGCCCTGATCATCGGTGAATTGGTGGATATCACACGAACGGGGAT  
 GTCGATGAATATGCGATCATGAAATATGGTCAGGAAAAAGGGTAGGCTGGCTCGC  
 ATGGTCCTGGTATGGCAATAGCTCGATTGAACTATTGGACTTGGCTACGGGACC  
 TAACGGAAGTTGACTAGCTTGGAAACACAGTCGTCAACGACACTTATGGAATCA  
 AAAATACTCAAAAAAAGCAGGGATCTAC.

**[0219]** The amino acid sequence of the PtuMan2 precursor protein is set forth as SEQ ID NO:23 (the predicted native signal peptide is shown in bold):

**MVKLQKCTVFTVIAALMLVILASAAPKASAATGFYVSGGKLYDSTGKAFVMRGVNH**  
 GHSWFKNDLNTAIPAIAKTGANTVRIVLSNGVQYTKDDLNSVKNIINVSVNKMIAVLE  
 VHDATGKDDYNSLDAAVNYWISIKEALIGKEDRVIVNIA NEWYGTWNGSAWADGYKK  
 AIPKLRNAGIKNTLIVDAAGWGQYPQSIVDYQGSVFAADSKNTVFSIHMYEYAGKDA  
 ATVKANMESVNLKGLALIIGEFGGYHTNGDVDEYAIMKYGQEKGWLAWSWYGN  
 SDLNYLDDLATGPNGSLTSFGNTVVNDTYGIKNTSKKAGIY.

**[0220]** The sequence of the fully processed mature PtuMan2 (303 amino acids) is set forth as SEQ ID NO:24:

ATGFYVSGGKLYDSTGKAFVMRGVNHHSWFKNDLNTAIPAIAKTGANTVRIVLSNGV  
 QYTKDDLNSVKNIINVSVNKMIAVLEVHDATGKDDYNSLDAAVNYWISIKEALIGKE  
 DRVIVNIA NEWYGTWNGSAWADGYKKAIIPKLRNAGIKNTLIVDAAGWGQYPQSIVDY  
 QGSVFAADSKNTVFSIHMYEYAGKDAATVKANMESVNLKGLALIIGEFGGYHTNGD  
 VDEYAIMKYGQEKGWLAWSWYGNSSDLNYLDDLATGPNGSLTSFGNTVVNDTYGI  
 KNTSKKAGIY.

## EXAMPLE 2

### Heterologous Expression of mannanases

**[0221]** The DNA sequences of the mature forms of *BciMan1*, *BciMan3*, *BciMan4*, *PpaMan2*, *PpoMan1*, *PpoMan2*, *PspMan4*, *PspMan5*, and *PspMan9* genes were synthesized and inserted into the *B. subtilis* expression vector p2JM103BBI (Vogtentanz, Protein Expr Purif, 55:40-52, 2007) by Generay Biotech (Shanghai, China), resulting in expression plasmids containing an aprE promoter, an aprE signal sequence used to direct target protein secretion in *B. subtilis*, an oligonucleotide AGK-proAprE that encodes peptide Ala-Gly-Lys to facilitate the secretion of the target protein, and the synthetic nucleotide sequence encoding the mature region of the gene of interest. A representative plasmid map for PspMan4 expression plasmid (p2JM-PspMan4) is depicted in Figure 1.

**[0222]** A suitable *B. subtilis* host strain was transformed with each of the expression plasmids and the transformed cells were spread on Luria Agar plates supplemented with 5ppm chloramphenicol. To produce each of the mannanases listed above, *B. subtilis* transformants containing the plasmids were grown in a 250ml shake flask in a MOPS based defined medium, supplemented with additional 5mM CaCl<sub>2</sub>.

**[0223]** The nucleotide sequence of the synthesized *BciMan1* gene in the expression plasmid p2JM-BciMan1 is set forth as SEQ ID NO:25 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in bold):

GTGAGAAGCAAAAATTGTGGATCAGCTTGTGCGTTAACGTTAACCTTTACG  
 ATGGCGTTCAGCAACATGAGCGCGCAGGCT**GCTGGAAAAGCAAGCGG**CTTTATGT  
 TTCAGGCACAAA**ACTGCTGGATGCAACAGGCCAACCGTT**TATGAGAGGCGTTA  
 ATCATGCACATA**CGTGGTATAAGATCAACTGTCAACAGCAATTCCGG**CAATCGCA  
 AAAACAGGCGCAA**ATACAATTAGAATTGTTCTGGCAATGGCC**ATAATGGACACT  
 GGATGATGTTAACACAGTCAACAATTCTGACACTGTGCGAACAGAATAACTGA  
 TTGCAGTTCTGGAAGTTCATGATGCGACAGGCTCAGATTCACTGTCAGATCTGGATA  
 ATGCAGTCAATTATTGGATCGGCATTAAATCAGCACTGATCGGAAAGAAGATCGC  
 GTCATTATTAACATTGCGAACGAATGGTATGGCACATGGATGGCGTTGCATGGGC  
 AAATGGCTATAAACAAAGCGATTCCGAA**ACTGAGAAATGCA**GGCCCTGACACATACAC  
 TGATTGTTGATT**CAGCAGGCTGGGACAATATCCGATT**CAGTAAAAACTATGGC  
 ACAGAAGTCTGAACGCAGATCCGCTGAAAA**ATACAGTCTT**AGCATCCACATGTA  
 CGAATATGCAGGCGGAA**ATGCATCAACAGT**GAAATCAAATATTGATGGCGTCTGA  
 ATAAAAAC**CTGGCACTGATTATTGGCAATTGGCGGACAACATACAA**ATGGCGAC  
 GTTGATGAAGCAACGATTATGTCATATAGCCAAGAAAAAGGCGTGGCTGGCTTGC  
 ATGGTCATGGAAAGGCAATT**CATCAGATCTGCATATCTGGATATGACGAATGATT**  
 GGGCAGGCAATAGCCTGACATCATTGGCAATACAGTGTCAATGGCAGCAATGGC  
 ATTAAAGCAACATCAGTTCTGTCAGGCATTGGCGGAGTTACACCGACATCATCA  
 CCGACAAGCACACCGACGTCAACACCTACATCAACGCCGACACCGACACCTAGCCC  
 GACACCTTCACCGGGAAATAATGGCACAATTCTGTATGATTGAAACAGGCACAC

AAGGCTGGTCAGGCAATAACATTCAAGCGGACCGTGGTTACAAATGAATGGAAA  
 GCGACAGGCGCACAAACACTGAAAGCAGATGTTCACTCAAAGCAATTCAACGCA  
 TAGCCTGTATATCACAAGCAATCAAATCTGAGCGGCAAATCAAGCCTGAAAGCAA  
 CAGTTAACATGCGAATTGGGGCAATATTGGAATGGAATTATGCGAAACTGTAC  
 GTTAAAACAGGCAGCGGCTGGACATGGTATGATTCAAGCGAAAATCTGATTCAAGTC  
 AAACGATGGAACAATCCTGACACTTCACCAAGCTCAAATAGCTCAGGCCAAAGCGCA  
 TTAAAGAAATTGGCGTCGAATTAGAGCAAGCTCAAATAGCTCAGGCCAAAGCGCA  
 ATTTATGTTGATAGCGTTCACTGCAG.

**[0224]** The amino acid sequence of the BciMan1 precursor protein expressed from the p2JM-BciMan1 plasmid is set forth as SEQ ID NO:26 (the predicted signal sequence is shown in *italics*, the three residue amino-terminal extension (AGK) is shown in **bold**):

*MRSKKLWISLLFALT***LIFTMAFSNMSA**QAGKASGFYVSGTKLLDATGQPFVMRGVNHA  
 HTWYKDQLSTAIPAIAKTGANTIRIVLANGHKWTLDDVNTVNNILTLCEQNKLIAVLEV  
 HDATGSDSLSLDNAVNYWIGIKSALIGKEDRVIINIANEWYGTWDGVAWANGYKQAI  
 PKLRNAGLTHTLIVDSAGWGQYPDSVKNYGTEVLNADPLKNTVFSIHMYEYAGGNAST  
 VKSNIDGVLNKNLALIIGEFGGQHTNGDVDEATIMSYSQEKGVGWLAWSWKGNSSDL  
 AYLDMTNDWAGNSLTSFGNTVVNGSNGIKATVLSGIFGGVPTSSPTSTPTSTPT  
 PTPSPTPSGNNGTILYDFETGTQGWSGNNISGGPVWTNEWKATGAQTLKADVSLQSNS  
 THSLYITSQNLSGKSSLKATVKHANWGNIGNGIYAKLYVKTGSGWTWYDSGENLIQS  
 NDGTILTSLSGISNLSSVKEIGVEFRASSNSSGQSAIYVDSVSLQ.

**[0225]** The amino acid sequence of the BciMan1 mature protein expressed from p2JM-BciMan1 plasmid is set forth as SEQ ID NO:27 (the three residue amino-terminal extension (AGK) based on the predicted cleavage site shown in **bold**):

**AGK**ASGFYVSGTKLLDATGQPFVMRGVNHAHTWYKDQLSTAIPAIAKTGANTIRIVLA  
 NGHKWTLDDVNTVNNILTLCEQNKLIAVLEVHDATGSDSLSLDNAVNYWIGIKSALI  
 GKEDRVIINIANEWYGTWDGVAWANGYKQAIPLRNAGLTHTLIVDSAGWGQYPDSV  
 KNYGTEVLNADPLKNTVFSIHMYEYAGGNASTVKSNIIDGVLNKNLALIIGEFGGQHTN  
 GDVDEATIMSYSQEKGVGWLAWSWKGNSSDLAYLDMTNDWAGNSLTSFGNTVVNGS  
 NGIKATVLSGIFGGVPTSSPTSTPTSTPTPSPTPSGNNGTILYDFETGTQGWS  
 GNNISGGPVWTNEWKATGAQTLKADVSLQSNSTHSLYITSQNLSGKSSLKATVKHAN  
 WGNIGNGIYAKLYVKTGSGWTWYDSGENLIQSNDGTILTSLSGISNLSSVKEIGVEFRA  
 SSNSSGQSAIYVDSVSLQ.

**[0226]** The amino acid sequence of the BciMan1 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:28:

ASGFYVSGTKLLDATGQPFVMRGVNHAHTWYKDQLSTAIPAIAKTGANTIRIVLANGH  
 KWTLDDVNTVNNILTLCEQNKLIAVLEVHDATGSDSLSLDNAVNYWIGIKSALIGKED

RVIINIANEWYGTWDGVAWANGYKQAIPKLRNAGLHTLIVDSAGWGQYPDSVKNYG  
 TEVLNADPLKNTVFSIHMYEYAGGNASTVKSNIQVNLNLALIIGEFGGQHTNGDVDE  
 ATIMSYSQEKGVGWLAWSWKGNSSDLAYLDMTNDWAGNSLTSFGNTVVNGSNGIKA  
 TSVLSGIFGGVTPTSSPTSTPTSTPTPSPTPSPGNNGTILYDFETGTQGWSGNISG  
 GPWVTNEWKATGAQTLKADVSLQSNSTHSLYITSQNLSGKSSLKATVKHANWGNIG  
 NGIYAKLYVKTGSGWTWYDSGENLIQSNDGTILTLSGISNLSSVKEIGVEFRASSNSS  
 GQSAIYVDSVSLQ.

**[0227]** The nucleotide sequence of the synthesized *BciMan3* gene in the p2JM-*BciMan3* plasmid is set forth as SEQ ID NO:29 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in bold):

GTGAGAAGCAAAAAATTGTGGATCAGCTTGTGTTGCGTTAACGTTAACCTTACG  
 ATGGCGTTTCAGCAACATGAGCGCGCAGGCT**GCTGGAAAAGCAACAGGCTTTATGT**  
 CAATGGCACGAAACTGTATGATAGCACAGGCAAAGCATTGTTATGAGAGGCGTTA  
 ATCATCCGCATACTGGTATAAAACGATCTGAATGCAGCAATTCCGGCTATTGCA  
 CAAACAGGCGCAAATACAGTTAGAGTTGTTCTGTCAAATGGCAGCCAATGGACAAA  
 AGATGATCTGAATAGCGTCAACAGCATTATTCACTGGTTAGCCAACATCAAATGAT  
 TGCAGTTCTGGAAGTTCATGATGCAACGGCAAAGATGAATATGCATCACTGGAAG  
 CAGCAGTCGATTATTGGATTCAATTAAAGGCGCACTGATCGGCAAAGAAGATAGA  
 GTCATTGTCATATTGCGAACGAATGGTATGCAATTGGAATTCATCAGGCTGGC  
 AGATGGCTATAAACAAAGCGATTCCGAAACTGAGAAATGCAGGCATTAAAACACA  
 CTGATTGTTGATGCAGCAGGCTGGGACAATATCCGAATCAATTGTCGATGAAGG  
 CGCAGCAGTTTGATCAGATCAACTGAAAAACACGGCTTACGATCCACATGTA  
 TGAATACGCTGGAAAAGATGCAGCAACAGTCAAAACAAATATGGATGACGTTCTGA  
 ATAAAGGCCCTGCCGCTGATTATTGGCGAATTGGCGGATATCATCAAGGCGCAGAT  
 GTTGATGAAATTGCGATTATGAAATACGCCAGCAAAAGAGGTTGGCTGGCTTGC  
 ATGGTCATGGTATGGAAACTCACCGGAACCTGAATGATCTGGATCTGGCAGCAGGAC  
 CGTCAGGCAATCTGACAGGATGGGCAATACAGTTGTCATGGCACAGATGGCATT  
 CAACAGACATCAAAAAAGCAGGCATCTAT.

**[0228]** The amino acid sequence of the *BciMan3* precursor protein expressed from the p2JM-*BciMan3* plasmid is set forth as SEQ ID NO:30 (the predicted signal sequence is shown in *italics*, the three residue amino-terminal extension (AGK) is shown in **bold**):

*MRSKKLWISLLFALTLIFTMAFSNMSAQ***AAGKATGFYVNGTKLYDSTGKAFVMRGVNHP**  
 HTWYKNDLNAJPAIAQTGANTVRVVLNSNGSQWTKDDLNSVNSIISLVSQHQMIAVLE  
 VHDATGKDEYASLEAADYWISIKGALIGKEDRVIVNIANEWYGNWNSSGWADGYKQ  
 AIPKLRNAGIKNTLIVDAAGWGQYPQSIVDEGAAVFASDQLKNTVFSIHMYEYAGKDA  
 ATVKTNMDDVLNKGPLIIGEFGGYHQGADVDEIAIMKYGQQKEVGWLAWSWYGNSP  
 ELNLDLAAAGPSGNLTGWGNTVVHGTDGIQQTSKKAGIY.

**[0229]** The amino acid sequence of the BciMan3 mature protein expressed from p2JM-BciMan3 is set forth as SEQ ID NO:31 (the three residue amino-terminal extension based on the predicted cleavage site shown in bold):

**AGK**ATGFYVNGTKLYDSTGKAFVMRGVNHPTHWYKNDLNAAIPAI**A**QTGANTVRVV  
 LSNGSQWTKDDLNSVNSIISLVSQHQ**MIAV**LEVHDATGK**DEY**ASLEAAVDYWISIKGAL  
 IGKEDRVIVNIA**NEWY**GNWNSSGWADGYK**Q**AIPKLRNAGIKNTLIVDAAGWGQYPQSI  
 VDEGAAVFASDQLKNTVFSI**HY**AGKDAATVKTNMDVLNKGLPLI**I**GEFGGYHQ  
 GADVDEIA**IMKY**GQQ**K**EVGWLAWSWYGN**SPEL**NDL**D**LAAGPSGNLTGWGNTVVHGT  
 DGIQQTSKKAGIY.

**[0230]** The amino acid sequence of the BciMan3 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:32:

ATGFYVNGTKLYDSTGKAFVMRGVNHPTHWYKNDLNAAIPAI**A**QTGANTVRVVL**SNG**  
 SQWTKDDLNSVNSIISLVSQHQ**MIAV**LEVHDATGK**DEY**ASLEAAVDYWISIKGAL**IGKE**  
 DRVIVNIA**NEWY**GNWNSSGWADGYK**Q**AIPKLRNAGIKNTLIVDAAGWGQYPQ**SIVDE**  
 GAAVFASDQLKNTVFSI**HY**AGKDAATVKTNMDVLNKGLPLI**I**GEFGGYHQ**GAD**  
 VDEIA**IMKY**GQQ**K**EVGWLAWSWYGN**SPEL**NDL**D**LAAGPSGNLTGWGNTVVHGT**DGI**  
 QQTTSKKAGIY.

**[0231]** The nucleotide sequence of the synthesized *BciMan4* gene in the expression plasmid p2JM-BciMan4 is set forth as SEQ ID NO:33 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in bold):

GTGAGAACAAAAATTGTGGATCAGCTTGTGTTGCGTTAACGTTAAC**GT**TTACG  
 ATGGCGTTAGCAACATGAGCGCGCAGG**CTG**TTGAAAGCAACAGGCTTTATGT  
 TAATGGCGAAA**ACT**GTATGATAGCACAGGCCAACCGTTTATATGCGTGGCATT  
 ATCATGGCCATAGCTGGTTAAA**ACG**ATCTGAATACAGCGATTCCGGCTATTGCA  
 AAAACAGGC**G**CAAATACAGTTAGAATTGTTCTGTCAAATGGCACGCAGTATCGAA  
 AGATGATCTGA**ACT**CACTAGTCAAAA**ACAT**CATCAATGTCGTCAACCG**GA**ACAAA**ATG**A  
 TTGCAGTCTGGAA**AGT**TCATGCAACGG**CA**AGATGATTCAATTCACTGGATG  
 CAGCAGT**CA**ACTATTGGATCTCAATTAAAGAAGCG**CTG**ATGG**CA**AGAAGATCGC  
 GTTATTGTTAATATTGCGAACGA**ATGGT**ATGGC**AC**ATGG**AA**ATGG**CTC**AGC**ATGG**  
 AGATGG**CT**ACAAAAAG**CA**ATT**CCG**AA**ACT**GGAGAGAT**GC**AGGC**AT**AAA**ACAC**  
 CTGATTGTTGAT**GC**GGC**AGG****CT**GGG**AC**AT**ATCCG**CA**AT**CA**ATTG**TTGATT**ATGG**  
 CAAAG**CG**TT**TC**GC**AG****CA****AG****AT**GC**CC****AG****AA****AA****AT**AC**AGC****GT****TT****AG****C****AT**CC**AC****AT**GT**TA**  
 TGAATAT**GC**GG**AA****AG****AT**GC**AG****CA****AC****AG****CT**AAA**AG****CA****AT**AT**GG****AA****AC****GT****CT****GT**  
 AATAAAGGC**CT**GG**CA****CT**GT**ATT**ATT**GG****CG****GA****AT**TT**GG****CG****GA****AT**TC**AT**AC**AA****AT****GG****CG****GA**

CGTTGACGAATATGCGATTATGAAATATGGCCTGGAAAAAGGCCTGGCTGGCTTG  
 CATGGTCATGGTATGGAAATTACATCAGGCCTTAATTATCTGGATCTGGCAACAGGAC  
 CGAATGGCAGCCTGACATCATATGGAATACAGTTGTCATGATACTGATGGCATC  
 AAAAATACGTCACAGAAAGCAGGCATCTT.

**[0232]** The amino acid sequence of the BciMan4 precursor protein expressed from plasmid p2JM-BciMan4 is set forth as SEQ ID NO:34 (the predicted signal sequence is shown in italics, the three residue amino-terminal extension (AGK) is shown in bold):

*MRSKKI*WISIILFAITLIFTMAFSNMSAQ**AAGK**ATGFYVNGGKLYDSTGKPFYMRGINHGH  
 SWFKNDLNTAIPAIAKTGANTVRIVLNSGTQYTKDDLNSVKNIINVVNANKMIAVLEVH  
 DATGKDDFNSLDAAVNYWISIKEALIGKEDRVIVNIA NEWYGTWNGSAWADGYKKAIP  
 KLRDAGIKNTLIVDAAGWGQYPQSIVDYQGSVFAADSQKNTAFSIHMYEYAGKDAAT  
 VKSNMENVLNKGLALIIGEFGGYHTNGDVDEYAIMKYGLEKGVGWLAWSWYGNSSG  
 LNYLDDLATGPNGSLTSYGNVVNDTYGIKNTSQKAGIF.

**[0233]** The amino acid sequence of the BciMan4 mature protein expressed from p2JM-BciMan4 is set forth as SEQ ID NO:35 (the three residue amino-terminal extension based on the predicted cleavage site shown in bold):

**AGK**ATGFYVNGGKLYDSTGKPFYMRGINHGH SWFKNDLNTAIPAIAKTGANTVRIVL  
 SGTQYTKDDLNSVKNIINVVNANKMIAVLEVHDATGKDDFN SLDAAVNYWISIKEALI  
 GKEDRVIVNIA NEWYGTWNGSAWADGYKKAIPKLRDAGIKNTLIVDAAGWGQYPQSI  
 VDYGGSVFAADSQKNTAFSIHMYEYAGKDAATVKS NMENVLNKGLALIIGEFGGYHT  
 NGDVDEYAIMKYGLEKGVGWLAWSWYGNSSGLNYLDDLATGPNGSLTSYGNVVNDT  
 YGIKNTSQKAGIF.

**[0234]** The amino acid sequence of the BciMan4 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:36:

ATGFYVNGGKLYDSTGKPFYMRGINHGH SWFKNDLNTAIPAIAKTGANTVRIVLNSGT  
 QYTKDDLNSVKNIINVVNANKMIAVLEVHDATGKDDFN SLDAAVNYWISIKEALIGKE  
 DRVIVNIA NEWYGTWNGSAWADGYKKAIPKLRDAGIKNTLIVDAAGWGQYPQSIVDY  
 QGSVFAADSQKNTAFSIHMYEYAGKDAATVKS NMENVLNKGLALIIGEFGGYHTNGD  
 VDEYAIMKYGLEKGVGWLAWSWYGNSSGLNYLDDLATGPNGSLTSYGNVVNDTYGI  
 KNTSQKAGIF.

**[0235]** The nucleotide sequence of the synthesized *PpaMan2* gene in plasmid p2JM-PpaMan2 is set forth as SEQ ID NO:37 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in bold):

GTGAGAAGCAAAAATTGTGGATCAGCTTGTGTTGCGTTAACGTTAACCTTTACG  
 ATCGCGCTTCAGCAAGATCAACCGCGACGCTGCTGCAAGACGCGACGCTTTATGT

ATGGGCTTCAGCAACATGAGGCGAGGCTGGTGGAAAAAGCAUCAGGCTTTATGTC  
 TTCAGGCAACAAAGCTGTATGATTCAACAGGAAAAGCATTGTTATGAGAGGGCGTTA  
 ATCATTCACATACATGGTTAAGAACGATCTTAATACAGCCATTCCGGCAATCGCGA  
 AGACAGGAGCAAATACAGTGAGAATTGTTCTTCAAACCGAACGCAATATAACAAA  
 GATGACCTGAACGCCGTTAAGAATATCATTAATCTGGTTCACAAAATAAGATGATT  
 GCAGTTCTGGAGGTTCATGATGCAACAGGCAAGGATGACTACAATAGCCTGGATGC  
 AGCGGTCAATTACTGGATTCAATTAAAGAACGACTTATTGGCAAAGAGGATAGAG  
 TTATTGTTAATATCGCAAATGAATGGTATGGAACGTGGAACGGCTCAGCATGGCA  
 GATGGCTACAAAAAAGCAATTCCGAAACTGAGAAATGCAGGAATCAAAATACAC  
 TGATTGTTGACGCCGAGGCTGGGACAATATCGCAAAGCATCGTTGATTATGGC  
 CAAAGCGTTTGCAGACGCACAGAAAAACACGGTTCTCAATTATGTAC  
 GAGTATGCTGGAAAGGATGCTGCAACGGTTAAAGCTAACATGGAAAATGTTCTGAA  
 TAAAGGCCTGGCACTGATCATTGGCAATTGGAGGCTATCACACAAATGGCATG  
 TTGATGAATACGCAATTATGAAATATGGACAAGAAAAAGGCCTGGATGGCTTGCA  
 TGGTCATGGTACGAAACAACCTGACACCTTAATTACCTGGACCTGGCTACGGGACC  
 GAATGGCACACTGACATCATTGGCAATACGGTCGTTATGACACGTATGGCATCA  
 AGAACACGAGCGTGAAAGCCGGATTAT.

**[0236]** The amino acid sequence of the PpaMan2 precursor protein expressed from plasmid p2JM-PpaMan2 is set forth as SEQ ID NO:38 (the predicted signal sequence is shown in *italics*, the three residue amino-terminal extension(AGK) is shown in **bold**):

*MRSKKLWISLLFALT*LIFTMAFSNMSAQ**AAGKAAGF**YVSGNKLYDSTGKAFVMRGVNHS  
 HTWFKNDLNTAIPAIAKTGANTVRIVLNGTQYTKDDLNAVKNIINLVSQNKMAVLEV  
 HDATGKDDYNSLDAAVNYWISIKEALIGKEDRVIVNIANEWYGTWNGSAWADGYKKA  
 IPKLRNAGIKNTLIVDAAGWGQYPQSVI**DYQ**GSVFAADAQKNTVFSIHMYEYAGKAA  
 TVKANMENVLNKGLALIIGEFGGYHTNGDVDEYAIMKYGQEKGVGWLAWSWYGNNS  
 DLNYLDLATGPNGTLTSFGNTVYDTYGIKNTSVKAGIY.

**[0237]** The amino acid sequence of the PpaMan2 mature protein expressed from p2JM-PpaMan2 is set forth as SEQ ID NO:39 (the three residue amino-terminal extension (AGK) based on the predicted cleavage site shown in **bold**):

**A**GKAAGF**YVSGNKLYDSTGKAFVMRGVNHS**HSTWFKNDLNTAIPAIAKTGANTVRIVL  
 SNGTQYTKDDLNAVKNIINLVSQNKMAVLEVHDATGKDDYNSLDAAVNYWISIKEAL  
 IGKEDRVIVNIANEWYGTWNGSAWADGYK**K**AI**P**KLRNAGIKNTLIVDAAGWGQYPQSVI  
**DYQ**GSVFAADAQKNTVFSIHMYEYAGKDAATVKANMENVLNKGLALIIGEFGGYHT  
 NGDVDEYAIMKYGQEKGVGWLAWSWYGNNSDLNYLDLATGPNGTLTSFGNTVYDT  
 YGIKNTSVKAGIY.

[0238] The amino acid sequence of the PpaMan2 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:40:

AAGFYVSGNKLYDSTGKAFVMRGVNHSHTWFKNLNTAIPAIAKTGANTVRIVLSNGT  
QYTKDDLNAVKNIINLVSQNKMIALEVHDAKGDDYNSLDAAVNYWISIKEALIGKE

DRVIVNIANEYWGTWNGSAWADGYKKAIPKLRNAGIKNTLIVDAAGWGQYPQSIVDY  
GQSVFAADAQKNTVFSIHMYEYAGKDAATVKANMENVLNKGLALIIGEFGGYHTNGD  
VDEYAIMKYGQEKGVGWLAWSWYGNNSDLNYLDLATGPNGTLTSFGNTVYDTYGI  
KNTSVKAGIY.

[0239] The nucleotide sequence of the synthesized *PpoMan1* gene in plasmid p2JM-*PpoMan1* is set forth as SEQ ID NO:41(the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in bold):

GTGAGAAGCAAAAAATTGTGGATCAGCTTGTGCGTTAACGTTAATCTTACG  
ATGGCGTTCAGCAACATGAGCGCGCAGGCTGCTGGAAAAGCAAGCGGCTTATGT  
TTCAGGCACAAAACGTATGATAGCACAGGCAAACCGTTATGAGAGGCCGTTA  
ATCATGCACATACGTGGTATAAAACGATCTGTATACGGCAATTCCGGCTATTGCAC  
AACAGGCGCAAATACAGTTAGAATTGTCTGAGCAATGGCAACCAGTATACGAAA  
GATGATATCAACAGCGTAAAAACATTATCAGCCTGGTCAGCAACTATAAAATGAT  
TGCAGTTCTGGAAGTCCATGATGCAACGGCAAAGATGATTATGCATCACTGGATG  
CAGCAGTCATTATTGGATTAGCATTAAAGATGCGCTGATGGCAAAGAAGATCGC  
GTTATTGTTAATATTGCGAACGAATGGTATGGCTCATGGAATGGCTCAGGCTGGC  
AGATGGCTATAAACAGCAATTCCGAAACTGAGAAATGCAGGCATTAAAACACA  
CTGATTGTTGATTGCGCAGGCTGGGACAATATCCGAATCAATTATGATTGGC  
AAAAGCGTTTGCAGCGGATAGCCTGAAAAATACAGTCTTAGCATCCATATGTAT  
GAATTGCGGGAAAAGATGCACAGACAGTCCGCACAAATATTGATAATGTCCTGAA  
TCAAGGCATCCCGCTGATTATTGGCGAATTGGCGGATATCATCAAGGGCAGATG  
TTGATGAAACAGAAATTGAGATACGGCCAATCAAAGGCCTGGCTGGCTTGC  
TGGTCATGGTATGGAAATTCAAGCAATCTGTCATATCTGGATCTGGTTACAGGACCG  
AATGGCAATCTTACAGATTGGGGCAAAACAGTTGTTAATGGCTCAAATGGCATCAA  
AGAAACGTCAAAAAAAGCAGGCATCTAT.

[0240] The amino acid sequence of the PpoMan1 precursor protein expressed from plasmid p2JM-PpoMan1 is set forth as SEQ ID NO:42 (the predicted signal sequence is shown in *italics*, the three residue amino-terminal extension(AGK) is shown in **bold**):

MRSKKLWISLLFALTTLIFTMAFSNMSAQAGKASGFYVSGTKLYDSTGKPVMRGVNHAHTWYKNDLYTAIPAIQATGANTVRIVLSNGNQYTKDDINSVKNIISLVSNSYKMIAVLEVHDATGKDDYASLDAAVNYWISIKDALIGKEDRVIVNIA NEWYGSWNGSGWADGYKQAPIKLRNAGIKNTLIVDCAGWGQYPQSINDFGKSVFAADSLKNTVFSIHMYEFAGKDAQTVRTNIDNVLNQGIPLIIGEFGGYHQGADVDETEIMRYGQSKGVGWLAWSWYGNSSNLS

YLDLVTGPNGNLTDWGKTVVNGSNGIKETSKKAGIY.

**[0241]** The amino acid sequence of the PpoMan1 mature protein expressed from p2JM-PpoMan1 is set forth as SEQ ID NO:43 (the three residue amino-terminal extension based on the predicted cleavage site shown in bold):

**AGK**ASGFYVSGTKLYDSTGKPFVMRGVNHAHTWYKNDLYTAIPAIAQTGANTVRIVLS  
NGNQYTKDDINSVKNIISLVSNYKMIAVLEVHDATGKDDYASLDAAVNYWISIKDALIG  
KEDRVIVNIANEWYGSWNGSGWADGYKQAIPKLRNAGIKNTLIVDCAGWGQYPQSIN  
DFGKSVFAADSLKNTVFSIHMYEFA**GK**DAQTVRTNIDNVLNQGIPLIIGEFGGYHQGAD  
VDETEIMRYGQSKGVGWLAWSWYGNSSNLSYLDLVTGPNGNLTDWGKTVVNGSNGI  
KETSKKAGIY.

**[0242]** The amino acid sequence of the PpoMan1 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:44:

ASGFYVSGTKLYDSTGKPFVMRGVNHAHTWYKNDLYTAIPAIAQTGANTVRIVLSNGN  
QYTKDDINSVKNIISLVSNYKMIAVLEVHDATGKDDYASLDAAVNYWISIKDALIGKED  
RVIVNIANEWYGSWNGSGWADGYKQAIPKLRNAGIKNTLIVDCAGWGQYPQSINDFG  
KSVFAADSLKNTVFSIHMYEFA**GK**DAQTVRTNIDNVLNQGIPLIIGEFGGYHQGADVDE  
TEIMRYGQSKGVGWLAWSWYGNSSNLSYLDLVTGPNGNLTDWGKTVVNGSNGIKETS  
KKAGIY.

**[0243]** The nucleotide sequence of the synthesized *PpoMan2* gene in plasmid p2JM-PpoMan2 is set forth as SEQ ID NO:45 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in bold):

GTGAGAACGAAAAATTGTGGATCAGCTTGTGTTGCGTTAACGTTAACCTTTACG  
ATGGCGTTCAGCAACATGAGCGCGCAGGCT**GCTGGAAAAGCAAGCGGCTTTATGT**  
TTCAGGCACAAATCTGTATGATAGCACAGGCCAACCGTTGTTATGAGAGGCGTTA  
ATCATGCACATACGTGGTATAAAACGATCTGTATACGGCAATTCCGGCAATCGCA  
AAAACAGGCGCAAATACAGTTAGAATTGTTCTGAGCAATGGCAACCAGTATCGAA  
AGATGATATCAACAGCGTAAAAACATTATCAGCCTGGTCAGCAACCATAAAATGA  
TTGCAGTTCTGGAAGTTCATGATGCAACGGCAAAGATGATTATGCATCACTGGAT  
GCAGCAGTCATTATTGGATTAGCATTAAAGATGCGCTGATGGCAAAGAAGATCG  
CGTTATTGTTAATTGCGAACGAATGGTATGGCTATGGAATGGCGGAGGCTGGG  
CAGATGGCTATAAACAAAGCAATTCCGAAACTGAGAAATGCAGGCATTAAAACAC  
ACTGATTGTTGATTGCGCAGGCTGGGACAATATCCGCAATCAATTATGATTTGG  
CAAAAGCGTTTGCGAGCGGATAGCCTGAAAAATACAGTCTTAGCATCCATATGTA  
TGAATTGCAAGGCAAAGACGTCCAAACAGTCCGCACAAATATTGATAATGTCCTGT  
ATCAAGGCCTGCCGCTGATTATTGGCGAATTGGCGGATATCATCAAGGCGCAGAT  
GTTGATGAAACAGAAATTATGAGATAACGGCCAGTCAGTTGGCTGGCTTGC

ATGGTCATGGTATGGAAATTCAAGCAATCTGAACATCTGGATCTGGTTACAGGAC  
CGAATGGCAATCTTACAGATTGGGGCAGAACAGTTGTTGAAGGCGCTAATGGAATT  
AAAGAAACGTCAAAAAAGCAGGCATTTT.

**[0244]** The amino acid sequence of the PpoMan2 precursor protein expressed from plasmid p2JM-PpoMan2 is set forth as SEQ ID NO:46 (the predicted signal sequence is shown in *italics*, the three residue amino-terminal extension (AGK) is shown in **bold**):

*MRSKKLWISLLFALT***LIFTMAFSNMSAQ****A**AGKASGFYVSGTNLYDSTGKPFVMRGVNHAH  
TWYKNDLYTAIPAIAKTGANTVRIVLSNGNQYT**K**DINSVKNIISLVSNHKMIAVLEVH  
DATGKDDYASLDAAVNYWISIKDALIGKEDRVIVNIA NEWYGSWNGGGWADGYKQAI  
PKLRNAGIKNTLIVDCAGWGQYPQSINDFGKSVFAADSLKNTVFSIHMYEFAGKDVQT  
VRTNIDNVLYQGLPLIIGEFGGYHQGADVDETEIMRYGQSKSVGWLAWSWYGNSSNLN  
YLDDLTGPNGLTDWGRTVVEGANGIKETSKKAGIF.

**[0245]** The amino acid sequence of the PpoMan2 mature protein expressed from p2JM-PpoMan2 is set forth as SEQ ID NO:47 (the three residue amino-terminal extension (AGK) based on the predicted cleavage site shown in **bold**):

**A**AGKASGFYVSGTNLYDSTGKPFVMRGVNHAHTWYKNDLYTAIPAIAKTGANTVRIVLS  
NGNQYT**K**DINSVKNIISLVSNHKMIAVLEVHDATGKDDYASLDAAVNYWISIKDALIG  
KEDRVIVNIA NEWYGSWNGGGWADGYKQAI**P**KLRNAGIKNTLIVDCAGWGQYPQSIN  
DFGKSVFAADSLKNTVFSIHMYEFAGKDVQTVRTNIDNVLYQGLPLIIGEFGGYHQGAD  
VDETEIMRYGQSKSVGWLAWSWYGNSSNLNYLDLVTGPNGLTDWGRTVVEGANGI  
KETSKKAGIF.

**[0246]** The amino acid sequence of the PpoMan2 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:48:

ASGFYVSGTNLYDSTGKPFVMRGVNHAHTWYKNDLYTAIPAIAKTGANTVRIVLSNGN  
QYT**K**DINSVKNIISLVSNHKMIAVLEVHDATGKDDYASLDAAVNYWISIKDALIG**K**  
RVIVNIA NEWYGSWNGGGWADGYKQAI**P**KLRNAGIKNTLIVDCAGWGQYPQSINDFG  
KSVFAADSLKNTVFSIHMYEFAGKDVQTVRTNIDNVLYQGLPLIIGEFGGYHQGADVDE  
TEIMRYGQSKSVGWLAWSWYGNSSNLNYLDLVTGPNGLTDWGRTVVEGANGIKETS  
KKAGIF.

**[0247]** The nucleotide sequence of the synthesized *PspMan4* gene in plasmid p2JM-PspMan4 is set forth as SEQ ID NO:49 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in **bold**):

GTGAGAAGCAAAAATTGTGGATCAGCTTGTGTTGCGTTAACGTTAACCTTACG  
ATGGCGTTCAGCAACATGAGCGCGCAGGCT**G**CTGGAAAATGGCGACAGGCTTT

ATGTTTCAGGCAACAAACTGTATGATAGCACAGGCAAACCGTTGTTATGAGAGGC  
 GTTAATCATGGCCATAGCTGGTTAAAAACGATCTGAATACAGCGATTCCGGCTATT  
 GCAAAAACAGGCGCAAATACAGTTAGAATTGTTCTGTCAAATGGCAGCCTGTATAC  
 GAAAGATGATCTGAATGCAGTCAAAACATCATCAATGTCGTCAACCAGAACAAAA  
 TGATTGCAGTTCTGGAAGTTCATGATGCAACGGCAAAGATGATTACAATTCACTG  
 GATGCAGCAGTCAACTATTGGATCTCAATTAAAGAAGCGCTGATGGCAAAGAAGA  
 TCGCGTTATTGTTAATATTGCGAACGAATGGTATGGCACATGGAATGGCTCAGCATG  
 GGCAGATGGCTACAAAAAAGCAATTCCGAAACTGAGAAATGCAGGCATCAAAAC  
 AACTGATTGTTGATGCGGCAGGCTGGGACAATTCCGAATCAATTGTTGATTAT  
 GGCACAAAGCGTTTGCAGCAGATGCCAGAAAAATACAGTCTTAGCATCCATAT  
 GTACGAATACGCTGGAAAAGATGCAGCAACAGTTAACAGCAATATGGAAAACGTC  
 CTGAATAAAGGCCTGGCACTGATTATTGGCAATTGGCGGATATCATAACAAATGG  
 CGACGTTGATGAATATGCGATTATGAGATATGCCAAGAAAAGGCCTGGCTGGC  
 TTGCATGGTCATGGTATGAAATTCACTCAGGCCCTAACTATCTGGATATGCCAACAG  
 GACCGAATGGATCACTGACATCATTGGCAATACAGTCGTCAATGATAACGTATGGA  
 ATCAAAATACGAGCCAGAAAGCTGGCATCTT.

**[0248]** The amino acid sequence of the *PspMan4* precursor protein expressed from plasmid p2JM-PspMan4 is set forth as SEQ ID NO:50 (the predicted signal sequence is shown in *italics*, the three residue amino-terminal extension (AGK) is shown in **bold**):

*MRSKKLWISLLFALT***LIFTMAFSNMSAQ**AAGKMATGFYVSGNKLYDSTGKPFVMRGVNH  
 GHSWFKNDLNTAIPAIAKTGANTVRIVLNSNGSLYTKDDLNAVKNIINVVNQNKMIAVLE  
 VHDATGKDDYNSLDAAVNYWISIKELIGKEDRVIVNIANEWYGTWNGSAWADGYKK  
 AIPKLRNAGIKNTLIVDAAGWGQFPQSIVDYGQSVFAADSKNTVFSIHMYEYAGKDA  
 ATVKANMENVLNKGLALIIGEFGGYHTNGDVDEYAIMRYGQEKGVGWLAWSWYGNS  
 SGLNYLDMATGPNGSLTSFGNTVVNDTYGIKNTSQKAGIF.

**[0249]** The amino acid sequence of the confirmed PspMan4 mature protein expressed from p2JM-PspMan4 is set forth as SEQ ID NO:51 (the three residue amino-terminal extension (AGK) based on the predicted cleavage site shown in **bold**):

**AGK**MATGFYVSGNKLYDSTGKPFVMRGVNHGSWFKNDLNTAIPAIAKTGANTVRIV  
 LSNGSLYTKDDLNAVKNIINVVNQNKMIAVLEVHDATGKDDYNSLDAAVNYWISIKEA  
 LIGKEDRVIVNIANEWYGTWNGSAWADGYKKAIPKLRNAGIKNTLIVDAAGWGQFPQS  
 IVDYGQSVFAADSKNTVFSIHMYEYAGKDAATVKANMENVLNKGLALIIGEFGGYHT  
 NGDVDEYAIMRYGQEKGVGWLAWSWYGNSGLNYLDMATGPNGSLTSFGNTVVNDT  
 YGIKNTSQKAGIF.

**[0250]** The amino acid sequence of the confirmed PspMan4 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:52:

MATGFYVSGNKL<sup>Y</sup>DSTGKPFVMRGVNHGHSWFKN<sup>D</sup>LNTAIPAI<sup>A</sup>KTGANTV<sup>R</sup>IVL<sup>S</sup>NG  
 SLYTKDDLN<sup>A</sup>VKN<sup>I</sup>INV<sup>V</sup>NQNK<sup>M</sup>IAVLEVHDATGKDDYNSLDAAVNYWISIKEALIGK  
 EDRVIVN<sup>I</sup>ANEWYGTWNGSAWADGYKKAIPKLRNAGIKNTLIVDAAGWGQFPQSIVD  
 YGQSVFAAD<sup>S</sup>QKNTVFSIHM<sup>Y</sup>AGKDAATVKANMENVLNKG<sup>L</sup>ALIIGEFGGYHTNG  
 DVDEYAIMRYGQEKGVGWL<sup>A</sup>WSWYGNSSGL<sup>N</sup>YLD<sup>M</sup>ATGPNGSLTSFGNTVVND<sup>T</sup>Y<sup>G</sup>  
 IKNTSQKAGIF.

**[0251]** The nucleotide sequence of the synthesized *PspMan5* gene in plasmid p2JM-PspMan5 is set forth as SEQ ID NO:53 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue amino-terminal extension (AGK) is shown in bold):

GTGAGAAGCAAAAATTGTGGATCAGCTGTTGCGTTAACGTTAAC<sup>A</sup>CTTACG  
 ATGGCGTT<sup>C</sup>AGCAACATGAGCGCGCAGGCTGCTGGAAAAGCAACAGGCTTTATGT  
 TTCAGGCACAACACTGTATGATTCAACAGGCAAACCGTTGTTATGAGAGGCGTTA  
 ATCATAGCCATACGTGGTTAAAACGATCTGAATGCAGCAATTCCGGCAATCGCA  
 AAAACAGGCGCAAATACAGTTAGAATTGTTCTGTCAAATGGCGTCCAGTATACAAG  
 AGATGATGTCAATAGCGTCAAAACATTATCAGCCTGGTCAACCAGAACAAAATGA  
 TTGCAGTTCTGGAAGTTCATGATGCGACAGGCAAAGATGATTATGCATCACTGGAT  
 GCAGCAGTCATTATTGGATTAGCATTAAAGATGCGCTGATGGCAAAGAAGATCG  
 CGTTATTGTTAATATTGCGAACGAATGGTATGGCACATGGAATGGCTCAGCATGGG  
 CAGATGGCTATAAACAAAGCGATTCCGAAACTGAGAAATGCAGGCATTAAAACAC  
 ACTGATTGTTGATGCGGCAGGCTGGGACAATGTCCGCAATCAATTGTTGATTATGG  
 CCAATCAGTTTGAGCGGATAGCCTGAAAAACACAATCTTAGCATCCATATGTA  
 TGAATATGCAGGCGGAACGGATGCAATTGTC<sup>A</sup>AGCAATATGGAAAACGT<sup>C</sup>CTGA  
 ATAAAGGCC<sup>T</sup>GCCGCTGATTATTGGCGAATTGGCGGACAACATACAAATGGCGAC  
 GTT<sup>G</sup>ATGAACATGCAATTATGAGATATGGCAACAAAAGGCGTGGCTGGCTTG  
 ATGGTCATGGTATGGAAATAATT<sup>C</sup>AGAACTGAGCTATCTGGATCTGGCAACAGGAC  
 CGGCAGGCTCACTGACATCAATTGGAAATACAATTGTGAACGATCCGTATGGCATT  
 AAAGCGACATCAAAAAAGCAGGCATT<sup>TTT</sup>.

**[0252]** The amino acid sequence of the PspMan5 precursor protein expressed from plasmid p2JM-PspMan5 is set forth as SEQ ID NO:54 (the predicted signal sequence is shown in italics, the three residue amino-terminal extension (AGK) is shown in bold):

*MRSKKLWISLLFALT*LIFTMAFSNMSAQAAGKATGFYVSGT<sup>T</sup>LYDSTGKPFVMRGVNHS<sup>H</sup>  
 TWFKNDLN<sup>A</sup>IPAI<sup>A</sup>KTGANTV<sup>R</sup>IVL<sup>S</sup>NGVQYTRDDVNSVK<sup>N</sup>IISLVNQNK<sup>M</sup>IAVLEVH  
 DATGKDDYASLDAAVNYWISIKDALIGKEDRVIVN<sup>I</sup>ANEWYGTWNGSAWADGYKQAI  
 PKLRNAGIKNTLIVDAAGWGQCPQSIVDYGQSVFAADSLKNTIFSIHM<sup>Y</sup>AGGTDAIV  
 KSNMENVLNKG<sup>L</sup>PLIIGEFGQQHTNGDVDEHAIMRYGQQKG<sup>V</sup>GWLAWSWYGNSEL  
 SYL<sup>D</sup>LATGPAGSLTSIGNTIVNDPYG<sup>I</sup>KATSKKAGIF.

[0253] The amino acid sequence of the PspMan5 mature protein expressed from p2JM-PspMan5 is set forth as SEQ ID NO:55 (the three residue amino-terminal extension (AGK) based on the predicted cleavage site shown in bold):

**AGK**ATGFYVSGTTLYDSTGKPFVMRGVNHSTWFKNLNAIPIAKTGANTVRIVLS  
NGVQYTRDDVNSVKNIISLVNQNKMIAVLEVHDATGKDDYASLDAAVNYWISIKDALI  
GKEDRVIVNIA NEWYGTWNGSAWADGYKQAPIKLRNAGIKNTLIVDAAGWGQCPQSI  
VDYGQSVFAADSLKNTIFSIHMYEYAGGTDAIVKSNMENVLNKGLPLIIGEFGGQHTNG  
DVDEHAIMRYGQQKGVGWLAWSWYGNNSELSYLDLATGPAGSLTSIGNTIVNDPYGI  
KATSKKAGIF.

[0254] The amino acid sequence of the PspMan5 mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:56:

ATGFYVSGTTLYDSTGKPFVMRGVNHSTWFKNLNAIPIAKTGANTVRIVLSNGV  
QYTRDDVNSVKNIISLVNQNKMIAVLEVHDATGKDDYASLDAAVNYWISIKDALIGKE  
DRVIVNIA NEWYGTWNGSAWADGYKQAPIKLRNAGIKNTLIVDAAGWGQCPQSIVDY  
GQSVFAADSLKNTIFSIHMYEYAGGTDAIVKSNMENVLNKGLPLIIGEFGGQHTNGDVD  
EHAIMRYGQQKGVGWLAWSWYGNNSELSYLDLATGPAGSLTSIGNTIVNDPYGIKATS  
KKAGIF.

[0255] The nucleotide sequence of the synthesized *PspMan9* gene in plasmid p2JM-PspMan9 is set forth as SEQ ID NO: 57 (the gene has an alternative start codon (GTG), the oligonucleotide encoding the three residue addition (AGK) is shown in bold):

GTGAGAACGAAAAATTGTGGATCAGCTTGTGTTGCGTTACGTTAACCTTACG  
ATGGCGTTTCAGCAACATGAGCGCGCAGGCTGCTGGAAAAGCAACAGGGTTTATGT  
TTCAGGAACAAACTTATGATAGCACGGAAAACCGTTGTGATGAGAGGGCGTTA  
ATCACTCACATACATGGTTAACGAAATGATCTGAATGCAGCTATCCCTGCGATTGCGA  
AGACAGGGCGAAACACCGTTAGAATTGTTCTGTCAAACGGCGTTCAATATACGAGA  
GATGATGTTAACATTCAAGAATATCATTCACTGGTGAATCAAATAAGATGATT  
GCAGTTCTGGAAGTTCATGATGCTACAGGAAAAGACGATTATGCATCACTGGATGC  
AGCAATTAACTATTGGATTCAATTAAAGATGCACTGATTGGCAAAGAAGATAGAG  
TTATTGTGAACATTGCAAATGAATGGTATGGCACATGGAATGGCTCAGCATGGCA  
GATGGATATAAACAGCTATTCTAAACTGAGAAATGCGGGCATAAAAATACGCT  
GATCGTGGATGCGGCTGGCTGGGCGAACATATCCGCAATCAATTGTTGATTACGGCC  
AGTCAGTTTGCGACAGATTCACTGAAGAACACAGTGTGTTAGCATCCATATGTATG  
AATATGCAGGCAGGACAGATGCAATGGTAAAGCTAATATGGAAGGAGTTCTGAAT  
AAAGGCCTGCCGCTGATTATTGGAGAATTGGCGGACAACATACAAATGGCGATGT  
TGACGAACCTGGCAATTATGAGATATGCCAACAAAAAGGCGTGGGATGGCTGGCAT  
GGTCATGGTACGGCAACACAGCGATCTGTATCTTGATCTGGCAACGGGACCG

AATGGATCACTGACAACGTTGGAAATACAGTGGTGAACGATACGAACGGAATTAA  
GGCAACGAGCAAGAAGGCGGGATTTCAA.

**[0256]** The amino acid sequence of the *PspMan9* precursor protein expressed from plasmid p2JM-*PspMan9* is set forth as SEQ ID NO:58 (the predicted signal sequence is shown in italics, the three residue amino-terminal extension (AGK) is shown in bold):

*MRSKKLWISLLFALT***LIFTMAFSNMSA**QAA**GKATGFYVSGTKLYDSTGKPFVMRGVNHS**H  
TWFKNDLNAAIPAIAKTGANTVRIVLSNGVQYTRDDVNSVKNIISLVNQNKMIAVLEVH  
DATGKDDYASLDAAINYWISIKDALIGKEDRVIVNIA NEWYGTWNGSAWADGYKQAIP  
KLRNAGIKNTLIVDAAGWGQYPQSIVDYGQSVFAADSLKNTVFSIHMYEYAGGTDAM  
VKANMEGVLNKGLPLIIGEFGGQHTNGDVDELAIMRYGQQKGVGWLAWSWYGNNSD  
LSYLDLATGPNGSLTTFGNTVVNDTNGIKATSKKAGIFQ

**[0257]** The amino acid sequence of the *PspMan9* mature protein expressed from p2JM-*PspMan9* is set forth as SEQ ID NO:59 (the three residue amino-terminal extension (AGK) based on the predicted cleavage site shown in bold):

**AGKATGFYVSGTKLYDSTGKPFVMRGVNHS**TWFNDLNAAIPAIAKTGANTVRIVLS  
NGVQYTRDDVNSVKNIISLVNQNKMIAVLEVHDATGKDDYASLDAAINYWISIKDALIG  
KEDRVIVNIA NEWYGTWNGSAWADGYKQAIPKLRNAGIKNTLIVDAAGWGQYPQSIV  
DYGQSVFAADSLKNTVFSIHMYEYAGGTDAMVKANMEGVLNKGLPLIIGEFGGQHTN  
GDVDELAIMRYGQQKGVGWLAWSWYGNNSDLSYLDLATGPNGSLTTFGNTVVNDT  
GIKATSKKAGIFQ.

**[0258]** The amino acid sequence of the *PspMan9* mature protein, based on the predicted cleavage of the naturally occurring sequence, is set forth as SEQ ID NO:60:

ATGFYVSGTKLYDSTGKPFVMRGVNHS  
QYTRDDVNSVKNIISLVNQNKMIAVLEVHDATGKDDYASLDAAINYWISIKDALIGKED  
RVIVNIA NEWYGTWNGSAWADGYKQAIPKLRNAGIKNTLIVDAAGWGQYPQSIVDYG  
QSVFAADSLKNTVFSIHMYEYAGGTDAMVKANMEGVLNKGLPLIIGEFGGQHTNGDV  
DELAIMRYGQQKGVGWLAWSWYGNNSDLSYLDLATGPNGSLTTFGNTVVNDTNGIK  
ATSKKAGIFQ.

### EXAMPLE 3

#### Purification of mannanases

**[0259]** BciMan1, BciMan4, and PspMan4 proteins were purified via two chromatography steps: anion-exchange and hydrophobic interaction chromatography. The concentrated and desalted crude protein samples were loaded onto a 70 ml Q-Sepharose High Performance column pre-equilibrated with buffer A (Tris-HCl, pH7.5, 20mM). After column washing, the proteins were eluted with a gradient of 0-50% buffer A with 1 M NaCl in 5 column volumes. The target protein was in the flowthrough. Ammonium sulfate was then added to the flowthrough to a final concentration of 0.8-1 M. The solution was loaded onto a Phenyl-Sepharose Fast Flow column pre-equilibrated with 20 mM Tris pH 7.5 with 0.8-1 M ammonium sulfate (buffer B). Gradient elution (0-100% buffer A) in 4 column volumes followed with 3 column volumes step elution (100% buffer A) was performed and the protein of interest was eventually eluted. The purity of the fractions was detected with SDS-PAGE and the results showed that the target protein had been completely purified. The active fractions were pooled and concentrated using 10 kDa Amicon Ultra-15 devices. The sample was above 90% pure and stored in 40% glycerol at -20°C to -80°C until usage.

**[0260]** BciMan3, PpoMan1, PpoMan2 proteins were purified using a three step anion-exchange, hydrophobic interaction chromatography and gel filtration purification strategy. The 700 mL crude broth from the shake flask was concentrated by VIVAFLOW 200 (cutoff 10 kDa) and buffer exchanged into 20 mM Tris-HCl (pH 7.5). The liquid was then loaded onto a 50 mL Q-Sepharose High Performance column which was pre-equilibrated with 20 mM Tris-HCl, pH 7.5 (buffer A). The column was eluted with a linear gradient from 0 to 50% buffer B (buffer A containing 1 M NaCl) in 3 column volumes, followed with 3 column volumes of 100% buffer B. The protein of interest was detected in the gradient elution part and the pure fractions were pooled. Subsequently, 3 M ammonium sulfate solution was added to the active fractions to an ultimate concentration of 1 M, and then the pretreated fraction was loaded onto a 50 mL Phenyl-Sepharose Fast Flow column equilibrated with 20 mM Tris-HCl (pH 7.5) containing 1 M ammonium sulfate. Four column volumes gradient elution (0-100% buffer A) followed with 3 column volumes step elution (100% buffer A) was performed and the relative pure fractions were pooled. The collected fraction was concentrated into 10 mL and loaded onto the HiLoad™ 26/60, Superdex-75 column (1 column volume = 320 mL) pre-equilibrated with 20 mM sodium phosphate buffer containing 0.15 M NaCl (pH 7.0). The pure fractions were pooled and concentrated using 10 kDa Amicon Ultra-15 devices. The purified sample was stored in 20 mM sodium phosphate buffer (pH 7.0) with 40% glycerol at -20°C until usage.

**[0261]** To purify PspMan5 and PspMan9 proteins, ammonium sulfate was added to the crude samples to a final concentration of 1 M. The solution was applied to a HiPrep™ 16/10 Phenyl FF column pre-equilibrated with 20 mM Tris (pH 8.5), 1M ammonium sulfate (buffer A). The target protein was eluted from the column with a linear salt gradient from 1 to 0 M ammonium sulfate. The active fractions were pooled, concentrated and buffer exchanged into 20 mM Tris (pH8.5) using a VivaFlow 200 ultra filtration device (Sartorius Stedim). The resulting solution was applied to a HiPrep™ Q XL 16/10 column pre-equilibrated with 20 mM Tris (pH8.5). The target protein was eluted from the column with a linear salt gradient from 0 to 0.6 M NaCl in buffer A. The resulting active protein fractions were then pooled and concentrated via 10 kDa Amicon Ultra devices, and stored in 40% glycerol at -20°C until usage.

**[0262]** PpaMan2 was purified using hydrophobic interaction chromatography and cation exchange chromatography. 800 mL crude broth was concentrated by VIVAFLOW 200 (cutoff 10 kDa) and ammonium sulfate was added to a final concentration of 0.8 M. The sample was then loaded onto a 50 mL Phenyl-Sepharose High Performance column which was pre-equilibrated with buffer A (20 mM sodium acetate containing 0.8 M ammonium sulfate, pH 5.5). The column was treated with a gradient elution of 0-100% buffer B (20 mM sodium acetate at pH 5.5) in 5 column volumes, followed with 3 column volumes of 100% buffer B. The relative pure active fractions were pooled and buffer exchanged into buffer B. The solution turned to be cloudy and was dispensed to 50 mL tubes, centrifuged at 3800 rpm for 20 min. The supernatant and the precipitant were collected. According to the SDS-PAGE gel analysis results, the target protein was identified in the supernatant which was then subjected onto an SP-Sepharose Fast Flow column, a linear gradient elution with 0-50% buffer C (20 mM sodium acetate containing 1M sodium chloride) in 4 column volumes followed with 3 column volumes' step elution (100% buffer C) was performed. The purity of the each fraction was evaluated with SDS-PAGE. Pure fractions were pooled and concentrated using 10 kDa Amicon Ultra-15 devices. The purified sample was stored in 20 mM sodium acetate buffer (pH 5.5) with 40% glycerol at -20°C.

#### EXAMPLE 4

#### Activity of mannanases

**[0263]** The beta 1-4 mannanase activity of the mannanases was measured using 0.5% locust bean gum galactomannan (Sigma G0753) and konjac glucomannan (Megazyme P-GLCML) as substrates. The assays were performed at 50°C for 10 minutes using two different buffer systems: 50 mM sodium acetate pH 5, and 50 mM HEPES pH 8.2. In both sets of assays, the released reducing sugar was quantified using a PAHBAH (p-Hydroxy benzoic acid hydrazide) assay (Lever, Anal Biochem, 47:248, 1972). A standard curve using mannose was created for each buffer, and was used to calculate enzyme activity units. In this assay, one mannanase unit is defined as the amount of enzyme required to generate 1 micromole of mannose reducing sugar equivalent per minute. The specific activities of the mannanases are summarized in Table 1.

**Table1. Specific activities (U/mg) of mannanases at pH 5.0 and pH 8.2 using different substrates**

Mannanase	pH5.0		pH8.2	
	Locust bean gum	Konjac glucomannan	Locust bean gum	Konjac glucomannan
BciMan1	25	70	328	363
BciMan3	17	35	377	414
BciMan4	160	221	590	681

**Table1. Specific activities (U/mg) of mannanases at pH 5.0 and pH 8.2 using different substrates**

Mannanase	pH5.0		pH8.2	
	Locust bean gum	Konjac glucomannan	Locust bean gum	Konjac glucomannan
PpaMan2	94	162	419	454
PpoMan1	148	205	616	601
PpoMan2	62	108	618	615
PspMan4	112	159	520	624
PspMan5	105	136	116	152
PspMan9	145	251	518	628

**EXAMPLE 5****pH Profile of Mannanases**

**[0264]** The pH profile of mannanases was determined by assaying for mannanase activity at various pH values ranging from 2 to 9 at 50°C for 10 min with locust bean gum as the substrate. The proteins were diluted in 0.005% Tween-80 to an appropriate concentration based on the dose response curve. The substrate solutions, buffered using sodium citrate/sodium phosphate buffers of different pH units, were pre-incubated in the thermomixer at 50°C for 5 min. The reaction was initiated by the addition of mannanases. The mixture was incubated at 50°C for 10 min, and then the reaction was stopped by transferring 10 microliters of reaction mixture to a 96-well PCR plate containing 100 microliters of the PAHBAH solution. The PCR plate was heated at 95°C for 5 minutes in a Bio-Rad DNA Engine. Then 100 microliters were transferred from each well to a new 96-well plate. The release of reducing sugars from the substrate was quantified by measuring the optical density at 410 nm in a spectrophotometer. Enzyme activity at each pH is reported as relative activity where the activity at the pH optimum was set to 100%. The pH optimum and range of  $\geq 70\%$  activity for the mannanases under these assay conditions is shown in Table 2.

**Table 2. Optimal pH and pH range of activity for mannanases**

Mannanase	pH Optimum	pH range of $\geq 70\%$ activity
BciMan1	7.0	6.0-8.5
BciMan3	7.0	6.5-8.5
BciMan4	7.0	5.5-8.5
PpaMan2	8.0	5.5-9.0*
PpoMan1	7.0	5.5-8.5

**Table 2. Optimal pH and pH range of activity for mannanases**

Mannanase	pH Optimum	pH range of $\geq 70\%$ activity
PpoMan2	7.0	6.0-8.5
PspMan4	7.5	5.5-9.0
PspMan5	6.0	4.5-7.5
PspMan9	6.0-8.0	5.5-9.0*

\*PpaMan2 and PspMan9 showed mannanase activity above pH9

#### EXAMPLE 6

##### Temperature Profile of mannanases

**[0265]** The temperature profile of mannanases was determined by assaying for mannanase activity with locust bean gum as the substrate at various temperatures for 10 min in 50 mM sodium citrate buffer at pH 6.0. The activity is reported as relative activity where the activity at the temperature optimum was set to 100%. The temperature optimum and temperature range of  $\geq 70\%$  activity for the mannanases under these assay conditions is shown in Table 3.

**Table 3. Optimal temperature and temperature range of activity for mannanases.**

Mannanase	Temperature Optimum (°C)	Temperature range of $\geq 70\%$ activity (°C)
BciMan1	60-65	45-70
BciMan3	55	40-65
BciMan4	55	50-60
PpaMan2	60	54-63
PpoMan1	55-58	45-65
PpoMan2	50-55	<35-60
PspMan4	55	47-60
PspMan5	50	40-55
PspMan9	58	48-62

#### EXAMPLE 7

##### Thermostability of *Paenibacillus* and *Bacillus* mannanases

**[0266]** The temperature stability of *Paenibacillus* and *Bacillus* mannanases was determined in 50 mM sodium citrate buffer at pH 6.0. The enzyme was incubated at temperatures ranging from 40°C to 90°C for 2 hours in a thermocycler. The remaining enzyme activity was measured using locust bean gum as the substrate. The activity of the sample kept on ice was defined as 100% activity. The temperatures at which the enzymes retain 50% activity ( $T_{50}$ ) after a 2-hour incubation period under these assay conditions are shown in Table 4.

**Table 4: Thermal Stability of Mannanases.**

Mannanase	$T_{50}$ (°C)
PspMan4	57
BciMan1	53
BciMan3	47
BciMan4	53
PpoMan1	54
PpoMan2	52
PspMan5	53
PspMan9	54
PpaMan2	58

## EXAMPLE 8

### Cleaning performance of mannanases

**[0267]** Cleaning performance was measured using a high throughput assay developed to measure galactomannan removal from technical soils. The assay measures the release of locust bean gum from the technical soils containing locust bean gum. The BCA reagent measures the reducing ends of oligosaccharides released in the presence of mannanase enzyme, as compared to a blank (no enzyme) control. This measurement correlates with the cleaning performance for the enzymes. As the mannanases hydrolyze galactomannans, oligosaccharides of varying lengths with new reducing ends are released from the cotton swatch. The bicinchoninic acid in the BCA reagent then allows for the highly sensitive colorimetric detection as  $\text{Cu}^{1+}$  is formed by the reduction of  $\text{Cu}^{2+}$ .

**[0268]** Two 5.5 cm diameter locust bean gum CS-73 microswatches (CFT, Vlaardingen, Holland) were placed into each well of a flat-bottom, non-binding 96-well assay plate. Enzymes

were diluted into 50 mM MOPS, pH 7.2, 0.005% Tween-80. Diluted enzyme and microswatch assay buffer (25 mM HEPES, pH 8, 2 mM CaCl<sub>2</sub>, 0.005% Tween-80) was added into each well for a combined volume of 100 microliters. Plates were sealed and incubated in an iEMS machine at 25°C with agitation at 1150 rpm for 20 minutes. To measure the new reducing ends produced, 100 microliters of the BCA assay reagent (Thermo Scientific Pierce, Rockford, IL) was pipetted into each well of a fresh PCR plate. 15 microliters of wash liquor was removed from each well of the microswatch assay plates after the incubation period was completed, and transferred to the plate containing the BCA reagent. Plates were sealed and incubated in a PCR machine at 95°C for 2-3 minutes. After the plate cooled to 25°C, 100 microliters of the supernatant was transferred to a fresh microtiter flat-bottom assay plate and absorbance was measured at 562 nm in a spectrophotometer. Figures 2A and 2B show the response of the mannanases in this assay. All mannanases tested exhibited galactomannan removal activity.

#### EXAMPLE 9

#### Identification of Homologous mannanases

**[0269]** Related proteins were identified by a BLAST search (Altschul et al., Nucleic Acids Res, 25:3389-402, 1997) against the NCBI non-redundant protein database using the mature protein amino acid sequence of PpaMan2 (SEQ ID NO:40), PspMan4 (SEQ ID NO:52), and PspMan9 (SEQ ID NO:60) and a subset of the results are shown on Tables 5A, 6A, and 7A, respectively. A similar search was run against the Genome Quest Patent database with search parameters set to default values using the mature protein amino acid sequence of PpaMan2 (SEQ ID NO:40), PspMan4 (SEQ ID NO:52), and PspMan9 (SEQ ID NO:60) as the query sequences, and a subset of the results are shown in Tables 5B, 6B, and 7B, respectively. 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. The column labeled "Sequence Length" refers to the length (in amino acids) of the protein sequences associated with the listed Accession Nos., while the column labeled "Aligned Length" refers to the length (in amino acids) of the aligned protein sequence used for the PID calculation.

**Table 5A: List of sequences with percent identity to PpaMan2 protein identified from the NCBI non-redundant protein database**

Accession #	PID	Organism	Sequence Length	Alignment Length
WP_024633848.1	95	<i>Paenibacillus</i> sp. MAEPY2J	326	296
ETT37549.1	94	<i>Paenibacillus</i> sp. FSL R5-192	326	296
WP_017688745.1	93	<i>Paenibacillus</i> sp. PAMC	326	296

**Table 5A: List of sequences with percent identity to PpaMan2 protein identified from the NCBI non-redundant protein database**

Accession #	PID	Organism	Sequence Length	Alignment Length
	26794			
ACU30843.1	93	<i>Paenibacillus</i> <i>sp. A1</i>	319	296
AAX87003.1	91	<i>B. circulans</i>	326	296
WP_017813111.1	88	<i>Paenibacillus</i> <i>sp. A9</i>	327	296
AEX60762.1	86	<i>Paenibacillus</i> <i>sp. CH-3</i>	327	296
YP_003868989.1/WP_013308634.1	81	<i>Paenibacillus</i> <i>polymyxa</i> <i>E681</i>	327	296
WP_016819573.1	81	<i>Paenibacillus</i> <i>polymyxa</i>	327	296
WP_017427981.1	81	<i>Paenibacillus</i> <i>sp.</i> <i>ICGEB2008</i>	327	296
YP_003944884.1/WP_013369280.1	80	<i>Paenibacillus</i> <i>polymyxa SC2</i>	327	296
WP_009593769.1	80	<i>Paenibacillus</i> <i>sp. HGF5</i>	326	296
AAX87002.1	81	<i>B. circulans</i>	327	296
BAA25878.1	71	<i>B. circulans</i>	516	297
WP_019912481.1	66	<i>Paenibacillus</i> <i>sp. FEW567</i>	547	294
YP_006190599.1/WP_014651264.1	66	<i>Paenibacillus</i> <i>mucilaginosus</i> <i>K02</i>	475	296

**Table 5B: List of sequences with percent identity to PpaMan2 protein identified from the Genome Quest database**

Patent ID #	PID	Organism	Sequence Length	Alignment Length
EP2260105-0418	91.6	<i>B. circulans</i>	326	296
EP2260105-0427	81.1	<i>B. circulans</i>	327	296
CN100410380-0004,	81.1	<i>B. circulans</i> <i>B48</i>	296	296
CN1904052-0003	80.4	<i>B. circulans</i> <i>B48</i>	327	296
US20090325240-0477	71.7	<i>B. circulans</i>	516	297

**Table 5B: List of sequences with percent identity to PpaMan2 protein identified from the Genome Quest database**

Patent ID #	PID	Organism	Sequence Length	Alignment Length
US20140199705-0388	68.4	<i>empty</i>	490	291
WO2014100018-0002	66	<i>Bacillus lentus</i>	299	297
WO2015022428-0015	63.1	<i>Bacillus sp.</i>	309	290
US20030203466-0004	62.8	<i>Bacillus sp.</i>	490	290
EP2260105-0445	62.1	<i>B. circulans</i>	493	290
EP2260105-0429	61.8	<i>Bacillus sp.</i> JAMB-602	490	296
US20030215812-0002	60.6	<i>Bacillus sp.</i>	493	297
US20030203466-0008	60.6	<i>Bacillus agaradhaerens</i>	468	297
US20030215812-0002	60.6	<i>Bacillus sp</i>	493	297

**Table 6A: List of sequences with percent identity to PspMan4 protein identified from the NCBI non-redundant protein database**

Accession #	PID	Organism	Sequence Length	Alignment Length
ACU30843.1	100	<i>Paenibacillus sp. A1</i>	319	297
ETT37549.1	99	<i>Paenibacillus sp. FSL R5-192</i>	326	296
WP_017688745.1	99	<i>Paenibacillus sp. PAMC 26794</i>	326	296
AAX87003.1	94	<i>B. circulans</i>	326	296
WP_024633848.1	94	<i>Paenibacillus sp. MAEPY2</i>	326	296
WP_017813111.1	89	<i>Paenibacillus sp. A9</i>	327	296
AEX60762.1	87	<i>Paenibacillus sp. CH-3</i>	327	296
YP_003868989.1/WP_013308634.1	81	<i>Paenibacillus polymyxa E681</i>	327	296
		<i>Paenibacillus</i>		

**Table 6A: List of sequences with percent identity to PspMan4 protein identified from the NCBI non-redundant protein database**

Accession #	PID	Organism	Sequence Length	Alignment Length
YP_003944884.1/WP_013369280.1	80	<i>polymyxa SC2</i>	327	296
WP_016819573.1	80	<i>Paenibacillus polymyxa</i>	327	296
WP_017427981.1	80	<i>Paenibacillus sp. ICGEB2008</i>	327	296
AAX87002.1	79	<i>B. circulans</i>	327	296
WP_009593769.1	78	<i>Paenibacillus sp. HGF5</i>	326	296
BAA25878.1	72	<i>B. circulans</i>	516	297
YP_006190599.1/WP_014651264.1	67	<i>Paenibacillus mucilaginosus K02</i>	475	296
WP_019912481.1	65	<i>Paenibacillus sp. FEW567</i>	547	294
BAD99527.1	62	<i>Bacillus sp. JAMB-602</i>	490	296
AGU71466.1	64	<i>Bacillus nealsonii</i>	353	297
WP_017426982.1	63	<i>Paenibacillus sp. ICGEB2008</i>	796	296
AAS48170.1	61	<i>Bacillus circulans</i>	493	296
AAT06599.1	60	<i>Bacillus sp. N16-5</i>	493	297
WP_018887458.1	63	<i>Paenibacillus massiliensis</i>	592	294
YP_006844719.1	60	<i>Amphibacillus xylanus NBRC 15112</i>	497	297

**Table 6B: List of sequences with percent identity to PspMan4 protein identified from the Genome Quest database**

Patent ID #	PID	Organism	Sequence Length	Alignment Length
EP2260105-0418	94.3	<i>B. circulans</i>	326	296
CN100410380-0004	79.1	<i>B. circulansB48</i>	296	296
EP2260105-0427	79.1	<i>B. circulans</i>	327	296

**Table 6B: List of sequences with percent identity to PspMan4 protein identified from the Genome Quest database**

Patent ID #	PID	Organism	Sequence Length	Alignment Length
CN1904052-0003	78.4	<i>B. circulans</i> B48	327	296
US20090325240-0477	72.1	<i>B. circulans</i>	516	297
EP2409981-0388	67.7	empty	490	297
WO2014100018-0002	66.3	<i>Bacillus lentinus</i>	299	297
WO2015022428-0015	62.5	<i>Bacillus</i> sp.	309	296
JP2006087401-0006	62.5	<i>Bacillus</i> sp.	458	296
US20090325240-0429	62.5	<i>Bacillus</i> sp. JAMB-602	490	296
EP2284272-0004	62.2	<i>Bacillus</i> sp.	476	296
EP2287318-0002	62.2	<i>Bacillus</i> sp. 1633	490	296
WO2014124927-0018	62.2	<i>Bacillus</i> sp. 1633	490	296
US20090325240-0445	61.5	<i>B. circulans</i>	493	296
US20030203466-0008	60.9	<i>Bacillus agaradhaerens</i>	468	297
US6964943-0002	60.9	<i>Bacillus</i> sp.	493	297

**Table 7A: List of sequences with percent identity to PspMan9 protein identified from the NCBI non-redundant protein database**

Accession #	PID	Organism	Sequence Length	Alignment Length
AEX60762.1	94	<i>Paenibacillus</i> sp. CH-3	327	296
WP_017813111.1	89	<i>Paenibacillus</i> sp. A9	327	296
ACU30843.1	88	<i>Paenibacillus</i> sp. A1	319	297
WP_024633848.1	88	<i>Paenibacillus</i> sp. MAEPY2J	326	296
ETT37549.1	88	<i>Paenibacillus</i> sp. FSL R5-192	326	296
WP_017688745.1	87	<i>Paenibacillus</i> sp. PAMC 26794	326	296
AAX87003.1	86	<i>B. circulans</i>	326	296

**Table 7A: List of sequences with percent identity to PspMan9 protein identified from the NCBI non-redundant protein database**

Accession #	PID	Organism	Sequence Length	Alignment Length
YP_003868989.1/WP_013308634.1	83	<i>Paenibacillus polymyxa</i> E681	327	296
WP_016819573.1	83	<i>Paenibacillus polymyxa</i>	327	296
WP_017427981.1	82	<i>Paenibacillus</i> sp. ICGEB2008	327	296
YP_003944884.1/WP_013369280.1	82	<i>Paenibacillus polymyxa</i> SC2	327	296
AAX87002.1	80	<i>B. circulans</i>	327	296
WP_009593769.1	79	<i>Paenibacillus</i> sp. HGF5	326	296
BAA25878.1	73	<i>B. circulans</i>	516	297
YP_006190599.1/ WP_014651264.1	68	<i>Paenibacillus mucilaginosus</i> K02	475	296
WP_019912481.1	66	<i>Paenibacillus</i> sp. HW567	547	294
AGU71466.1	68	<i>B. nealsonii</i>	353	297
WP_018887458.1	65	<i>Paenibacillus massiliensis</i>	592	294
WP_019687326.1	64	<i>Paenibacillus polymyxa</i>	796	296
WP_006037399.1	64	<i>Paenibacillus curdlanolyticus</i>	707	297

**Table 7B: List of sequences with percent identity to PspMan9 protein identified from the Genome Quest database**

Patent ID #	PID	Organism	Sequence Length	Alignment Length
EP2260105-0418	86.2	<i>B. circulans</i>	326	296
CN100410380-0004	80.4	<i>B. circulans</i> B48	296	296
EP2260105-0427	80.4	<i>B. circulans</i>	327	296
CN1904052-0003	79.7	<i>B. circulans</i> B48	327	296
EP2260105-0477	73.4	<i>B. circulans</i>	516	297
US20140199705-0388	68.4	empty	490	297
WO2014100018-0002	68	<i>Bacillus lenthus</i>	299	297

**Table 7B: List of sequences with percent identity to PspMan9 protein identified from the Genome Quest database**

Patent ID #	PID	Organism	Sequence Length	Alignment Length
JP2006087401-0001	62.8	<i>Bacillus sp.</i>	458	296
WO2015022428-0015	62.5	<i>Bacillus sp.</i>	309	296
US20030203466-0004	62.2	<i>Bacillus sp.</i>	490	296
JP2006087401-0005	62.8	<i>Bacillus sp.</i>	490	296
US20090325240-0429	62.8	<i>Bacillus sp.</i> JAMB-602	490	296
EP2287318-0004	62.2	<i>Bacillus sp.</i>	476	296
EP2260105-0445	61.5	<i>B. circulans</i>	493	296

#### EXAMPLE 10

#### Analysis of Homologous Sequences

**[0270]** An alignment of the amino acid sequences of the mature BciMan1 (SEQ ID NO:28), BciMan3 (SEQ ID NO:32), BciMan4 (SEQ ID NO:36), PamMan2 (SEQ ID NO:17), PpaMan2 (SEQ ID NO:40), PpoMan1 (SEQ ID NO:44), PpoMan2 (SEQ ID NO:48), PspMan4 (SEQ ID NO:52), PspMan5 (SEQ ID NO:56), PspMan9 (SEQ ID NO:60), and PtuMan2 (SEQ ID NO:24) mannanases with some of the sequences of the mature forms of mannanases from Tables 5A, 6A, and 7A (identified from NCBI searches) is shown in Figure 3. The full-length, untrimmed sequences were aligned using CLUSTALW software (Thompson et al., Nucleic Acids Research, 22:4673-4680, 1994) with the default parameters, wherein Figure 3 displays the alignment of amino acids 1-300 and not the alignment of the entire full-length, untrimmed sequences.

**[0271]** A phylogenetic tree for amino acid sequences of the mannanases aligned in Figure 3 was built, and is shown on Figure 4. The full-length, untrimmed sequences were entered in the Vector NTI Advance suite and a Guide Tree was created using the Neighbor Joining (NJ) method (Saitou and Nei, Mol Biol Evol, 4:406-425, 1987). The tree construction was calculated using the following parameters: Kimura's correction for sequence distance and ignoring positions with gaps. AlignX displays the calculated distance values in parenthesis following the molecule name displayed on the tree shown in Figure 4.

#### EXAMPLE 11

#### Unique features of the NDL-Clade mannanases

**[0272]** When the mannanases described in Example 10 were aligned common features were shared among BciMan3, BciMan4, PamMan2, PpaMan2, PpoMan1, PpoMan2, PspMan4, PspMan5, PspMan9, and PtuMan2 mannanases. In one case, there is a common pattern of conserved amino acids between residues Trp30 and Ile39, wherein the amino acid positions of the polypeptide are numbered by correspondence with the amino sequence set forth in SEQ ID NO:32. The NDL mannanases share features to create a clade, subsequently termed NDL-Clade, where the term NDL derives from the complete conserved residues NDL near the N-terminus (Asn-Asp-Leu 33-35). The numbering of residues for the mannanases shown is the consecutive linear sequence and are numbered by correspondence with the amino acid sequence set forth in SEQ ID NO:32. The pattern of conserved amino acids related to the NDL-Clade is highlighted in Figure 5, and can be described as  $WX_aKNDLXXAI$ , where  $X_a$  is F or Y and X is any amino acid;  $WX_aKNDLXbX_cAI$ , where  $X_a$  is F or Y,  $X_b$  is N, Y or A, and  $X_c$  is A or T; or  $WF/YKNDLX_1T/AAI$ , where  $X_1$  is N, Y or A.

**[0273]** The phylogenetic tree described in Example 10 shows a differentiation between the NDL-Clade mannanases and other mannanases. The clade further differentiates into NDL-Clade 1 and NDL-Clade 2 where NDL-Clade 1 includes PtuMan2, PamMan2, PspMan4, BciMan4, PpaMan2, PspMan9 and PspMan5 while NDL-Clade 2 includes BciMan3, PpoMan2 and PpoMan1.

**[0274]** All members of the NDL-Clade have a conserved motif with the key feature of a deletion which is not present in the *Bacillus* sp. JAMB-602 and other reference mannanase sequences (hereinafter the "deletion motif"). The deletion motif starts at position 262 in the conserved linear sequence of the amino acid sequences set forth in Figure 6 and includes the sequence LDXXXGPXGXLT, where X is any amino acid or LDM/LV/AT/AGPX<sub>1</sub>GX<sub>2</sub>LT, where X<sub>1</sub> is N, A or S and X<sub>2</sub> is S, T or N. The sequence further differentiates into LDM/LATGPN/AGS/TLT for NDL-Clade 1 mannanases; LDLA/VA/TGPS/NGNLT for NDL-Clade 2 mannanases; and LDL/VS/AT/NGPSGNLT for NDL-Clade 3 mannanases. All members of the NDL-Clade have a conserved deletion motif not seen in the *Bacillus* sp. JAMB-602\_BAD99527.1, *B\_nealsonii*\_AGU71466.1, and *Bciman1\_B\_circulans*\_BAA25878.1 mannanase sequences. The NDL-Clade deletion motif (i.e., LDM/LV/AT/AGPX<sub>1</sub>GX<sub>2</sub>LT, where X<sub>1</sub> is N, A or S and X<sub>2</sub> is S, T or N) set forth in Figure 6 occurs between the conserved residues Leu262-Asp263 (LD) and Leu272-Thr273 (LT).

**[0275]** The closest related structure to the NDL-Clade mannanases is that from *Bacillus* sp. JAMB-602 (1WKY.pdb) and thus this will be used as a reference to understand the probable consequences of the differentiating characteristics of the NDL-Clade mannanases. Figure 7 shows the structure of *Bacillus* sp. JAMB-602 (black) and models of the NDL-Clade mannanases PspMan4, PspMan9 and PpaMan2 (gray). The structures of PspMan4, PspMan9 and PpaMan2 were modelled using the "align" option in the Molecular Operating Environment

(MOE) software (Chemical Computing Group, Montreal, Quebec, Canada) to look for structural similarities. The alignment applies conserved structural motifs as an additional guide to conventional sequence alignment. This alignment was performed using standard program defaults present in the 2012.10 distribution of MOE. The deletion motif segment is designated with an arrow. This deletion motif is located in a loop in the structure in the C-terminus. The C-terminal region of the *Bacillus sp.* JAMB-602 mannanase is thought to be important to understanding how these mannanases interact in alkaline environments (Akita et al., *Acta Cryst*, 60:1490-1492, 2004). It is postulated that the deletion impacts the structure, length and flexibility of this loop which then impacts the activity and performance of the NDL-Clade mannanases.

## REFERENCES CITED IN THE DESCRIPTION

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### Patent documents cited in the description

- [EP2287318A \[0002\]](#)
- [WO2014100018A1 \[0002\]](#)
- [WO2012149333A1 \[0002\]](#)
- [WO2012149325A \[0002\]](#)
- [CN1904052A \[0002\]](#)
- [US6602842B \[0024\] \[0147\]](#)
- [WO9535362A1 \[0024\] \[0147\]](#)
- [WO9934011A \[0036\]](#)
- [US6605458B \[0036\] \[0123\] \[0165\] \[0169\] \[0171\] \[0171\]](#)
- [US6566114B \[0036\] \[0147\] \[0148\] \[0148\] \[0148\] \[0148\] \[0148\] \[0148\] \[0148\]](#)
- [WO2010044786A \[0092\]](#)
- [WO9000609A \[0094\]](#)
- [WO9516782A \[0094\]](#)
- [EP238023A \[0099\] \[0161\]](#)
- [WO9206154A \[0117\]](#)
- [EP2100949A \[0122\] \[0174\] \[0179\] \[0184\] \[0185\] \[0186\] \[0190\]](#)
- [US6610642B \[0123\] \[0165\] \[0169\] \[0169\] \[0170\] \[0171\] \[0171\]](#)
- [US5705464A \[0123\] \[0165\]](#)
- [US5710115A \[0123\] \[0165\]](#)
- [US5698504A \[0123\] \[0165\]](#)

- [US5695679A \[0123\]](#) [\[0165\]](#)
- [US5686014A \[0123\]](#) [\[0165\]](#)
- [US5646101A \[0123\]](#) [\[0165\]](#)
- [WO9711151A \[0131\]](#)
- [EP0922499A \[0131\]](#)
- [US4977252A \[0131\]](#)
- [US5354559A \[0131\]](#)
- [US5935826A \[0131\]](#)
- [US6060299A \[0147\]](#) [\[0148\]](#)
- [WO9964573A1 \[0147\]](#) [\[0148\]](#)
- [WO9964619A1 \[0147\]](#) [\[0148\]](#) [\[0148\]](#) [\[0148\]](#) [\[0148\]](#) [\[0148\]](#) [\[0148\]](#) [\[0148\]](#)
- [WO2015022428A \[0147\]](#)
- [US6376445B \[0149\]](#)
- [WO9523221A \[0152\]](#)
- [WO9221760PCT \[0152\]](#)
- [US20080090747A \[0152\]](#)
- [US5801039A \[0152\]](#)
- [US5340735A \[0152\]](#)
- [US5500364A \[0152\]](#)
- [US5855625A \[0152\]](#)
- [USRE34606E \[0152\]](#)
- [US5955340A \[0152\]](#)
- [US5700676A \[0152\]](#)
- [US6312936B \[0152\]](#)
- [US6482628B \[0152\]](#)
- [WO07044993A \[0152\]](#)
- [GB1296839A \[0153\]](#)
- [JP56131376B \[0156\]](#)
- [JP56068393B \[0156\]](#)
- [JP59066588B \[0156\]](#)
- [JP63042938B \[0156\]](#)
- [JP73006557B \[0156\]](#)
- [US4435307A \[0160\]](#)
- [EP0495257A \[0160\]](#)
- [US5874276A \[0160\]](#)
- [EP258068A \[0161\]](#)
- [EP305216A \[0161\]](#)
- [EP214761A \[0161\]](#)
- [EP218272A \[0161\]](#)
- [EP331376A \[0161\]](#)
- [GB1372034A \[0161\]](#)
- [JP64744992B \[0161\]](#)
- [WO9116422A \[0161\]](#)
- [WO8809367A \[0161\]](#)

- [WO9009446A \[0161\]](#)
- [WO9412621A \[0163\]](#)
- [WO9501426A \[0163\]](#)
- [WO05056782A \[0164\]](#)
- [US6376450B \[0169\] \[0170\] \[0170\] \[0171\] \[0171\] \[0171\]](#)
- [GB400898A1 \[0172\] \[0172\]](#)
- [GB514276A1 \[0172\] \[0172\]](#)
- [EP0011340A \[0172\] \[0172\]](#)
- [EP0026528A \[0172\] \[0172\]](#)
- [EP0242919A \[0172\] \[0172\]](#)
- [EP0299575A \[0172\] \[0172\]](#)
- [EP0313146A \[0172\] \[0172\]](#)
- [US5019292A \[0172\] \[0172\]](#)
- [US5379584A \[0173\] \[0191\]](#)
- [US5691297A \[0173\] \[0191\]](#)
- [US5574005A \[0173\] \[0191\]](#)
- [US5569645A \[0173\] \[0191\]](#)
- [US5565422A \[0173\]](#)
- [US5516448A \[0173\] \[0191\]](#)
- [US5489392A \[0173\] \[0191\]](#)
- [US5486303A \[0173\] \[0191\]](#)
- [WO02102955A \[0174\]](#)
- [US4765916A \[0174\]](#)
- [US4972017A \[0174\]](#)
- [WO04111178A \[0174\]](#)
- [EP2100947A \[0174\]](#)
- [WO2013165725A \[0174\]](#)
- [US5576282A \[0175\] \[0187\]](#)
- [US6306812B \[0175\]](#)
- [US6326348B \[0175\]](#)
- [WO07145964A \[0183\]](#)
- [US4246612A \[0186\]](#)
- [US5227084A \[0186\]](#)
- [US4810410A \[0186\]](#)
- [WO9906521A \[0186\]](#)
- [US4430243A \[0187\]](#)
- [US5597936A \[0187\]](#)
- [US5595967A \[0187\]](#)
- [WO200032601A \[0189\]](#)
- [US6225464B \[0189\]](#)
- [WO9426860A \[0190\]](#)
- [WO9426859A \[0190\]](#)
- [EP22601050418A \[0269\] \[0269\] \[0269\]](#)
- [EP22601050427A \[0269\] \[0269\] \[0269\]](#)

- CN1004103800004 [0269] [0269] [0269]
- CN19040520003 [0269] [0269] [0269]
- US200903252400477A [0269] [0269]
- US201401997050388A [0269] [0269]
- WO20141000180002A [0269] [0269] [0269]
- WO20150224280015A [0269] [0269] [0269]
- US200302034660004A [0269] [0269]
- EP22601050445A [0269] [0269]
- EP22601050429A [0269]
- US200302158120002A [0269] [0269]
- US200302034660008A [0269] [0269]
- EP24099810388A [0269]
- JP20060874010006B [0269]
- US200903252400429A [0269] [0269]
- EP22842720004A [0269]
- EP22873180002A [0269]
- WO20141249270018A [0269]
- US200903252400445A [0269]
- US69649430002A [0269]
- EP22601050477A [0269]
- JP20060874010001B [0269]
- JP20060874010005B [0269]
- EP22873180004A [0269]

#### Non-patent literature cited in the description

- YEOMAN et al. *Adv Appl Microbiol* Elsivier [0002]
- MOREIRAFILHO *Appl Microbiol Biotechnol*, 2008, vol. 79, 165- [0002]
- LEVER *Anal. Biochem*, 1972, vol. 47, 248- [0024]
- ALTSCHUL et al. *J. Mol. Biol.*, 1990, vol. 215, 403-410 [0056]
- HENIKOFF et al. *Proc. Natl. Acad. Sci. USA*, 1989, vol. 89, 10915- [0056]
- KARIN et al. *Proc. Natl. Acad. Sci USA*, 1993, vol. 90, 5873- [0056]
- HIGGINS et al. *Gene*, 1988, vol. 73, 237-244 [0056]
- PEARSON et al. *Proc. Natl. Acad. Sci. USA*, 1988, vol. 85, 2444-2448 [0056]
- CHANGCOHEN *Mol. Gen. Genet.*, 1979, vol. 168, 111-115 [0068]
- SMITH et al. *Appl. Env. Microbiol.*, 1986, vol. 51, 634- [0068]
- FERRARI et al. *Harwood, Bacillus* Plenum Publishing Corporation 1989000057-72 [0068]
- SINGLETON SAINSBURY *Dictionary of Microbiology and Molecular Biology* John Wiley and Sons 19940000 [0069]
- HALE MARHAM *The Harper Collins Dictionary of Biology* Harper

Perennial19910000 [0069]

- **TOMME et al.** Eur J Biochem, 1988, vol. 170, 575-581 [0094]
- **GILKES et al.** J Biol Chem, 1988, vol. 263, 10401-10407 [0094]
- Cellulose-binding domains: classification and properties **TOMME et al.** Enzymatic Degradation of Insoluble Polysaccharides American Chemical Society 19950000142-163 [0094]
- **BORASTON et al.** Biochem J, 2004, vol. 382, 769-81 [0094]
- **TANG et al.** Appl. Environ. Microbiol., 2001, vol. 67, 2298-2303 [0149]
- **CIVAS et al.** Biochem. J., 1984, vol. 219, 857-863 [0149]
- **CHRISTGAU et al.** Biochem. Mol. Biol. Int., 1994, vol. 33, 917-925 [0149]
- **SETATI et al.** Protein Express Purif, 2001, vol. 21, 105-114 [0149]
- **PUCHART et al.** Biochimica et biophysica Acta, 2004, vol. 1674, 239-250 [0149]
- **ADEMARK et al.** J. Biotechnol., 1998, vol. 63, 199-210 [0149]
- **REGALADO et al.** J. Sci. Food Agric., 2000, vol. 80, 1343-1350 [0149]
- **CHEN et al.** J. Biotechnol., 2007, vol. 128, 3452-461 [0149]
- **HUANG et al.** Wei Sheng Wu Xue Bao, 2007, vol. 47, 2280-284 [0149]
- **AKINO et al.** Arch. Microbiol., 1989, vol. 152, 10-15 [0149]
- **ARAUJOWARDJ.** Appl. Bacteriol., 1990, vol. 68, 253-261 [0149] [0149]
- **YOSHIDA et al.** Biosci. Biotechnol. Biochem., 1998, vol. 62, 3514-520 [0149]
- **HATADA et al.** Extremophiles, 2005, vol. 9, 497-500 [0149]
- **CHEN et al.** Wei Sheng Wu Xue Bao., 2000, vol. 40, 62-68 [0149]
- **YANHE et al.** Extremophiles, 2004, vol. 8, 447-454 [0149]
- **TALBOTSYGUSCH** Appl. Environ. Microbiol., 1990, vol. 56, 3505-3510 [0149]
- **MENDOZA et al.** World J. Microbiol. Biotechnol., 1994, vol. 10, 51-54 [0149]
- **LI et al.** Z. Naturforsch (C), 2006, vol. 61, 840-846 [0149]
- **CUI et al.** Wei Sheng Wu Xue Bao., 1999, vol. 39, 160-63 [0149]
- **SUN et al.** Sheng Wu Gong Cheng Xue Bao., 2003, vol. 19, 3327-330 [0149]
- **HELOWKHATTAB** Acta Microbiol. Immunol. Hung., 1996, vol. 43, 289-299 [0149]
- **GHERARDINI et al.** J. Bacteriol., 1987, vol. 169, 2038-2043 [0149]
- **MATSUSHITA et al.** J. Bacteriol., 1991, vol. 173, 6919-6926 [0149]
- **SUNNA et al.** Appl. Environ. Microbiol., 2000, vol. 66, 664-670 [0149]
- **MORRIS et al.** Appl. Environ. Microbiol., 1995, vol. 61, 2262-2269 [0149]
- **BICHO et al.** Appl. Microbiol. Biotechnol., 1991, vol. 36, 337-343 [0149]
- **STOLL et al.** Appl. Environ. Microbiol., 1999, vol. 65, 62598-2605 [0149]
- **NAKAJIMAMATSUURA** Biosci. Biotechnol. Biochem., 1997, vol. 61, 1739-1742 [0149]
- **PERRET et al.** Biotechnol. Appl. Biochem., 2004, vol. 40, 255-259 [0149]
- **KATAOKATOKIWA** J. Appl. Microbiol., 1998, vol. 84, 357-367 [0149]
- **HALSTEAD et al.** Microbiol., 1999, vol. 145, 3101-3108 [0149]
- **GIBBS et al.** Curr. Microbiol., 1999, vol. 39, 6351-357 [0149]
- **ZAKARIA et al.** Biosci. Biotechnol. Biochem., 1998, vol. 62, 655-660 [0149]
- **CHARRIERROU** Land J. Expt. Zool., 2001, vol. 290, 125-135 [0149]
- **YAMAMURA et al.** Biosci. Biotechnol. Biochem., 1996, vol. 60, 674-676 [0149]
- **FILICHKIN et al.** Plant Physiol., 2000, vol. 134, 1080-1087 [0149]
- **PASONRATANAKHANOKCHAI** Appl. Environ. Microbiol., 2006, vol. 72, 2483-

2490 [0149]

- HAN **et al.** Appl. Microbiol Biotechnol., 2006, vol. 73, 3618-630 [0149]
- WYMELENBERG **et al.** J. Biotechnol., 2005, vol. 118, 17-34 [0149]
- FANUTTI **et al.** J. Biol. Chem., 1995, vol. 270, 4929314-29322 [0149]
- YAMAMURA **et al.** Biosci. Biotechnol. Biochem., 1993, vol. 7, 1316-1319 [0149]
- BRAITHWAITE **et al.** Biochem J., 1995, vol. 305, 1005-1010 [0149]
- POLITZ **et al.** Appl. Microbiol. Biotechnol., 2000, vol. 53, 6715-721 [0149]
- SACHSLEHNER **et al.** J. Biotechnol., 2000, vol. 80, 127-134 [0149]
- KANSOHNAGIEB Anton. van. Leeuwenhoek., 2004, vol. 85, 103-114 [0149]
- ARCAND **et al.** J. Biochem., 1993, vol. 290, 857-863 [0149]
- CANN **et al.** J. Bacteriol., 1999, vol. 181, 1643-1651 [0149]
- HILGE **et al.** Structure, 1998, vol. 6, 1433-1444 [0149]
- PARKER **et al.** Biotechnol. Bioeng., 2001, vol. 75, 3322-333 [0149]
- DUFFAUD **et al.** Appl. Environ. Microbiol., 1997, vol. 63, 169-177 [0149]
- FRANCO **et al.** Biotechnol Appl. Biochem., 2004, vol. 40, 255-259 [0149]
- STALBRAND **et al.** J. Biotechnol., 1993, vol. 29, 229-242 [0149]
- TAMARU **et al.** J. Ferment. Bioeng., 1997, vol. 83, 201-205 [0149]
- World J. Microbiol. Microbiotechnol., 1992, vol. 8, 2115-120 [0156]
- Agr. Biol. Chem., 1972, vol. 36, 2285-93 [0156]
- VINCKEN **et al.** Plant Physiol., 1994, vol. 104, 99-107 [0158]
- HAYASHI **et al.** Plant. Physiol. Plant Mol. Biol., 1989, vol. 40, 139-168 [0158]
- DARTOIS **et al.** Biochem. Biophys. Acta, 1993, vol. 1131, 253-260 [0161]
- YAMAGUCHI **et al.** Gene, 1991, vol. 103, 61-67 [0161]
- SCHIMADA **et al.** J. Biochem., 1989, vol. 106, 383-388 [0161]
- HASS **et al.** Gene, 1991, vol. 109, 117-113 [0161]
- KUGIMIYA **et al.** Biosci. Biotech. Biochem., 1992, vol. 56, 716-719 [0161]
- VOGTENTANZ Protein Expr Purif, 2007, vol. 55, 40-52 [0221]
- LEVERAnal Biochem, 1972, vol. 47, 248- [0263]
- ALTSCHUL **et al.** Nucleic Acids Res, 1997, vol. 25, 3389-402 [0269]
- THOMPSON **et al.** Nucleic Acids Research, 1994, vol. 22, 4673-4680 [0270]
- SAITOUNEIMol Biol Evol, 1987, vol. 4, 406-425 [0271]
- AKITA **et al.** Acta Cryst., 2004, vol. 60, 1490-1492 [0275]

## PAENIBACILLUS- OG BACILLUS SPP. MANNANASER

## PATENTKRAV

1. Rengøringssammensætning, der omfatter et overfladeaktivt middel og et endo- $\beta$ -mannanasepolypeptid, der omfatter en aminosyresekvens med mindst 90 % identitet med en aminosyresekvens, der er udvalgt fra SEQ ID NO: 8, 10, 12, 42, 43, 44, 46, 47, 48, 50, 51 og 52, eller et aktivt fragment deraf, hvor polypeptidet eller det aktive fragment deraf har mannanaseaktivitet i tilstedevarsel af et overfladeaktivt middel.
2. Rengøringssammensætning ifølge krav 1, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf indeholder Asn33-Asp-34-Leu35, hvor polypeptidets aminosyrepositioner er nummereret i overensstemmelse med aminosekvensen ifølge SEQ ID NO: 32 og er baseret på den bevarede lineære sekvensnummerering.
3. Rengøringssammensætning ifølge krav 1 eller krav 2, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf endvidere omfatter et  $WX_aKNDLXXAI$ -mønster ved positionerne 30-38, hvor  $X_a$  er F eller Y og X er en hvilken som helst aminosyre, hvor polypeptidets aminosyrepositioner er nummereret i overensstemmelse med aminosekvensen ifølge SEQ ID NO: 32 og er baseret på den bevarede lineære sekvensnummerering.
4. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf endvidere omfatter et  $WX_aKNDLX_bX_cAI$ -mønster ved positionerne 30-38, hvor  $X_a$  er F eller Y,  $X_b$  er N, Y eller A, og  $X_c$  er A eller T, hvor polypeptidets aminosyrepositioner er nummereret i overensstemmelse med aminosekvensen ifølge SEQ ID NO: 32 og er baseret på den bevarede lineære sekvensnummerering.
5. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf endvidere omfatter et  $L_{262}D_{263}XXXGPXGXL_{272}T_{273}$ -mønster ved positionerne 262-273, hvor X er en hvilken som helst aminosyre, og hvor polypeptidets aminosyrepositioner er nummereret i overensstemmelse med aminosekvensen ifølge SEQ ID NO: 32 og er baseret på den bevarede lineære sekvensnummerering.
6. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf endvidere omfatter et  $L_{262}D_{263}M/LV/AT/AGPX_1GX_2L_{272}T_{273}$ -mønster ved positionerne 262-273, hvor  $X_1$  er N, A eller S og  $X_2$  er S, T eller N, og hvor polypeptidets aminosyrepositioner er nummereret i overensstemmelse med aminosekvensen ifølge SEQ ID NO: 32 og er baseret på den bevarede lineære sekvensnummerering.
7. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet er et NDL-Clade-1-polypeptid, der endvidere omfatter et LDM/LATGPA/NGS/TLT-mønster ved positionerne 262-273, hvor polypeptidets aminosyrepositioner er nummereret i overensstemmelse med aminosekvensen ifølge SEQ ID NO: 32 og er baseret på den bevarede lineære sekvensnummerering.
8. Rengøringssammensætning ifølge et hvilket som helst af krav 1 til 6, hvor endo- $\beta$ -mannanasepolypeptidet er et NDL-Clade-2-polypeptid, der endvidere omfatter et LDLA/VA/TGPS/NGNLT-mønster ved positionerne 262-273, hvor polypeptidets aminosyrepositioner er

nummereret i overensstemmelse med aminosekvensen ifølge SEQ ID NO:32 og er baseret på den bevarede lineære sekvensnummerering.

9. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf har mannanaseaktivitet på johannesbrødkernemel-galactomannan eller konjacglucomannan.

10. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf bevarer mindst 70 % af dets maksimale mannanaseaktivitet ved et pH-interval fra 4,5-9,0; bevarer mindst 70 % af dets maksimale mannanaseaktivitet ved et pH-interval fra 5,5-8,5; bevarer mindst 70 % af dets maksimale mannanaseaktivitet ved et pH-interval fra 6,0-7,5; bevarer mindst 70 % af dets maksimale mannanaseaktivitet ved et temperaturinterval fra 40 °C til 70 °C; bevarer mindst 70 % af dets maksimale mannanaseaktivitet ved et temperaturinterval fra 45 °C til 65 °C; og/eller bevarer mindst 70 % af dets maksimale mannanaseaktivitet ved et temperaturinterval fra 50 °C til 60 °C.

11. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf er i stand til at hydrolyser et substrat, der er udvalgt fra gruppen bestående af guargummi, johannesbrødkernemel og kombinationer deraf.

12. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor endo- $\beta$ -mannanasepolypeptidet eller det aktive fragment deraf endvidere ikke omfatter et carbohydrate-bindingsmodul.

13. Rengøringssammensætning ifølge et hvilket som helst foregående krav, der endvidere omfatter; mindst én hjælpemiddelbestanddel; og/eller et enzym, der er udvalgt fra gruppen bestående af acyltransferaser, amylaser, alpha-amylaser, beta-amylaser, alpha-galactosidaser, arabinaser, arabinosidaser, arylesteraser, beta-galactosidaser, beta-glucanaser, carrageenaser, catalaser, cellobiohydrolaser, cellulaser, chondroitinaser, cutinaser, endo-beta-1,4-glucanaser, endo-beta-mannanaser, exo-beta-mannanaser, esteraser, exo-mannanaser, galactanaser, glucoamylaser, hemicellulaser, hyaluronidaser, keratinaser, laccaser, lactaser, ligninaser, lipaser, lipolytiske enzymer, lipoxygenaser, mannanaser, oxidaser, pectatlyaser, pectinacetyl esteraser, pectinaser, pentosanaser, perhydrolaser, peroxidaser, phenoloxidaser, phosphataser, phospholipaser, phytaser, polygalacturonaser, proteaser, pullulanaser, reduktaser, rhamnogalacturonaser, beta-glucanaser, tannaser, transglutaminaser, xylanacetyl-esteraser, xylanaser, xyloglucanaser, xylosidaser, metalloproteaser og kombinationer deraf.

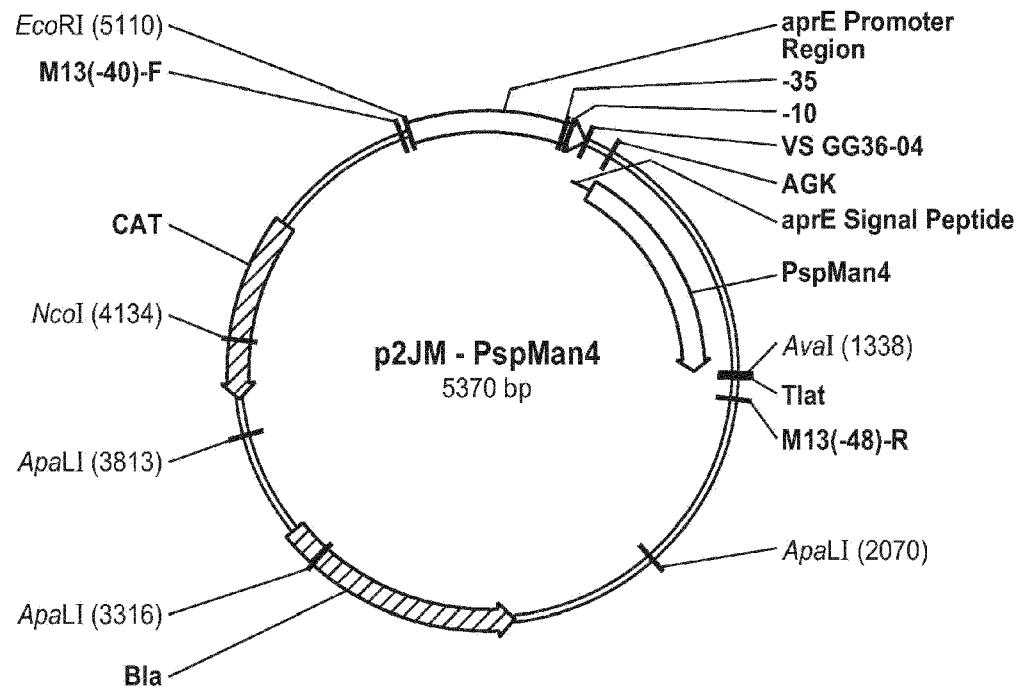
14. Rengøringssammensætning ifølge et hvilket som helst foregående krav, hvor rengøringssammensætningen:

- (i) er en vaskemiddelsammensætning, der er udvalgt fra gruppen bestående af et tojvaskemiddel, et stofblodgøringsmiddel, et maskinopvaskemiddel og et vaskemiddel til hårde overflader;
- (ii) er i en form, der er udvalgt fra gruppen bestående af en væske, et pulver, et granuleret faststof, en tablet, et lag og en enhedsdosis;
- (iii) er phosphat-fri; eller
- (iv) er bor-fri.

- 3 -

15. Fremgangsmåde til tekstilrengøring, der omfatter at bringe et tilsmudset tekstil i kontakt med rengøringssammensætningen ifølge et hvilket som helst af de foregående krav med henblik på fremstilling af et rent tekstil.

# DRAWINGS



**FIG. 1**

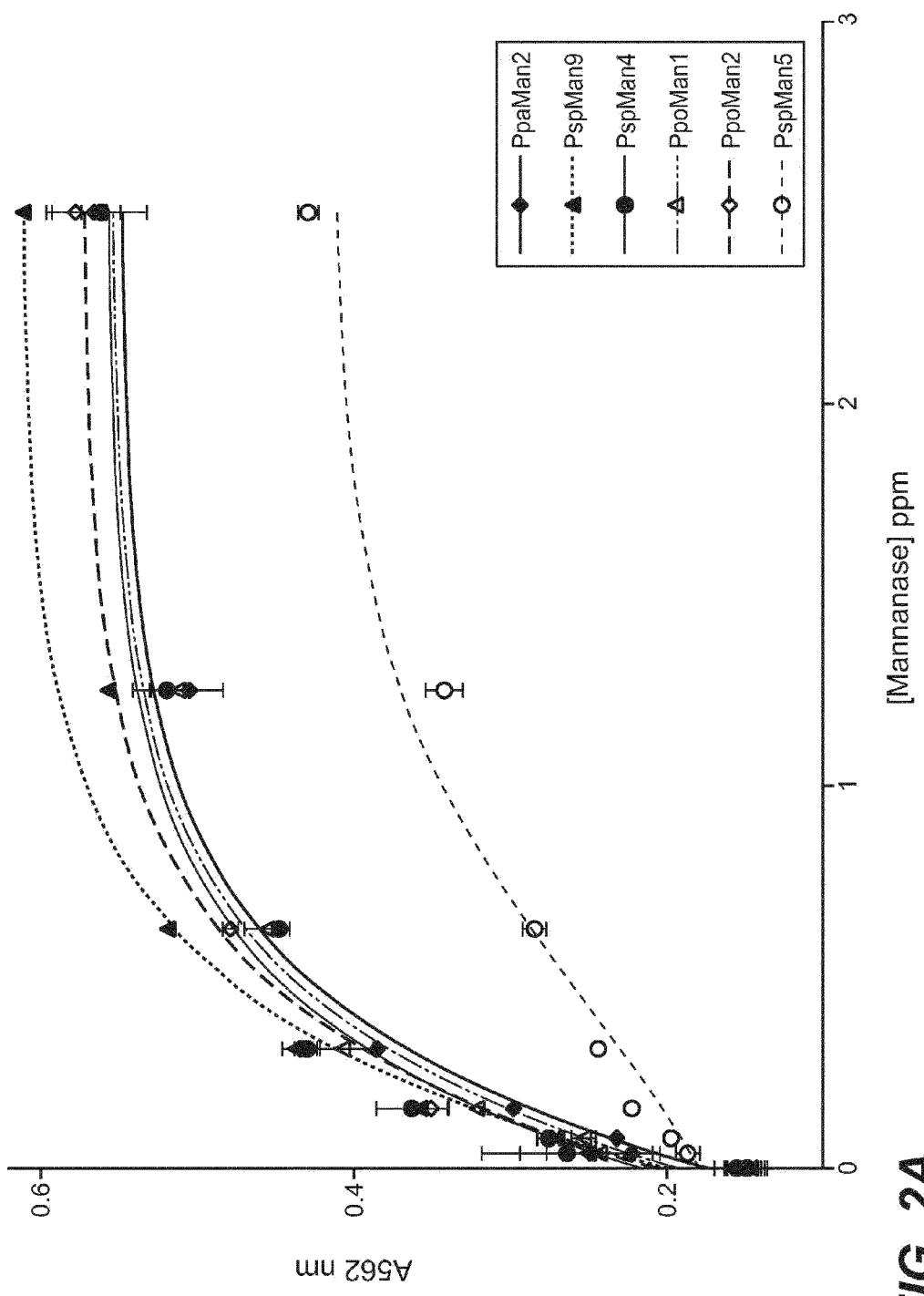


FIG. 2A

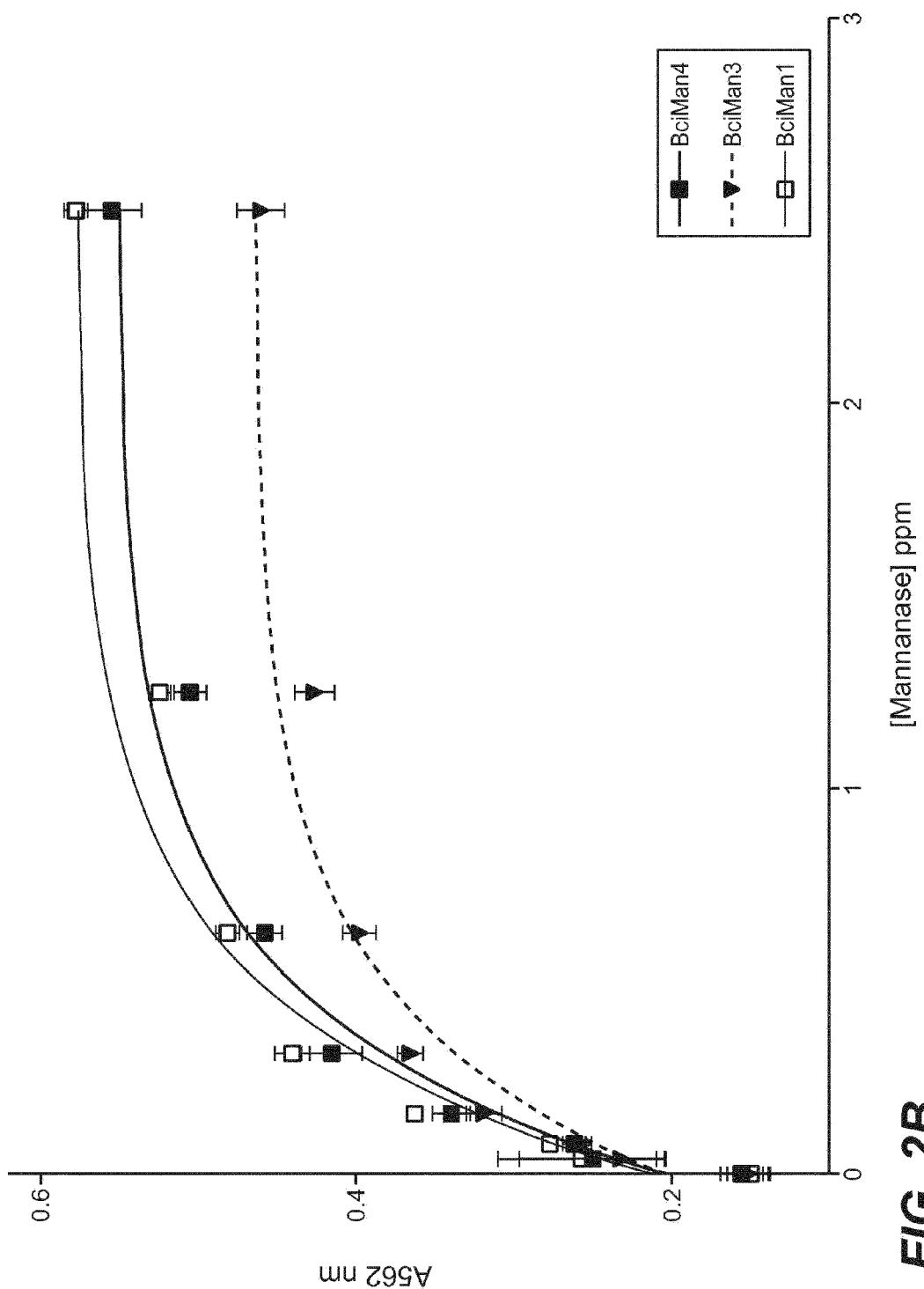


FIG. 2B

		50
1		
PamMan2	(1)	-A-G-G-Y-V-S-G-N-K-L-Y-D-S-T-G-K-F-V-M-R-G-V-N-E-G-H-S-W-F-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T
PtuMan2	(1)	-A-G-G-Y-V-S-G-G-K-L-Y-D-S-T-G-K-A-F-V-M-R-G-V-N-E-G-H-S-W-F-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T
RpaMan2	(1)	-A-A-G-G-Y-V-S-G-N-K-L-Y-D-S-T-G-K-A-F-V-M-R-G-V-N-E-S-H-T-W-F-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T
PspMan9	(1)	-A-A-G-G-Y-V-S-G-T-K-L-Y-D-S-T-G-K-P-F-V-M-R-G-V-N-E-S-H-T-W-F-K-N-D-I-N-A-I-P-A-I-A-K-T-G-A-N-T
PspMan4_Pae_sp_ACU30843.1	(1)	M-A-G-G-Y-V-S-G-N-K-L-Y-D-S-T-G-K-P-F-V-M-R-G-V-N-E-G-H-S-W-F-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T
PspMan5_Pae_sp_CH-3_AEX60762.1	(1)	-A-A-G-G-Y-V-S-G-T-L-Y-D-S-T-G-K-P-F-V-M-R-G-V-N-E-S-H-T-W-F-K-N-D-I-N-A-I-P-A-I-A-K-T-G-A-N-T
Pae_sp_PAMC26794_WP_017688745.1	(1)	-A-A-G-G-Y-V-S-G-N-K-L-Y-D-S-T-G-K-A-F-V-M-R-G-V-N-E-G-H-S-W-F-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T
BciMan4_B_circulans_AAX87003.1	(1)	-A-A-G-G-Y-V-N-G-G-K-L-Y-D-S-T-G-K-P-F-Y-M-R-G-I-N-E-G-H-S-W-F-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T
Pae_sp_A9_WP_017813111.1	(1)	-A-A-G-G-Y-V-S-G-T-K-L-Y-D-S-T-G-K-P-F-A-M-R-G-I-N-E-H-T-W-Y-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T
BciMan3_B_circulans_AAX87002.1	(1)	-A-A-G-G-Y-V-N-G-T-K-L-Y-D-S-T-G-K-A-F-V-M-R-G-V-N-E-P-H-T-W-Y-K-N-D-I-N-A-I-P-A-I-A-Q-T-G-A-N-T
PpoMan1_P_polymyxaE681_WP_003868989.1	(1)	-A-S-G-G-Y-V-S-G-T-K-L-Y-D-S-T-G-K-P-F-V-M-R-G-V-N-E-H-T-W-Y-K-N-D-I-Y-T-A-I-P-A-I-A-Q-T-G-A-N-T
Pae_sp_HGF5_WP_009593769.1	(1)	-A-A-G-G-Y-V-N-G-T-K-L-Y-D-S-T-G-K-A-F-V-M-R-G-V-N-E-P-H-T-W-Y-K-N-D-I-N-A-I-P-A-I-A-Q-T-G-A-N-T
Pae_sp_ICGEE2008_WP_017427981.1	(1)	-A-S-G-G-Y-V-S-G-T-K-L-Y-D-S-T-G-N-P-F-V-M-R-G-V-N-E-H-T-W-Y-K-N-D-I-Y-T-A-I-P-A-I-A-K-T-G-A-N-T
PpoMan2_P_polymyxa_SC2_WP_003944884.1	(1)	-A-S-G-G-Y-V-S-G-T-N-L-Y-D-S-T-G-K-P-F-V-M-R-G-V-N-E-H-T-W-Y-K-N-D-I-Y-T-A-I-P-A-I-A-K-T-G-A-N-T
Pae_sp_HW567_WP_019912481.1	(1)	-V-R-G-G-Y-V-S-G-T-K-L-Y-D-A-T-G-S-P-F-V-M-R-G-V-N-E-H-A-T-T-W-Y-K-N-D-I-A-T-A-I-P-A-I-A-T-G-S-N-T
P_muclaginosusk02_WP_006190599.1	(1)	-A-A-G-M-Y-V-S-G-T-V-Y-D-A-N-G-K-P-F-V-M-R-G-I-N-E-P-H-A-W-Y-K-N-D-I-A-T-A-I-P-A-I-A-T-G-A-N-S
Bciman1_B_circulans_BAA25878.1	(1)	-A-S-G-G-Y-V-S-G-T-K-L-L-D-A-T-Q-Q-F-F-V-M-R-G-V-N-E-H-A-T-T-W-Y-K-D-O-L-S-T-A-I-P-A-I-A-K-T-G-A-N-T
B_nealsonii_AGU71466.1	(1)	-A-S-G-G-Y-V-S-G-T-T-L-Y-D-A-T-G-K-F-F-T-M-R-G-V-N-E-H-A-S-W-F-K-E-D-S-A-A-I-P-A-I-A-T-G-A-N-T
B_sp_JAMB-602_BAD99527.1	(1)	-N-S-G-G-Y-V-S-G-T-T-L-Y-D-A-N-G-N-P-F-F-V-M-R-G-I-N-E-G-H-A-W-Y-K-D-O-A-T-T-A-I-E-G-I-A-N-T-G-A-N-T
Consensus	(1)	A-A-G-G-Y-V-S-G-T-K-L-Y-D-S-T-G-K-P-F-V-M-R-G-V-N-E-H-A-T-T-W-Y-K-N-D-I-N-T-A-I-P-A-I-A-K-T-G-A-N-T

FIG. 3A-1

		100
PamMan2	(50)	VR=VL SNG SLY TKE DLN AVKN IIN VV N QNK MIA VLE VHD A= G K D D Y N S D
PtuMan2	(50)	VR=VL SNG V Q Y T K C D L N S V K N I I N V V S V N K M I A V L E V H D A= G K D D Y N S D
PpaMan2	(50)	VR=VL SNG T Q Y T K E D L N A V K N I I N L V S O N K M I A V L E V H D A= G K D D Y N S D
PspMan9	(50)	VR=VL SNG V Q Y T R E D V N S V K N I I S L V N Q N K M I A V L E V H D A= G K D D Y A S S D
PspMan4_Pae. spA1_ACU30843.1	(51)	VR=VL SNG SLY T K E D L N A V K N I I N V V N Q N K M I A V L E V H D A= G K D D Y N S D
PspMan5_Pae. sp_CH-3_AEX60762.1	(50)	VR=VL SNG V Q Y T R E D V N S V K N I I S L V N Q N K M I A V L E V H D A= G K D D Y A S S D
Pae. sp_PAMCC6794_WP_017688745.1	(50)	VR=VL SNG S Q W T K E D L N A V K N I I N V V N Q N K M I A V L E V H D A= G K D D Y N S D
BciMan4_B_circulans_AAX87003.1	(50)	VR=VL SNG T Q Y T K E D L N S V K N I I N V V N A N K M I A V L E V H D A= G K D D F N S D
PpoMan1_P_polymyxaE661_WP_003868989.1	(50)	VR=VL SNG M Q Y T K E D V N S V K N I I S L V N Q N K M I A V L E V H D A= G K D D Y N S D
BciMan3_B_circulans_AAX87002.1	(50)	VR=VL SNG S Q W T K E D L N S V N S I I S L V S Q H Q M I A V L E V H D A= G K D D Y A S S E
Pae. sp_A9_WP_017813111.1	(50)	VR=VL SNG N Q Y T K E D L N S V K N I I S L V S N Y K M I A V L E V H D A= G K D D Y A S S D
Pae. sp_HGF5_WP_009593769.1	(50)	VR=VL SNG S Q W K I K D D I N V A N S I I S L V S Q H Q M I A V L E V H D A= G K D D D A S S E
Pae. sp_ICGEB2008_WP_017427981.1	(50)	VR=VL SNG T Q Y T K E D D I N S V K N I I S L V T S Y K M I P V L E V H D A= G K D D Y A S S D
PpoMan2_P_polymyxa_SC2_WP_003944884.1	(50)	VR=VL SNG N Q Y T K E D I N S V K N I I S L V S N H K M I A V L E V H D A= G K D D Y A S S D
Pae. sp_HW567_WP_019912481.1	(50)	IR=VL SNG S Q W S K D S L A S I G N I I A L C D Q Y K I T A M L E V H D A= G S D N A S D N
P_muclaginosusk02_WP_006190599.1	(50)	VR=VL SNG S Q W S K D S L A S I G N I I A L C Q Y R M I A I L E V H D A= G S D S Y T A D D
BciMan1_B_circulans_BAA25878.1	(50)	IR=VL A N G H K W T L C D V N N I I L C E Q N K I A V L E V H D A= G S D S L S D D
B_nealsonii_AGU71466.1	(50)	VR=VL S D G G Q Y T K E D I N T V K S I I S L A E K I N I H S G V M T H R K - - D D V E S S N
B_sp_JAMB-602_BAD99527.1	(50)	VR=VL S D G G Q W T K E D I Q T V R N I I S L A E D N N I V A V L E V H D A= G Y D S I A S S N
Consensus	(51)	VR=VL S NG S Q Y T K E D L N S V K N I I S L V Q N K M I A V L E V H D A= G K D D Y A S S D

FIG. 3A-2

150

10-

PamMan2 (100) A V N Y W I S I K E A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K K A I F K L R N  
 PtuMan2 (100) A V N Y W I S I K E A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K K A I F K L R N  
 PpaMan2 (100) A V N Y W I S I K E A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K K A I F K L R N  
 PspMan9 (100) A A V N Y W I S I K D A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K Q A I F K L R N  
 PspMan4\_Pae\_spA1\_ACU30843.1 (101) A V N Y W I S I K E A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K K A I F K L R N  
 PspMan5\_Pae\_sp\_CH-3\_AEX60762.1 (100) A V N Y W I S I K D A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K Q A I F K L R N  
 Pae\_sp\_PAMC26794\_WP\_017688745.1 (100) A V N Y W I S I K E A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K K A I F K L R N  
 BciMan4\_B\_circulans\_AAX87003.1 (100) A V N Y W I S I K E A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K K A I F K L R D  
 Pae\_sp\_A9\_WP\_017813111.1 (100) A V N Y W I S I K D A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K Q A I F K L R N  
 BciMan3\_B\_circulans\_AAX87002.1 (100) A V D Y W I S I K G A L T G K E D R V I V N = A N E W Y G N N S S G W A D G Y K Q A I F K L R N  
 PpoMan1\_P\_polymyxaE681\_WP\_003868989.1 (100) A V V Y W I S I K D A L T G K E D R V I V N = A N E W Y G S W A D G Y K Q A I F K L R N  
 Pae\_sp\_HGF5\_WP\_009593769.1 (100) A V D Y W I G I K E A L T G K E D R V I V N = A N E W Y G N N S S G W A E G Y K Q A I F K L R N  
 Pae\_sp\_ICGEE2008\_WP\_017427981.1 (100) A V V Y W I S I K D A L T G K E D R V I V N = A N E W Y G S W A D G Y K Q A I F K L R N  
 PpoMan2\_P\_polymyxa\_SC2\_WP\_003944884.1 (100) A V V Y W I S I K D A L T G K E D R V I V N = A N E W Y G S W A D G Y K Q A I F K L R N  
 Pae\_sp\_HW567\_WP\_019912481.1 (100) A V V Y W I S I K D A L T G K E D R V I V N = A N E W F G S W G T A S W A S A V Q S A I F A R A  
 P\_muclaginosusk02\_WP\_006190599.1 (100) N A V V Y W I E M K S A L T G K E R T V I V N = A N E W Y G T D A S G W A N G Y K Q A I F K L R S  
 Bciman1\_B\_circulans\_BAA25878.1 (100) N A V V Y W I C I K S A L T G K E D R V I V N = A N E W Y G T W D G V A W A N G Y K Q A I F K L R N  
 B\_nealsonii\_AGU71466.1 (97) R A V D Y W I S I K D T L T G K E D R V I V N = A N E W Y G T W D G A A W A A G Y K Q A I F K L R N  
 B\_sp\_JAMB-602\_BAD99527.1 (100) R A V D Y W I E M R S A L T G K E D R V I V N = A N E W F G S W D G A A W A D G Y K Q A I F K L R N  
 Consensus (101) A V N Y W I S I K D A L T G K E D R V I V N = A N E W Y G T W N G S A W A D G Y K Q A I F K L R N


**FIG. 3B-1**

200  
150  
PamMan2 (150) AG=KNTL=VDAAGWGQFPQSIIVDYGQCSVFATDSQKNTVFS=HMYEYAGKD  
PtuMan2 (150) AG=KNTL=VDAAGWGQYPQSIIVDYGQCSVFAADDSQKNTVFS=HMYEYAGKD  
PpaMan2 (150) AG=KNTL=VDAAGWGQYPQSIIVDYGQCSVFAADDAQKNTVFS=HMYEYAGKD  
PspMan9 (150) AG=KNTL=VDAAGWGQYPQSIIVDYGQCSVFAADDSLKNNTVFS=HMYEYAGGT  
PspMan4\_Pae\_sp\_CH-3\_AEX60762.1 (151) AG=KNTL=VDAAGWGQFPQSIIVDYGQCSVFAADDSQKNTVFS=HMYEYAGKD  
Pae\_sp\_WP\_017813111.1 (150) AG=KNTL=VDAAGWGQCPQSIIVDYGQCSVFAADDSLKNNTVFS=HMYEYAGGT  
BciMan3\_B\_circulans\_AAX87002.1 (150) AG=KNTL=VDAAGWGQYPQSIIVDYGQCSVFAADDSQKNTVFS=HMYEYAGKD  
BciMan4\_B\_circulans\_AAX87003.1 (150) AG=KNTL=VDAAGWGQYPQSIIVDYGQCSVFAADDSQKNTAFAFS=HMYEYAGKD  
Pae\_sp\_A9\_WP\_017688745.1 (150) AG=KNTL=VDAAGWGQYPQSIIVDYGQCSVFAADDSQKNTVFS=HMYEYAGKD  
BciMan1\_P\_polymyxaE681\_WP\_0038688989.1 (150) AG=KNTL=VDCAGWGQYPQSIINDFGKSVFAADDSLKNNTVFS=HMYEYAGKD  
Pae\_sp\_HGF5\_WP\_009593769.1 (150) AG=KNTL=VDAAGWGQYPQSIIVDEGAFAVFS=DQLKNNTVFS=HMYEYAGKD  
Pae\_sp\_JCGB2008\_WP\_017427981.1 (150) AG=KNTL=VDCAGWGQYPQSIINDFGKSVFAADDSLKNNTVFS=HMYEYAGKD  
PpoMan2\_P\_polymyxa\_SC2\_WP\_003944884.1 (150) AG=KNTL=VDCAGWGQYPQSIINDFGKSVFAADDSLKNNTVFS=HMYEYAGKD  
Pae\_sp\_HW567\_WP\_019912481.1 (150) AG=KNTLIVVDAAGWGQYPTSIFTSGNAVENSDFLRNTNTIFS=HMYEYAGGT  
P\_muclaginosusk02\_WP\_006190599.1 (150) AGLDHDLVDAAGWGQYPASIIHTMGRKEVLAADPRKNTMFS=HMYEYAGGT  
Bciman1\_B\_circulans\_BAA25878.1 (150) AGLHTHLEVDSAGWGQYPDSVKNYC3EVLNADPLKNNTVFS=HMYEYAGGN  
B\_nealsonii\_AGU71466.1 (147) AGLNHTL=IDSAGWGQYPASIIHNYGKEVNAADPLKNNTMFS=HMYEYAGGD  
B\_sp\_JAMB-602\_BAD99527.1 (150) AGLNNTLYIDAAGWGQFPQSIIDYGREVENADPQRNTMFS=HMYEYAGGN  
Consensus (151) AG=KNTL=VDAAGWGQYPQSIIVDYGQCSVFAADDSLKNNTVFS=HMYEYAGKD

FIG. 3B-2

		250
PamMan2	(200)	A = V X A N Y E N V L N K G L A L I I G E F S G Y I T N G D V D E V A I M R Y G Q E K G V G W = A
PtuMan2	(200)	A = V X A N Y E S V L N K G L A L I I G E F S G Y I T N G D V D E V A I M K Y G Q E K G V G W = A
PpaMan2	(200)	A = V X A N Y E N V L N K G L A L I I G E F S G Y I T N G D V D E V A I M K Y G Q E K G V G W = A
PspMan9	(200)	D A V V X A N Y E G V L N K G L P L I I G E F S G Q I T N G D V D E L A I M R Y G Q Q K G V G W = A
PspMan5_Pae_sp_CH-3_AEX60762.1	(200)	D A = V X S N Y E N V L N K G L P L I I G E F S G Q I T N G D V D E H A I M R Y G Q Q K G V G W = A
Pae_sp_PAMC26794_WP_017688745.1	(200)	A = V X A N Y E N V L N K G L A L I I G E F S G Y I T N G D V D E V A I M R Y G Q E K G V G W = A
BciMan4_B_circulans_AAX87003.1	(200)	A = V X S N Y E N V L N K G L A L I I G E F S G Y I T N G D V D E V A I M K Y G L E K G V G W = A
Pae_sp_A9_WP_017813111.1	(200)	A = V X A N = D G V L N K G L P L I I G E F S G Y I T N G D V D E V A I M R Y G Q E K G T I G W = A
BciMan3_B_circulans_AAX87002.1	(200)	A = V X T N Y = D V L N K G L P L I I G E F S G Y I Q G A D V D E V A I M K Y G Q Q K E V G W = A
PpoMan1_P_polymyxaE681_WP_003868989.1	(200)	A Q = V X T N = D N V L N Q G I P L I I G E F S G Y I Q G A D V D E E I M R Y G Q S K G V G W = A
Pae_sp_HGF5_WP_009593769.1	(200)	A A = V X T N Y = D V L N K G L P L I I G E F S G Y I Q G A D V D E V A I M K Y G Q Q K E V G W = A
Pae_sp_ICGEE2008_WP_017427981.1	(200)	V Q = V X T N = D N V L N Q G I P L I I G E F S G Y I Q G A D V D E E I M R Y G Q S K G I G W = A
PpoMan2_P_polymyxa_SC2_WP_003944884.1	(200)	V Q = V X T N = D N V L Y Q G L P L I I G E F S G Y I Q G A D V D E E I M R Y G Q S K S V G W = A
Pae_sp_HW567_WP_019912481.1	(200)	A = V X S N = D N A L A = G V P V I V G E F S K R I T G = D V D E A I M S Y S Q E V D E A I M S Y S Q Q K G V G W = A
P_muclaginosusk02_WP_006190599.1	(200)	A D Q V X S N = D G V L N Q G L A V V V G E F S P K H S N G E V D E A I M S Y S Q Q K G V G W = V
Bciman1_B_circulans_BAA25878.1	(200)	A S = V X S N = D G V L N K N L A L I I G E F S G C H T N G D V D E A I M S Y S Q Q K G V G W = A
B_nealsonii_AGU71466.1	(197)	A A = V X S N = D G V L N Q G G L A L I I G E F S Q K H T N G D V D E A I M S Y S Q Q K N I G W = A
B_sp_JAMB-602_BAD99527.1	(200)	A S Q V X T N = D R V L N Q D L A L V I G E F S H R H T N G D V D E S I I M S Y S E Q R G V G W = A
Consensus	(201)	A A = V X A N Y E N V L N K G L A L I I G E F S G Y I T N G D V D E A I M R Y G Q K G V G W = A

FIG. 3C-1

251 (250) WSWYGNSSGLNYLDMAZG2NGS-1-SEGK2VNVNDTYSIIXTISQXAG-E- SEQ ID NO:17  
 PtuMan2 (250) WSWYGNSSDLNYLDLADG2NGS-1-SEGK2VNDTYSIIXNTSKKAG-E- SEQ ID NO:24  
 PpoMan2 (250) WSWYGNSSDLNYLDLADG2NGT-1-SEGK2VVDTYSIIXNTSKKAG-E- SEQ ID NO:40  
 PspMan9 (250) WSWYGNNSDSYLSDYLSDLADG2NGS-1-SEGK2VNDTNSIKATISKKAG-E-FQ- SEQ ID NO:60  
 PspMan4\_Pae\_sp1\_ACU35843.1 (251) WSWYGNSSGLNYLDMAZG2NGS-1-SEGK2VNDTYSIIXNTSKKAG-E- SEQ ID NO:52  
 PspMan5\_Pae\_sp\_CH-3\_AEX6762.1 (250) WSWYGNNSELSYLDLADG2AGS-1-SEGK2VNDTYSIIXNTSKKAG-E- SEQ ID NO:56  
 Pae\_sp\_PAMC26794\_WP\_017688745.1 (250) WSWYGNSSGLNYLDMAZG2NGS-1-SEGK2VNDTYSIIXNTSKKAG-E- SEQ ID NO:69  
 BciMan4\_B\_circulans\_AAX87003.1 (250) WSWYGNSSGLNYLDLADG2NGS-1-SEGK2VNDTYSIIXNTSKKAG-E- SEQ ID NO:36  
 Pae\_sp\_A9\_WP\_017813111.1 (250) WSWYGNSTNLYLDLADG2NGS-1-SEGK2VNDTSSIKATISKKAG-E- SEQ ID NO:71  
 BciMar3\_B\_circulans\_AAX87002.1 (250) WSWYGNSEELNDLADG2SGN-1-SEGK2VNDTQIQTISKKAG-E- SEQ ID NO:32  
 PpoMan1\_P\_polymyxaE681\_WP\_WP\_00386989.1 (250) WSWYGNSSNLSDLDLADG2NGN-1-SEGK2VNDTWSKAG-E- SEQ ID NO:44  
 Pae\_sp\_HGF5\_WP\_WP\_009593769.1 (250) WSWYGNSEELNDLADG2SGN-1-SEGK2VNDTWSKAG-E- SEQ ID NO:73  
 Pae\_sp\_ICGEB2008\_WP\_WP\_017427981.1 (250) WSWYGNSSNLSDLDLADG2SGN-1-SEGK2VNDTWSKAG-E- SEQ ID NO:72  
 PpoMan2\_P\_polymyxa\_SC2\_WP\_WP\_003944884.1 (250) WSWYGNSSNLNDLADG2PGN-1-SEGK2VNDTWSKAG-E- SEQ ID NO:48  
 Pae\_sp\_HW567\_WP\_WP\_019972481.1 (250) WSWYGNSSGVEYLDLADG2SGN-1-SEGK2VNDTWSKAG-E- SEQ ID NO:74  
 P\_mucilaginosusK02\_WP\_WP\_00619599.1 (250) WSWYGNSSDLNYLDLADG2SGS-1-SEGK2VNDTWSKAG-E- SEQ ID NO:81  
 BciMan1\_B\_circulans\_BAA25878.1 (250) WSWKGNSSDLAYLDMINDWAGNS12SEGK2VNDTWSKAG-E- SEQ ID NO:28  
 B\_neasoriensis\_AGU71466.1 (247) WSWKGMNSTDWSYLDLSDWAGNS12WGK2VNDTWSKAG-E- SEQ ID NO:76  
 B\_sp\_JAMB-6022\_BAD9527.1 (250) WSWKGNGEEW3YLDLSDWAGN12WGK2VNDTWSKAG-E- SEQ ID NO:77  
 Consensus (251) WSWYGNSSDLNYLDLADG2NGS-1-SEGK2VNDTWSKAG-E- SEQ ID NO:82

300  
**FIG. 3C-2**

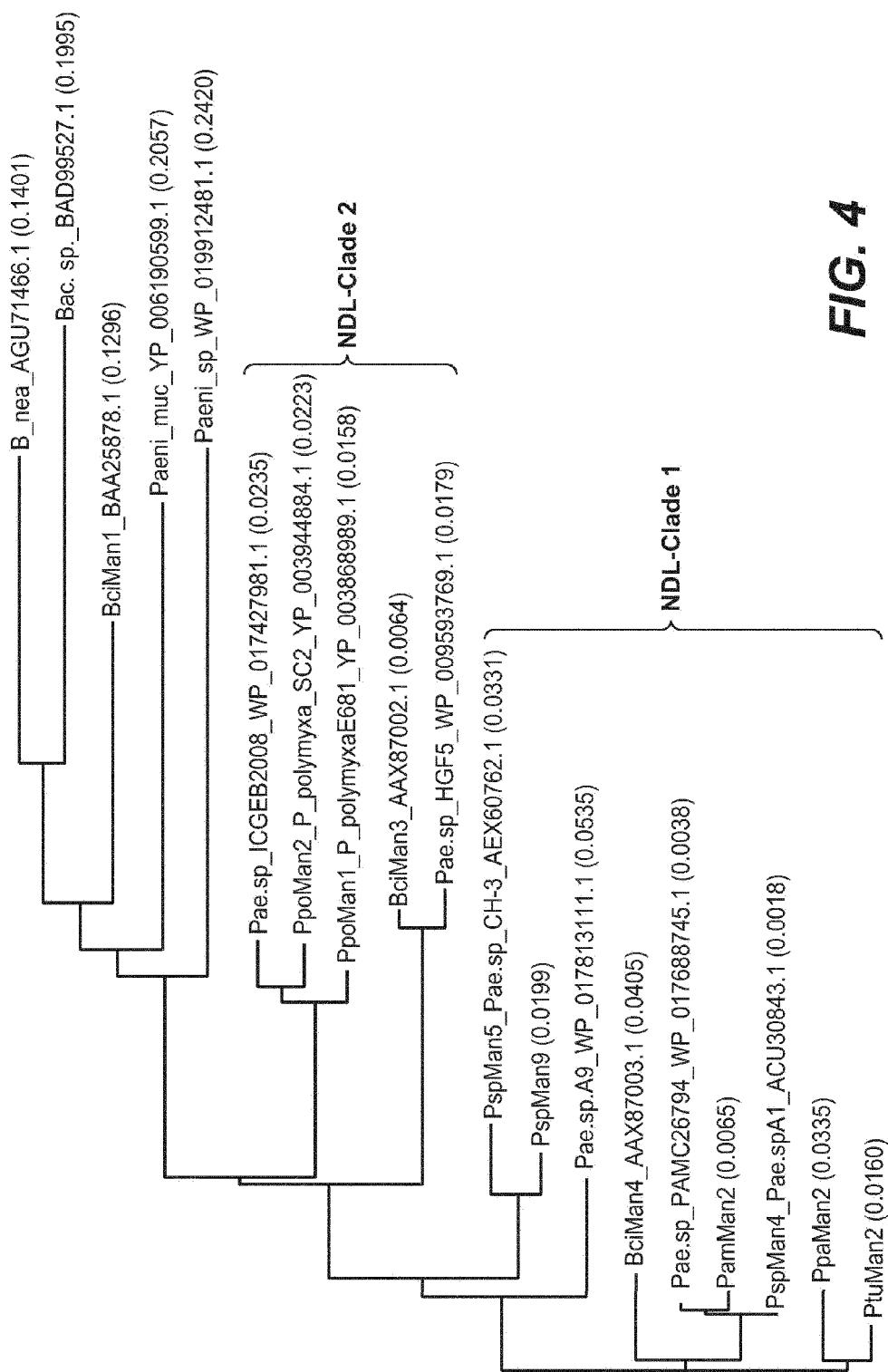


FIG. 4

PamMan2	(1)	-ATGFFYVSSGNYLVDSTGKATVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:83
PtuMan2	(1)	-ATGFFYVSSGGRYLVDSTGKATVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:84
PpaMan2	(1)	-AGFFYVSSGNYLVDSTGKATVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:85
PspMan9	(1)	-ATGFFYVSSTRLYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:86
PspMan4_Pae. spa1_ACU30643.1	(1)	MAIGFFYVSSGNYLVDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:87
PspMan5_Pae. sp. CH-3_AEX60762.1	(1)	-ATGFFYVSSGTYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:88
Pae. sp. PAMC26794_WP_017688745.1	(1)	-ATGFFYVSSGNYLVDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:89
BciMan4_B_circulans_AAX87003.1	(1)	-ATGFFYVSSGKGRLVDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:90
Pae. sp. A9_WP_017813111.1	(1)	-ATGFFYVSSCTKLYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:91
BciMan3_B_circulans_AAX87002.1	(1)	-ATGFFYVSSGTYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:92
PpoMan1_P_polymyxaE581_YP_003858989.1	(1)	-ASGFFYVSSGKATVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:93
Pae. sp. HGF5_WP_009563769.1	(1)	-ATGFFYVSSGTYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:94
Pae. sp. ICGEB2008_WP_017427981.1	(1)	-ASGFFYVSSCTKLYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:95
PpoMan2_P_polymyxa_SC2_YP_003944884.1	(1)	-ASGFFYVSSCTNLYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:96
Pae. sp. HW867_WP_019912481.1	(1)	-VRGFFYVSSTRLYDATGSPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:97
P_mucilaginosuk02_YP_006190599.1	(1)	-ATGMYVSSGTYDANGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:98
Bciman1_B_circulans_BAA25878.1	(1)	-ASGFFYVSSCTKLDATGQPFFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:99
B_reasonii_AGU1466.1	(1)	-ASGFFYVSSCTLYDATGKPFVIMRGCVNHNHGSWPKEDSAAIIPAIAKTGAN <sup>7</sup> SEQ ID NO:100
B_sp. JAMB-602_BAD9527.1	(1)	-NSGFFYVSSCTLYDANGNKPFFVVMRGCVNHNHGSWPKDQATTAEGLIANTGANSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:101
Consensus	(1)	ATGFFYVSSCTKLYDSTGKPFVVMRGCVNHNHGSWPKNDLNTAIPAIAKTGAN <sup>7</sup> SEQ ID NO:102

NDL-Clade motif

WX<sub>a</sub>KNDLX<sub>b</sub>X<sub>c</sub>AI, where X<sub>a</sub> is F or Y and X is any Amino Acid

or

WX<sub>a</sub>KNDLX<sub>b</sub>X<sub>c</sub>AI, where X<sub>a</sub> is F or Y; X<sub>b</sub> is N, Y or A; and X<sub>c</sub> is A or T

FIG. 5

251

300

PamMan2 (251)	SWYGNSSCLKYLDMATGPNCS-L	SEGNTVVNDTGEKTSQKAGI?	---	SEQ ID NO:103
PluMan2 (251)	SWYGNSSDLKYLDATGPNCS-L	SEGNTVVNDTGEKNTSKKAGI?	---	SEQ ID NO:104
PpmMan2 (251)	SWYGNSSDLKYLDATGPNCT-L	SEGNTVVNDTGEKNTSKKAGI?	---	SEQ ID NO:105
PspMan9 (251)	SWYGNNSDSYLDDATGPNCS-L	TTFGNTVVNDTNGEKATSKKAGI?	Q	SEQ ID NO:106
PspMan5_Pae_sp_CH-3_AEX60762.1 (251)	SWYGNSSCLKYLDMATGPNCS-L	SEGNTVVNDTGEKNTSQKAGI?	---	SEQ ID NO:107
Pae_sp_PAMC26794_WP_017688745.1 (251)	SWYGNSSCLKYLDMATGPNCS-L	SEGNTIVNDTGEKNTSQKAGI?	---	SEQ ID NO:108
BciMan4_B_circulans_AAX87003.1 (251)	SWYGNSSCLKYLDATGPNCS-L	SEGNTVVNDTGEKNTSQKAGI?	---	SEQ ID NO:109
Pae_sp_ZA9_WP_017813111.1 (251)	SWYGNSSCLKYLDATGPNCS-L	SEGNTVVNDTGEKNTSQKAGI?	---	SEQ ID NO:110
BciMan3_B_circulans_AAX87002.1 (251)	SWYGNSSPEINDLDAAGPSCN-L	SEGNTVVNDTGEKNTSQKAGI?	---	SEQ ID NO:111
PpoMan1_P_polymyxaE681_YP_00386989.1 (251)	SWYGNSSNSLSYLDIYTGPNCN-L	DDWGGKTVVNGNSNGEKETSKKAGI?	---	SEQ ID NO:103
Pae_sp_HGF5_WP_00953769.1 (251)	SWYGNSSPEINDLDAAGPSCN-L	GGGNTIVVHGIDGQ	---	SEQ ID NO:102
Pae_sp_ICGEB2008_WP_017427981.1 (251)	SWYGNSSNSLSYLDIYTGPNCN-L	DAGRTIVVGETNGEKETSKKAGI?	---	SEQ ID NO:104
PpoMan2_P_polymyxa_SC2_YP_003944884.1 (251)	SWYGNSSCLKYLDATGPNCS-L	DAGRTIVVGETNGEKETSKKAGI?	---	SEQ ID NO:105
Pae_sp_HW867_WP_019912481.1 (251)	SWYGNKCGGVEYLDISNGPSCN-L	DAGRTIVVGETNGEKETSKKAGI?	---	SEQ ID NO:106
P_mucilaginosusK02_YP_006190599.1 (251)	SWYGNSSCLKYLDATGPNCS-L	SWGNTIVVNGTNGEKATSALASV	GT	SEQ ID NO:107
BciMan1_B_circulans_BAA23878.1 (251)	SWKGNSSCLAYLDNTKWA	NGTNGEKATSVLSGI	GGV	SEQ ID NO:108
B_nealsonii_AGU71466.1 (248)	SWKGNSSCLDSNWSNSL	GGTNGEKATSKLSGV	GS-	SEQ ID NO:110
B_sp_JAMB-602_BAD96527.1 (251)	SWKGNNGPEWEYLDISN	AGNTIVVNG2YGLRETSKL	STVZ-G-	SEQ ID NO:111
Consensus (251)	SWYGNSSDLKYLDATGPNCS-L	SWGNTIVVNGT	G-K	SEQ ID NO:112

FIG. 6A

NDL-Clade motif

$L_{262}D_{263}XXXGPXGX_{272}T_{273}$ , where X is any Amino Acid

or

$L_{262}D_{263}M/LV/AT/AGPX_1GX_2L_{272}T_{273}$ , where  $X_1$  is N, A or S and  $X_2$  is S, T or N, where the  $L_{262}D_{263}$  and  $L_{272}T_{273}$  are Conserved Residues

or

NDL-Clade 1 motif  
 $LDM/LATGPN/AGSTLT$

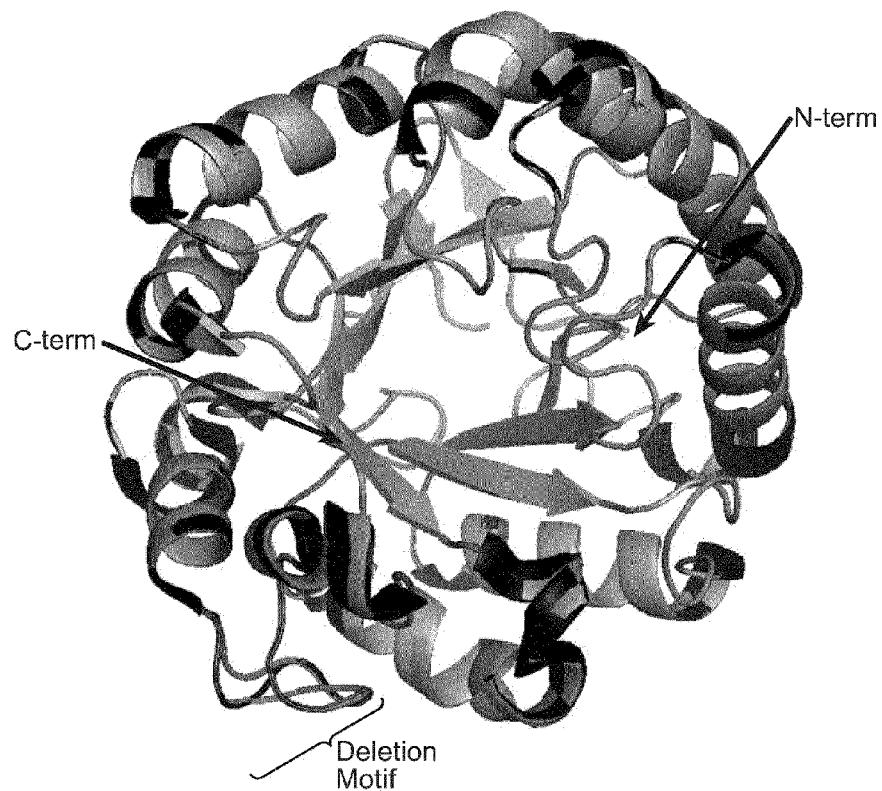
or

NDL-Clade 2 motif  
 $LDA/VATGPS/NGNLT$

or

NDL-Clade 3 motif  
 $LDL/VS/AT/NGPSGNLT$

**FIG. 6B**



**FIG. 7**