(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 June 2017 (15.06.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/097861 A1

(51) International Patent Classification:
C12N 9/42 (2006.01) *C12N 5/10* (2006.01)
C11D 3/386 (2006.01) *C12N 15/52* (2006.01)

(21) International Application Number:
PCT/EP2016/080150

(22) International Filing Date:
7 December 2016 (07.12.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
15198282.4 7 December 2015 (07.12.2015) EP
15198277.4 7 December 2015 (07.12.2015) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2017/097861 A1

(54) **Title**: DISHWASHING COMPOSITIONS COMPRISING POLYPEPTIDES HAVING BETA-GLUCANASE ACTIVITY AND USES THEREOF

(57) **Abstract**: The present invention relates to dish washing compositions comprising polypeptides having beta-glucanase activity, catalytic domains, beta-glucan binding domains and polynucleotides encoding the polypeptides, catalytic domains or beta-glucan binding domains. The invention further relates to dish washing compositions comprising polypeptides exhibiting beta-glucanase activity and one or more amylases and/or one or more proteases and uses thereof in dish wash applications and dish wash processes.

DISHWASHING COMPOSITIONS COMPRISING POLYPEPTIDES HAVING BETA-GLUCANASE ACTIVITY AND USES THEREOF

Reference to a Sequence Listing

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

Background of the Invention

Field of the Invention

The present invention relates to dish washing compositions comprising polypeptide(s) having beta-glucanase activity, catalytic domains, beta-glucan binding domains. The invention further relates to dish washing compositions comprising polypeptides exhibiting beta-glucanase activity and one or more amylases and/or one or more proteases and uses thereof in dish wash applications and dish wash processes.

Description of the Related Art

Beta-glucans are polysaccharides consisting of glucose units linked by beta-glycosidic bonds. Cellulose is one type of beta-glucan, in which all of the glucose units are linked by beta-1,4-glucosidic bonds. This feature results in the formation of insoluble cellulose micro-fibrils. Enzymatic hydrolysis of cellulose to glucose requires the use of endo beta-glucanases (*e.g.* EC 3.2.1.4), cellobiohydrolases (*e.g.* EC 3.2.1.91) and beta-glucosidases (*e.g.* EC 3.2.1.21).

Beta-glucans can also be linked by beta-1,3-glucosidic bonds (*e.g.,* as found in the cell walls of baker's yeast, *Saccharomyces cerevisiae*), beta-1,6-glucosidic bonds as well as combinations of beta-1,3-, beta-1,4- and beta-1,6-glucosidic bonds. The combination of beta-1,3- and beta-1,4-glucosidic bonds can be found, *e.g.* in the soluble fibre from cereals such as oats and barley. A subgroup of beta-glucanases, also known as a licheninases (or lichenases) (EC 3.2.1.73), can be used to catalyse the hydrolysis of the beta-1,4-glucosidic bonds to give beta-glucans. Licheninases (or lichenases) (*e.g.* EC 3.2.1.73) hydrolyse (1,4)-beta-D-glucosidic linkages in beta-D-glucans containing (1,3)- and (1,4)-bonds and can act on lichenin and cereal beta-D-glucans, but not on beta-D-glucans containing only 1,3- or 1,4-bonds. Other beta-glucanases (*e.g.* EC 3.2.1.4) can, for example, perform endohydrolysis of (1,4)-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans and will also hydrolyze 1,4-linkages in beta-D-glucans containing 1,3-linkages.

The removal of cereal stains as oat and barley containing stains in dish wash is a recognised problem, and there is a considerable interest in finding enzymes that can degrade the beta-glucans found therein. Various *Bacillus* species like *e.g. B. amyloliquefaciens* express a beta-glucanase, but these enzymes are generally not very suitable for alkaline applications, *e.g.*

at pH 7.5 or above.

The present invention provides polypeptides of glycoside hydrolyase family 16 (GH16) having beta-glucanase activity (*e.g.* comprising or consisting of licheninase (EC 3.2.1.73) activity) and polynucleotides encoding said polypeptides, which are highly active in degrading different types of beta-glucans (*e.g.* beta-D-glucans, beta-1,3-1,4 glucans, mix-linkage beta-glucans, barley beta-glucans and oatmeal beta-glucans), *e.g.* under alkaline conditions (*e.g.* at pH 7.5 or above), and therefore could be used in the aforementioned applications, *e.g.* in cleaning or detergent applications and processes such as dish washing. The existing products comprising beta-glucanases have very low effect on this type of beta-glucan as their main enzymatic substrate is cellulose. Therefore, the present invention provides novel beta-glucanases with improved properties (*e.g.* with significant improvement of performance and/or stability under alkaline conditions; beta-glucanases without cellulase activity (*e.g.* not having endo-cellulase activity on β-1,4 linkages between D-glucose units) (*e.g.* EC 3.2.1.73). A difference between use of cellulases and lichenases on textile in laundry is that the lichenases do not degrade the fibers of the textile.

Furthermore, some particular solid detergents have pH above 10. The known beta-glucanases are not suitable for these very high pH detergents. Thus, for example, known beta-glucanases from *Bacillus amyloliquefaciens* and *Bacillus subtilis* quickly lose their activity under alkaline conditions as has been demonstrated in Example 8 herein. The present invention provides novel beta-glucanases with improved properties (*e.g.* with significant improvement of performance and/or stability under alkaline conditions).

An uncharacterized protein from *Bacillus halodurans* (uniprot:Q9K7X6) is 88.4% identical to the beta-glucanase shown in SEQ ID NO: 7.

An uncharacterized protein from *Bacillus cellulosilyticus* (uniprot:E6TRB0) is 80.7% identical to the beta-glucanase shown in SEQ ID NO: 3.

An uncharacterized protein from *Bacillus akibai* (uniprot:W4QVK7) is 98.2% identical to the beta-glucanase shown in SEQ ID NO: 5.

An uncharacterized protein from *Bacillus subtilis subsp. niger.* (uniprot:A0A080UVP7) is 97.9% identical to the beta-glucanase shown in SEQ ID NO: 9.

Summary of the Invention

In one aspect, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising polypeptide(s) having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency

conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity; wherein said cleaning or detergent composition further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases,

preferably said polypeptide having beta-glucanase activity and said one or more amylases (e.g., SEQ ID NO: 12) (and/or said one or more proteases) have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In another aspect, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising polypeptide(s) having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 81% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 81% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

wherein said cleaning or detergent composition further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases,

preferably said polypeptide having beta-glucanase activity and said one or more amylases (e.g., SEQ ID NO: 12) (and/or said one or more proteases) have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In another aspect, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a composition comprising polypeptide(s) having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 99% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 99% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

wherein said cleaning or detergent composition further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases,

preferably said polypeptide having beta-glucanase activity and said one or more amylases (e.g., SEQ ID NO: 12) (and/or said one or more proteases) have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about

40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In another aspect, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising polypeptide(s) having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 89% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 89% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

wherein said cleaning or detergent composition further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases,

preferably said polypeptide having beta-glucanase activity and said one or more amylases (e.g., SEQ ID NO: 12) (and/or said one or more proteases) have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In another aspect, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising polypeptide(s) having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 98% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the

group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 98% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

wherein said cleaning or detergent composition further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases,

preferably said polypeptide having beta-glucanase activity and said one or more amylases (e.g., SEQ ID NO: 12) (and/or said one or more proteases) have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In another aspect, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising polypeptide(s) having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having 100% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

wherein said cleaning or detergent composition further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases,

preferably said polypeptide having beta-glucanase activity and said one or more amylases (e.g., SEQ ID NO: 12) (and/or said one or more proteases) have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In another aspect, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase of the invention together with one or more alpha-amylases (and/or said one or more proteases). In another aspect, the present invention relates to a cleaning or detergent composition comprising a beta-glucanase together with one or more amylases and one or more further enzymes selected from the group comprising of proteases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases, mannanases, or any mixture thereof. In another aspect, the present invention relates to a cleaning or detergent composition of the invention having an enzyme detergency benefit or improved wash performance in cleaning or detergent applications.

In another aspect, the present invention relates to use of a beta-glucanase of the invention together with one or more proteases, and optionally one or more further enzymes such as proteases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases, mannanases, or any mixture thereof, for dish wash.

In another aspect, the present invention relates to a dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one or more polypeptide(s) having beta-glucanase activity. In another aspect, the present invention relates to dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one ore more polypeptide(s) having beta-glucanase activity with improved wash performance and/or improved stability at alkaline conditions (e.g. at pH 7.5 or above). In another aspect, the present invention relates to dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one or more polypeptide(s) having beta-glucanase activity selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity.

In another aspect, the present invention relates to dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one or more polypeptide(s) having beta-glucanase activity selected from the group consisting of:

(a) a polypeptide having at least 81% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 81% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity.

In another aspect, the present invention relates to dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one or more polypeptide(s) having beta-glucanase activity selected from the group consisting of:

(a) a polypeptide having at least 99% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 99% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity.

The present invention further relates to dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one or more polypeptide(s) having beta-glucanase activity selected from the group consisting of:

(a) a polypeptide having at least 89% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 89% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity.

In another aspect, the present invention relates to dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one or more polypeptide(s) having beta-glucanase activity selected from the group consisting of:

(a) a polypeptide having at least 98% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 98% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group

consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity.

In another aspect, the present invention relates to dishwashing composition, especially a dishwash cleaning or detergent composition, comprising one or more polypeptide(s) having beta-glucanase activity selected from the group consisting of:

(a) a polypeptide having at least 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 100% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity.

In another aspect, the present invention relates to cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising the polypeptide of the present invention and the use of polypeptides of the present invention in degrading a beta-glucan (e.g. beta-D-glucan, beta-1,3-1,4 glucan, a mix-linkage beta-glucan, barley beta-glucan, oatmeal beta-glucan), for cleaning dishware; methods for degrading beta-glucan comprising applying a composition comprising the polypeptide of the present invention to the beta-glucan.

In another aspect, the present invention relates to a difference between the use of cellulases and lichenases of the present invention on textile in laundry is that the lichenases of the present invention do not degrade the fibers of the textile.

In another aspect, the present invention relates to methods of dish washing including automated dish wash (ADW) and hand dish wash (HDW) using a polypeptide or a composition (*e.g.* cleaning or detergent composition) of the invention. In another aspect, the present invention relates to dish washing composition, said composition comprising polypeptide(s) of the invention. In another aspect, the present invention relates to a cleaning or detergent composition, wherein said composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide of the invention and one or more amylases (and/or said one or more proteases).

In another aspect, the present invention relates to use of polypeptide(s) of the present invention in dish washing for preventing, reducing or removing a biofilm from an item.

In another aspect, the present invention relates to use of polypeptide(s) or detergent composition of the invention for reducing or preventing soil redeposition in dishwasing.

Overview of Sequence Listing

SEQ ID NO: 1 is the DNA sequence of the beta-glucanase as isolated from a strain of a *Bacillus* sp.

SEQ ID NO: 2 is the amino acid sequence of the beta-glucanase as automatically deduced from SEQ ID NO: 1.

SEQ ID NO: 3 is the amino acid sequence of the beta-glucanase as deduced from SEQ ID NO: 1 taking into account that the first amino acid (position -28) in the polypeptide shown in SEQ ID NO: 2 and encoded by the polynucleotide shown in SEQ ID NO:1 should be Met, not Val. When the first codon is gtg a Met is inserted though gtg normally codes for V.

SEQ ID NO: 4 is the DNA sequence of the beta-glucanase as isolated from a strain of a *Bacillus akibai.*

SEQ ID NO: 5 is the amino acid sequence of the beta-glucanase as deduced from SEQ ID NO: 4.

SEQ ID NO: 6 is the DNA sequence of the beta-glucanase as isolated from a strain of a *Bacillus agaradhaerens.*

SEQ ID NO: 7 is the amino acid sequence of the beta-glucanase as deduced from SEQ ID NO: 6.

SEQ ID NO: 8 is the DNA sequence of the beta-glucanase as isolated from a strain of a *Bacillus mojavensis.*

SEQ ID NO: 9 is the amino acid sequence of the beta-glucanase as deduced from SEQ ID NO: 8.

SEQ ID NO: 10 is a polypeptide secretion signal *Bacillus clausii.*

SEQ ID NO: 11 is an artificial N-terminal poly-histidine affinity purification tag sequence.

SEQ ID NO: 12 is alpha-amylase protein sequence from *Bacillus sp.* (Stainzyme).

SEQ ID NO: 13 is a polypeptide corresponding to SEQ ID NO: 2 of WO 95/10603.

SEQ ID NO: 14 is a polypeptide corresponding to SEQ ID NO: 6 in WO 02/010355.

SEQ ID NO: 15 is a polypeptide corresponding to a hybrid polypeptide comprising residues 1-33 of SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 of WO 2006/066594.

SEQ ID NO: 16 is a polypeptide corresponding to SEQ ID NO: 6 of WO 02/019467.

SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19 are polypeptides respectively corresponding to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873.

SEQ ID NO: 20 is a polypeptide corresponding to SEQ ID NO: 2 of WO 08/153815

SEQ ID NO: 21 is a polypeptide corresponding to SEQ ID NO: 10 of WO 01/66712.

SEQ ID NO: 22 is a polypeptide corresponding to SEQ ID NO: 2 of WO 09/061380.

SEQ ID NO: 23 is an amylase protein sequence from *Bacillus sp.*

SEQ ID NO: 24 is an amylase protein sequence from *Bacillus sp.*

SEQ ID NO: 25 is an amylase protein sequence from *Bacillus sp.*

SEQ ID NO: 26 is an amylase protein sequence from *Cytophaga sp.*

SEQ ID NO: 27 is an amylase protein sequence from *Bacillus sp.*

SEQ ID NO: 28 is an amylase protein sequence from *Bacillus sp.*

SEQ ID NO: 29 is an amylase protein sequence from *Bacillus halmapalus.*

SEQ ID NO: 30 is an artificial amylase protein sequence.

SEQ ID NO: 31 is an amylase protein sequence from *Bacillus sp.*

SEQ ID NO: 32 is a beta-glucanase protein sequence from *Bacillus amyloliquefaciens*.

SEQ ID NO: 33 is a beta-glucanase protein sequence from *Bacillus subtilis*.

SEQ ID NO: 34 is a protease protein sequence from *Bacillus Lentus.*

SEQ ID NO: 35 is an artificial protease protein sequence.

SEQ ID NO: 36 is an artificial protease protein sequence.

SEQ ID NO: 37 is His-tagged recombinant mature beta-glucanase protein from Bacillus sp-62449.

SEQ ID NO: 38 is His-tagged recombinant mature beta-glucanase protein from Bacillus akibai.

SEQ ID NO: 39 is His-tagged recombinant mature beta-glucanase protein from Bacillus agaradhaerens.

SEQ ID NO: 40 is His-tagged recombinant mature beta-glucanase protein from Bacillus mojavensis.

Definitions

Anti-redeposition: The term “anti-redeposition” or “anti-redeposition effect” means the reduction or prevention of soil from depositing back onto the hard surface such as dishware. The anti-redeposition effect can be determined using the Mini-LOM or Mini-TOM wash assay as described in the examples herein (e.g., as in example 14).

Synergistic effect: The term “synergistic effect” means a cooperative action of polypeptides such that a total combined effect of said polypeptides is greater than the sum of the individual enzymatic effects of said polypeptides. Non-limiting examples of synergistic effect include REM synergistic effect of a beta-glucanase polypeptide of the invention and one or more alpha-amylase (and/or one or more proteases).

REM synergistic effect: REM synergistic effect of polypeptides as used herein can be measured based on the analysis of stain removal carried out by using any suitable wash

performance methodology (*e.g.* Wascator bottle wash method). A preferred method for determining the REM synergistic effect is disclosed in examples disclosed herein, e.g. Example 7.

Beta-glucanase: The term "beta-glucanase" as used herein means an endo beta-1,4-glucanase activity (*e.g.* endo-1,4-β-D-glucanase) that catalyzes the hydrolyses of a beta-1,4-bonds connecting two glucosyl residues in a beta-glucan. Non-limiting examples of beta-glucanases as defined herein include cellulases (*e.g.* EC 3.2.1.4, *e.g.* having endo-cellulase activity on β-1,4 linkages between D-glucose units and licheninases (or lichenases) (*e.g.* EC 3.2.1.73) hydrolysing (1,4)-beta-D-glucosidic linkages in beta-D-glucans containing (1,3)- and (1,4)-bonds. Beta-glucanases (*e.g.* EC 3.2.1.4) can, for example, perform endohydrolysis of (1,4)-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans and will also hydrolyze 1,4-linkages in beta-D-glucans containing 1,3-linkages. For purposes of the present invention, beta-glucanase activity is determined according to the procedure described in the Examples. In one aspect of the invention, the polypeptides of the present invention have at least 20%, *e.g.*, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the beta-glucanase activity of the polypeptide having the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9. Beta-glucanase activity can suitably be measured using barley beta-glucan as substrate. A preferred assay for determining beta-glucanase activity is disclosed in Example 1 (AZCL-Barley beta-glucan assay). A further subgroup of beta-glucanases as defined herein, also known as a licheninases (or lichenases) (*e.g.* EC 3.2.1.73), can also be used to catalyse the hydrolysis of the beta-1,4-glucosidic bonds to give beta-glucans. Licheninases (or lichenases) (*e.g.* EC 3.2.1.73) hydrolyse (1,4)-beta-D-glucosidic linkages in beta-D-glucans containing (1,3)- and (1,4)-bonds and can act on lichenin and cereal beta-D-glucans, but not on beta-D-glucans containing only 1,3- or 1,4-bonds. As used herein the term "beta-glucanase activity" comprises licheninase (or lichenases) (*e.g.* EC 3.2.1.73) activity.

Beta-glucan: The term "beta-glucan" as used herein means a polysaccharide that only contain glucose as structural components, and in which the glucose units are linked by beta-glycosidic bonds. Non-limiting examples of beta-glucans include beta-D-glucans, beta-1,3-1,4 glucans, mix-linkage beta-glucans, barley beta-glucans, oatmeal beta-glucans.

Allelic variant: The term "allelic variant" means any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequences. An allelic variant of a polypeptide is a polypeptide encoded by an allelic variant of a gene.

Biofilm: The term "biofilm" means any group of microorganisms in which cells stick to each other on a surface, such as dishware. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS is a polymeric

conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.

Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One effect of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community.

Carbohydrate binding module: The term “carbohydrate binding module” means the region within a carbohydrate-active enzyme that provides carbohydrate-binding activity. A majority of known carbohydrate binding modules (CBMs) are contiguous amino acid sequences with a discrete fold. The carbohydrate binding module (CBM) is typically found either at the N-terminal or at the C-terminal extremity of an enzyme. Some CBMs are known to have specificity for cellulose.

Catalytic domain: The term “catalytic domain” means the region of an enzyme containing the catalytic machinery of the enzyme.

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

Cellulolytic enzyme or cellulase: The term “cellulolytic enzyme” or “cellulase” means one or more (*e.g.*, several) enzymes that hydrolyze a cellulosic material. Such enzymes include endoglucanase(s) (*e.g.* EC 3.2.1.4), cellobiohydrolase(s), beta-glucosidase(s), or combinations thereof. The two basic approaches for measuring cellulolytic enzyme activity include: (1) measuring the total cellulolytic enzyme activity, and (2) measuring the individual cellulolytic enzyme activities (endoglucanases, cellobiohydrolases, and beta-glucosidases). Total cellulolytic enzyme activity can be measured using insoluble substrates, including Whatman №1 filter paper, microcrystalline cellulose, bacterial cellulose, algal cellulose, cotton, pretreated lignocellulose, *etc*. The most common total cellulolytic activity assay is the filter paper assay using Whatman №1 filter paper as the substrate.

Cellulolytic enzyme activity can be determined by measuring the increase in production/release of sugars during hydrolysis of a cellulosic material by cellulolytic enzyme(s) under the following conditions: 1-50 mg of cellulolytic enzyme protein/g of cellulose in pretreated corn stover (PCS) (or other pretreated cellulosic material) for 3-7 days at a suitable temperature such as 40°C-80°C, *e.g.*, 50°C, 55°C, 60°C, 65°C, or 70°C, and a suitable pH such as 4-9, *e.g.*, 5.0, 5.5, 6.0, 6.5, or 7.0, compared to a control hydrolysis without addition of cellulolytic enzyme

protein. Typical conditions are 1 ml reactions, washed or unwashed PCS, 5% insoluble solids (dry weight), 50 mM sodium acetate pH 5, 1 mM $MnSO_4$, 50°C, 55°C, or 60°C, 72 hours, sugar analysis by AMINEX® HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Cellulosic material: The term "cellulosic material" means any material containing cellulose. The predominant polysaccharide in the primary cell wall of biomass is cellulose, the second most abundant is hemicellulose, and the third is pectin. The secondary cell wall, produced after the cell has stopped growing, also contains polysaccharides and is strengthened by polymeric lignin covalently cross-linked to hemicellulose. Cellulose is a homopolymer of anhydrocellobiose and thus a linear beta-(1-4)-D-glucan, while hemicelluloses include a variety of compounds, such as xylans, xyloglucans, arabinoxylans, and mannans in complex branched structures with a spectrum of substituents. Although generally polymorphous, cellulose is found in plant tissue primarily as an insoluble crystalline matrix of parallel glucan chains. Hemicelluloses usually hydrogen bond to cellulose, as well as to other hemicelluloses, which help stabilize the cell wall matrix.

Cellulose is generally found, for example, in the stems, leaves, hulls, husks, and cobs of plants or leaves, branches, and wood of trees. The cellulosic material can be, but is not limited to, agricultural residue, herbaceous material (including energy crops), municipal solid waste, pulp and paper mill residue, waste paper, and wood (including forestry residue). It is understood herein that the cellulose may be in the form of lignocellulose, a plant cell wall material containing lignin, cellulose, and hemicellulose in a mixed matrix. In one aspect, the cellulosic material is any biomass material. In another aspect, the cellulosic material is lignocellulose, which comprises cellulose, hemicelluloses, and lignin.

In an embodiment, the cellulosic material is agricultural residue, herbaceous material (including energy crops), municipal solid waste, pulp and paper mill residue, waste paper, or wood (including forestry residue).

In another embodiment, the cellulosic material is arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, rice straw, switchgrass, or wheat straw.

In another embodiment, the cellulosic material is aspen, eucalyptus, fir, pine, poplar, spruce, or willow.

In another embodiment, the cellulosic material is algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose (*e.g.*, AVICEL®), or phosphoric-acid treated cellulose.

In another embodiment, the cellulosic material is an aquatic biomass. As used herein the term "aquatic biomass" means biomass produced in an aquatic environment by a photosynthesis process. The aquatic biomass can be algae, emergent plants, floating-leaf plants, or submerged plants.

The cellulosic material may be used as is or may be subjected to pretreatment, using conventional methods known in the art, as described herein. In a preferred aspect, the cellulosic material is pretreated.

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

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

Detergent composition: the term "detergent composition" refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles, dishes, and hard surfaces. The detergent composition may be used to e.g. clean textiles, dishes and hard surfaces for both household cleaning and industrial cleaning. The terms encompass any materials/compounds selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, gel, powder, granulate, paste, or spray compositions) and includes, but is not limited to, detergent compositions (e.g., liquid and/or solid laundry detergents and fine fabric detergents; hard surface cleaning formulations, such as for glass, wood, plastic, ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; fabric fresheners; fabric softeners; and textile and laundry pre-spotters, as well as dish wash detergents). In addition to containing a GH16 beta-glucanase of the invention, the detergent formulation may contain one or more additional enzymes (such as amylases, proteases, peroxidases, cellulases, betaglucanases, xyloglucanases, hemicellulases, xanthanases, xanthan lyases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo-beta-mannanases, exo-beta-mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof, or any mixture thereof), and/or components such as surfactants, builders, chelators or chelating agents, bleach system or bleach components, polymers, fabric conditioners, foam boosters, suds suppressors, dyes, perfume, tannish inhibitors, optical brighteners, bactericides, fungicides, soil suspending agents, anti corrosion agents, enzyme inhibitors or stabilizers, enzyme activators, transferase(s), hydrolytic enzymes, oxido reductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers.

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

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

Fragment: The term “fragment” means a polypeptide or a catalytic or carbohydrate binding module having one or more (*e.g.*, several) amino acids absent from the amino and/or carboxyl terminus of a mature polypeptide or domain; wherein the fragment has beta-glucanase or carbohydrate binding activity. In one aspect, a fragment contains at least 340 amino acid residues, or at least 230 amino acid residues, or at least 210 amino acid residues or at least 200 amino acid residues, or at least 180 amino acid residues, wherein the fragment has beta-glucanase activity.

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

Hemicellulolytic enzyme or hemicellulase: The term “hemicellulolytic enzyme” or “hemicellulase” means one or more (*e.g.*, several) enzymes that hydrolyze a hemicellulosic material. Hemicellulases are key components in the degradation of plant biomass. Examples of hemicellulases include, but are not limited to, an acetylmannan esterase, an acetylxylan esterase, an arabinanase, an arabinofuranosidase, a coumaric acid esterase, a feruloyl esterase, a galactosidase, a glucuronidase, a glucuronoyl esterase, a mannanase, a mannosidase, a xylanase, and a xylosidase. The substrates for these enzymes, hemicelluloses, are a heterogeneous group of branched and linear polysaccharides that are bound via hydrogen bonds to the cellulose microfibrils in the plant cell wall, crosslinking them into a robust network. Hemicelluloses are also covalently attached to lignin, forming together with cellulose a highly complex structure. The variable structure and organization of hemicelluloses require the concerted action of many enzymes for its complete degradation. The catalytic modules of hemicellulases are either glycoside hydrolases (GHs) that hydrolyze glycosidic bonds, or carbohydrate esterases (CEs), which hydrolyze ester linkages of acetate or ferulic acid side groups. These catalytic modules, based on homology of their primary sequence, can be assigned into GH and CE families. Some families, with an overall similar fold, can be further grouped into clans, marked alphabetically (*e.g.*, GH-A). A most informative and updated classification of these

and other carbohydrate active enzymes is available in the Carbohydrate-Active Enzymes (CAZy) database. Hemicellulolytic enzyme activities can be measured according to Ghose and Bisaria, 1987, *Pure & Appl. Chem.* 59: 1739-1752, at a suitable temperature such as 40°C-80°C, *e.g.*, 50°C, 55°C, 60°C, 65°C, or 70°C, and a suitable pH such as 4-9, *e.g.*, 5.0, 5.5, 6.0, 6.5, or 7.0.

Isolated: The term "isolated" means a substance in a form or environment that does not occur in nature. Non-limiting examples of isolated substances include (1) any non-naturally occurring substance, (2) any substance including, but not limited to, any enzyme, variant, nucleic acid, protein, peptide or cofactor, that is at least partially removed from one or more or all of the naturally occurring constituents with which it is associated in nature; (3) any substance modified by the hand of man relative to that substance found in nature; or (4) any substance modified by increasing the amount of the substance relative to other components with which it is naturally associated (*e.g.*, recombinant production in a host cell; multiple copies of a gene encoding the substance; and use of a stronger promoter than the promoter naturally associated with the gene encoding the substance). A fermentation broth produced by culturing a recombinant host cell expressing the polynucleotide of the invention will comprise the polypeptide of the invention in an isolated form.

Lichenase activity: The term "lichenase activity" means enzymes that hydrolysis beta-1,3, beta-1,4-glucans (e.g. EC 3.2.1.73).

Mature polypeptide: The term "mature polypeptide" means a polypeptide in its final form following translation and any post-translational modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc. In one aspect, the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9. The amino acids -28 to -1 of SEQ ID NO: 2 are a signal peptide. The amino acids -28 to -1 of SEQ ID NO: 3 are a signal peptide. The amino acids -31 to -1 of SEQ ID NO: 5 are a signal peptide. The amino acids -15 to -1 of SEQ ID NO: 7 are a signal peptide. The amino acids -29 to -1 of SEQ ID NO: 9 are a signal peptide.

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

Mature polypeptide coding sequence: The term "mature polypeptide coding sequence" means a polynucleotide that encodes a mature polypeptide having beta-glucanase activity. In one aspect, the mature polypeptide coding sequence is selected from the group consisting of: nucleotides 85 to 1137 of SEQ ID NO: 1, nucleotides 94 to 828 of SEQ ID NO: 4, nucleotides 46 to 711 of SEQ ID NO: 6, nucleotides 88 to 729 of SEQ ID NO: 8. The nucleotides 1 to 84 of SEQ

ID NO: 1 encode a signal peptide. The nucleotides 1 to 93 of SEQ ID NO: 4 encode a signal peptide. The nucleotides 1 to 45 of SEQ ID NO: 6 encode a signal peptide. The nucleotides 1 to 87 of SEQ ID NO: 8 encode a signal peptide.

Malodor: The term ”malodor” means an odor which is not desired on clean items. The cleaned item should smell fresh and clean without malodors adhered to the item. One example of malodor is compounds with an unpleasant smell, which may be produced by microorganisms. Another example of malodor can be the smell from spices, for example curry or other exotic spices adhering to an item such as a piece of textile. One way of measuring the ability of an item to adhere malodor is by using the Malodor Assay.

Pretreated corn stover: The term “Pretreated Corn Stover” or “PCS” means a cellulosic material derived from corn stover by treatment with heat and dilute sulfuric acid, alkaline pretreatment, neutral pretreatment, or any pretreatment known in the art.

Sequence identity: The relatedness between two amino acid sequences is described by the parameter “sequence identity”. For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package , preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled “longest identity” (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm as implemented in the Needle program of the EMBOSS package, preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled “longest identity” (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

Stringency conditions: The different stringency conditions are defined as follows.

The term “very low stringency conditions” means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 1.6X SSC, 0.2% SDS at 60°C.

The term “low stringency conditions” means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml

sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 0.8X SSC, 0.2% SDS at 60°C.

The term "medium stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 35% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 0.8X SSC, 0.2% SDS at 65°C.

The term "medium-high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 35% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 0.4X SSC, 0.2% SDS at 65°C.

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

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

Subsequence: The term "subsequence" means a polynucleotide having one or more (*e.g.*, several) nucleotides absent from the 5' and/or 3' end of a mature polypeptide coding sequence; wherein the subsequence encodes a fragment having beta-glucanase activity. In one aspect, a subsequence contains at least 1052 nucleotides of SEQ ID NO: 1 or the cDNA sequence thereof, at least 1037 nucleotides of SEQ ID NO: 1 or the cDNA sequence thereof, or 1022 nucleotides of SEQ ID NO: 1 or the cDNA sequence thereof).

Variant: The term "variant" means a polypeptide having beta-glucanase activity comprising an alteration, *i.e.*, a substitution, insertion, and/or deletion of one or more (several) amino acid residues at one or more (several) positions. A substitution means a replacement of an amino acid occupying a position with a different amino acid; a deletion means removal of an amino acid occupying a position; and an insertion means adding 1-3 amino acids adjacent to an amino acid occupying a position. The variants of the present invention have at least 20%, *e.g.*, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the beta-glucanase activity of the polypeptide of sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9

or the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9.

Wild-type beta-glucanase: The term "wild-type" beta-glucanase means a beta-glucanase expressed by a naturally occurring microorganism, such as a bacterium, yeast, or filamentous fungus found in nature.

Wash performance: The term "wash performance" is defined herein as the ability of an enzyme or a blend of enzymes to remove stains present on an object to be cleaned during e.g. wash or hard surface cleaning, such as dish washing, relative to the wash performance without one or more on the enzymes present.

Detailed Description of the Invention

Dishwashing compositions comprising one or more polypeptide(s) having beta-glucanase activity

This invention provides the use of novel beta-glucanases and one or more amylases (and/or one or more proteases) for cleaning or detergent compositions which have a benefit in removing stains and which can be used in cleaning or detergent applications, such as dishwashing or for processes such as dish wash. The invention also provides the use of beta-glucanases that are wash stable in detergent formulations in the presence of amylases. The beta-glucanases of the invention may show synergistic effect with one or more amylases (and/or one or more proteases) (*e.g.* wherein a preferred method for determining the REM synergistic effect is disclosed in examples disclosed herein, e.g. Example 7).

In an embodiment, the present invention relates to a cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, the composition comprising polypeptide(s) having beta-glucanase activity, wherein said polypeptides having a sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9; at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, which have beta-glucanase activity; and one or more amylases (and/or one or more proteases), preferably said polypeptide having beta-glucanase activity and said one or more amylases (and/or one or more proteases) have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In an embodiment, the present invention relates to cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprise polypeptide(s) having a sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9; at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, which have beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one aspect, the polypeptide(s) differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment of the present invention is a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 81% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 82% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 83% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 84% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 85% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 86% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 87% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 88% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 89% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 90% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 91% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 92% identity to the mature polypeptide of sequence

selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 93% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 94% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 95% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 96% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 97% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 98% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having at least 99% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to a cleaning or detergent composition

wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase having 100% identity to the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and one or more amylases (and/or one or more proteases).

In another embodiment of the inventionthe polypeptide having beta-glucanase activity and one or more amylases (and/or one or more proteases) have a synergistic effect; preferably said synergistic effect is a REM synergistic effect, further preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

In another embodiment REM synergistic effect is of more than 1.4 (such as 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, or 9.0) at about 40°C (or 35°C, 45°C, 50°C, 55°C, 60°C) for about 30 minutes (or 15 min, 20 min, 25 min, 35 min, 40 min) at pH of about 7.0 (or 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5), e.g. in Wascator bottle wash in Model detergent A1 at 40°C, 30 min (pH 7.7), or Wascator bottle wash in Model detergent X1 at 40°C, 30 min (pH 10.1), or Wascator bottle wash in ADW Model detergent A1 at 40°C, 30 min (pH 10.2) (e.g. see Example 7).

In another embodiment a pH optimum of a polypeptide of the present invention is selected in the range from about 6 to about 9. In another embodiment a pH optimum of a polypeptide of the present invention is selected from the group consisting of: 6, 6.5, 7, 7.5, 8, 8.5, 9. In another embodiment a pH optimum of a polypeptide of the present invention is at least 6 (or at least 6.5, or at least 7, or at least 7.5, or at least 8, or at least 8.5, or at least 9). In another embodiment a pH optimum of a polypeptide of the present invention is more than 6 (or more than 6.5, or more than 7, or more than 7.5, or more than 8, or more than 8.5, or more than 9).

In another embodiment a pH optimum of a polypeptide of the present invention is selected in the range from about 6 to about 9, wherein said polypeptide has a significantly higher relative activity at pH 10 ranging from 23-90% compared to a beta-glucanase from *Bacillus subitilis* or *Bacillus amyloliquefaciens*. In another embodiment a pH optimum of a polypeptide of the present invention is selected from the group consisting of: 6, 6.5, 7, 7.5, 8, 8.5, 9, wherein said polypeptide has a significantly higher relative activity at pH 10 ranging from 23-90% compared to a beta-glucanase from *Bacillus subitilis* or *Bacillus amyloliquefaciens*. In another embodiment a pH optimum of a polypeptide of the present invention is at least 6 (or at least 6.5, or at least 7, or at least 7.5, or at least 8, or at least 8.5, or at least 9), wherein said polypeptide has a significantly

higher relative activity at pH 10 ranging from 23-90% compared to a beta-glucanase from *Bacillus subitilis* or *Bacillus amyloliquefaciens*. In another embodiment a pH optimum of a polypeptide of the present invention is more than 6 (or more than 6.5, or more than 7, or more than 7.5, or more than 8, or more than 8.5, or more than 9), wherein said polypeptide has a significantly higher relative activity at pH 10 ranging from 23-90% compared to a beta-glucanase from *Bacillus subitilis* or *Bacillus amyloliquefaciens*.

In one aspect, the polypeptides differ by no more than thirty amino acids, *e.g.*, by twenty five amino acids, by twenty amino acids, by fifteen amino acids, by twelve amino acids, by ten amino acids, by nine amino acids, by eight amino acids, by seven amino acids, by six amino acids, by five amino acids, by four amino acids, by three amino acids, by two amino acids, and by one amino acid from the polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In an embodiment, the polypeptide has been isolated. A polypeptide of the present invention preferably comprises or consists of the amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. In another aspect, the polypeptide comprises or consists of the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment of the present invention is a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprise said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another aspect, the polypeptide comprises or consists of amino acids amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 214 of SEQ ID NO: 9. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising wherein said cleaning or detergent composition is a dish washing composition, said composition said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment beta-glucanase of the present invention is not an endo-cellulase having activity on β-1,4 linkages between D-glucose units of cellulose. In another embodiment beta-glucanase of the present invention have licheninase (EC 3.2.1.73) enzymatic activity having activity on β-1,3 β-1,4 glucans. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment beta-glucanase of the present invention comprises alkaline beta-

glucanase activity (*e.g.* beta-glucanase activity in an aqueous solution at pH 7.5 or above, *e.g.* beta-glucanase activity at pH selected from the group consisting of 7.5, 8, 9, 10, 11, 12, 13, 13.5, *e.g.* beta-glucanase activity at pH in the range from about 7.5 to about 13.5, wherein said aqueous solution optionally comprises a bleaching agent, preferably said pH is selected in the range from about 7.5 to about 12.5, further preferably said pH is selected in the range from about 8.5 to about 11.5, most preferably said pH is selected in the range from about 9.5 to about 10.5). An embodiment of the present invention is a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment a beta-glucanase of the present invention is capable of:

i) having beta-glucanase activity for at least 15 minutes in an aqueous solution with a pH selected in the range from about 7.5 to about 13.5, wherein said aqueous solution optionally comprises a bleaching agent, preferably said pH is selected in the range from about 7.5 to about 12.5, further preferably said pH is selected in the range from about 8.5 to about 11.5, most preferably said pH is selected in the range from about 9.5 to about 10.5; and/or

ii) having beta-glucanase activity for at least 15 minutes in an aqueous solution at a temperature selected in the range from about 20°C to about 75°C, wherein said aqueous solution optionally comprises a bleaching agent.

An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment a beta-glucanase of the present invention is capable of having beta-glucanase activity in an aqueous solution at a temperature selected in the range from about 20°C to about 75°C, wherein said aqueous solution optionally comprises a bleaching agent, preferably said temperature is selected in the range from about 40°C to about 60°C. In another embodiment a beta-glucanase of the present invention is capable of having beta-glucanase activity in an aqueous solution at a temperature selected from the group consisting of: 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C, 37°C, 38°C, 39°C, 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, 80°C, 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C, 90°C, 90°C. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment a beta-glucanase of the present invention is capable of having beta-glucanase activity for at least 15 minutes, preferably at least 30 minutes. In another embodiment a beta-glucanase of the present invention is capable of having beta-glucanase

activity for a period of time selected from the group consisting of: at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30 minutes, e.g. in combination with any single or multiple embodiments as disclosed herein. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment, a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprises a beta-glucanase polypeptide and one or more amylases, wherein said amylase is an alpha-amylase.

In another embodiment, a cleaning or detergent composition of the invention wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide and one or more amylases, wherein said alpha-amylase is selected from the group consisting of:

(a) a polypeptide having at least 90% sequence identity to SEQ ID NO: 13 (corresponding to SEQ ID NO: 2 of WO 95/10603);

(b) a polypeptide having at least 90% sequence identity to SEQ ID NO: 13 (corresponding to SEQ ID NO: 2 in WO 95/10603) wherein the polypeptide comprises a substitution in one or more of positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and/or 444;

(c) a polypeptide having at least 90% sequence identity to SEQ ID NO: 14 (corresponding to SEQ ID NO: 6 in WO 02/010355);

(d) a polypeptide having at least 90% sequence identity to the hybrid polypeptide of SEQ ID NO: 15 (comprising residues 1-33 of SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 of WO 2006/066594);

(e) a polypeptide having at least 90% sequence identity to the hybrid polypeptide of SEQ ID NO: 15 (comprising residues 1-33 of SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 of WO 2006/066594), wherein the hybrid polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 48, 49, 107, 156, 181, 190, 197, 201, 209 and/or 264;

(f) a polypeptide having at least 90% sequence identity to SEQ ID NO: 16 (corresponding to SEQ ID NO: 6 of WO 02/019467);

(g) a polypeptide having at least 90% sequence identity to SEQ ID NO: 16 (corresponding to SEQ ID NO: 6 of WO 02/019467), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 181, 182, 183, 184, 195, 206, 212, 216 and/or 269;

(h) a polypeptide having at least 90% sequence identity to SEQ ID NO: 17, SEQ ID

NO: 18 or SEQ ID NO: 19 (corresponding to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873)

(i) a polypeptide having at least 90% sequence identity to SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19 (corresponding to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 140, 183, 184 195, 206, 243, 260, 304 and/or 476;

(j) a polypeptide having at least 90% sequence identity to SEQ ID NO: 20 (corresponding to SEQ ID NO: 2 of WO 08/153815);

(k) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21 (corresponding to SEQ ID NO: 10 of WO 01/66712);

(l) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21 (corresponding to SEQ ID NO: 10 of WO 01/66712), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 176, 177, 178, 179, 190, 201, 207, 211 and/or 264;

(m) a polypeptide having at least 90% sequence identity to SEQ ID NO: 22 (corresponding to SEQ ID NO: 2 of WO 09/061380);

(n) a polypeptide having at least 90% sequence identity to SEQ ID NO: 22 (corresponding to SEQ ID NO: 2 of WO 09/061380), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 87, 98, 125, 128, 131, 165, 178, 180, 181, 182, 183, 201, 202, 225, 243, 272, 282, 305, 309, 319, 320, 359, 444 and/or 475;

(o) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21, wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 28, 118, 174; 181, 182, 183, 184, 186, 189, 195, 202, 298, 299, 302, 303, 306, 310, 314; 320, 324, 345, 396, 400, 439, 444, 445, 446, 449, 458, 471 and/or 484;

(p) a polypeptide having at least 90% sequence identity to SEQ ID NO: 12;

(q) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO:23 having alterations G182* + D183*;

(r) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T;

(s) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K;

(t) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K

(u) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100%

sequence identity) to a variant of SEQ ID NO: 27 having alteration M202L;

(v) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K;

(w) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K;

(x) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K;

(y) a polypeptide having at least 90% sequence identity (e.g., at least 95% or 100% sequence identity) to a variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K.

In another embodiment, a cleaning or detergent composition of the invention wherein said cleaning or detergent composition is a dish washing composition, said composition comprises a beta-glucanase polypeptide and one or more proteases, wherein said protease is selected from the group consisting of:

a) a polypeptide having protease activity, which has at least 60% sequence identity (e.g., at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to SEQ ID NO: 34;

b) a polypeptide having protease activity, which has at least 60% sequence identity (e.g., at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to SEQ ID NO: 35;

c) a polypeptide having protease activity, which has at least 60% sequence identity (e.g., at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least

74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to SEQ ID NO: 36.

In another embodiment, the present invention relates to polypeptide(s) having beta-glucanase activity encoded by a polynucleotide that hybridizes under very low stringency conditions, low stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions, or very high stringency conditions with (i) the mature polypeptide coding sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii) (. In an embodiment, the polypeptide has been isolated. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

The polynucleotide of sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or a subsequence thereof, as well as the polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 or a fragment thereof may be used to design nucleic acid probes to identify and clone DNA encoding polypeptides having beta-glucanase activity from strains of different genera or species according to methods well known in the art. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In particular, such probes can be used for hybridization with the genomic DNA or cDNA of a cell of interest, following standard Southern blotting procedures, in order to identify and isolate the corresponding gene therein. Such probes can be considerably shorter than the entire sequence, but should be at least 15, *e.g.*, at least 25, at least 35, or at least 70 nucleotides in length. Preferably, the nucleic acid probe is at least 100 nucleotides in length, *e.g.*, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 600 nucleotides, at least 700 nucleotides, at least 800 nucleotides, or at least 900 nucleotides in length. Both DNA and RNA probes can be used. The probes are typically labeled for detecting the corresponding gene (for example, with ^{32}P, ^{3}H, ^{35}S, biotin, or avidin). Such probes are encompassed by the present invention. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

A genomic DNA or cDNA library prepared from such other strains may be screened for DNA that hybridizes with the probes described above and encodes a polypeptide having beta-

glucanase activity. Genomic or other DNA from such other strains may be separated by agarose or polyacrylamide gel electrophoresis, or other separation techniques. DNA from the libraries or the separated DNA may be transferred to and immobilized on nitrocellulose or other suitable carrier material. In order to identify a clone or DNA that hybridizes with sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or a subsequence thereof, the carrier material is used in a Southern blot. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

For purposes of the present invention, hybridization indicates that the polynucleotide hybridizes to a labeled nucleic acid probe corresponding to (i) sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8; (ii) the mature polypeptide coding sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8; (iii) the cDNA sequence thereof; (iv) the full-length complement thereof; or (v) a subsequence thereof; under very low to very high stringency conditions. Molecules to which the nucleic acid probe hybridizes under these conditions can be detected using, for example, X-ray film or any other detection means known in the art. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one aspect, the nucleic acid probe is nucleotides 85 to 1137 or nucleotides 1 to 1137 of SEQ ID NO: 1. In one aspect, the nucleic acid probe is nucleotides 1 to 828 or nucleotides 94 to 828 of SEQ ID NO: 4. In one aspect, the nucleic acid probe is nucleotides 1 to 711 or nucleotides 46 to 711 of SEQ ID NO: 6. In one aspect, the nucleic acid probe is nucleotides 1 to 729 or nucleotides 88 to 729 of SEQ ID NO: 8. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another aspect, the nucleic acid probe is a polynucleotide that encodes the polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9; the mature polypeptide thereof; or a fragment thereof. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another aspect, the nucleic acid probe is a sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or

detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment, the present invention relates to an polypeptide having beta-glucanase activity encoded by a polynucleotide having a sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In a further embodiment, the polypeptide has been isolated.

In another embodiment, the present invention relates to variants of the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the mature polypeptide of sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). The amino acid changes may be of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of 1-30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

Examples of conservative substitutions are within the groups of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine,

alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In*, *The Proteins*, Academic Press, New York. Common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

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

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

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed in WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (*e.g.*, U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis ().

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

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

The polypeptide may be a fusion polypeptide or cleavable fusion polypeptide in which another polypeptide is fused at the N-terminus or the C-terminus of the polypeptide of the present invention. A fusion polypeptide is produced by fusing a polynucleotide encoding another polypeptide to a polynucleotide of the present invention. Techniques for producing fusion polypeptides are known in the art, and include ligating the coding sequences encoding the polypeptides so that they are in frame and that expression of the fusion polypeptide is under control of the same promoter(s) and terminator. Fusion polypeptides may also be constructed using intein technology in which fusion polypeptides are created post-translationally ().

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

Sources of Polypeptides Having Beta-glucanase Activity

A polypeptide having beta-glucanase activity of the present invention may be obtained from microorganisms of any genus (e.g. genus *Bacillus*). For purposes of the present invention, the term "obtained from" as used herein in connection with a given source shall mean that the polypeptide encoded by a polynucleotide is produced by the source or by a strain in which the polynucleotide from the source has been inserted. In one aspect, the polypeptide obtained from a given source is secreted extracellularly.

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

In one aspect, the polypeptide is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus sp., Bacillus akibai, Bacillus agaradhaerens, Bacillus mojavensis* or *Bacillus thuringiensis* polypeptide.

In another aspect, the polypeptide is not a fungal polypeptide (e.g. a polypeptide of the present invention excludes fungal polypeptides). An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

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

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

The polypeptide may be identified and obtained from other sources including microorganisms isolated from nature (*e.g.*, soil, composts, water, etc.) or DNA samples obtained directly from natural materials (*e.g.*, soil, composts, water, etc.) using the above-mentioned probes. Techniques for isolating microorganisms and DNA directly from natural habitats are well known in the art. A polynucleotide encoding the polypeptide may then be obtained by similarly

screening a genomic DNA or cDNA library of another microorganism or mixed DNA sample. Once a polynucleotide encoding a polypeptide has been detected with the probe(s), the polynucleotide can be isolated or cloned by utilizing techniques that are known to those of ordinary skill in the art.

In preferred embodiments a polypeptide of the present invention is a bacterial polypeptide (preferably isolated from a bacterium/bacteria from genus *Bacillus*). In further preferred embodiments a polypeptide of the present invention belongs to Glycoside Hydrolase Family 16 (GH16) (*e.g.* has Glycoside hydrolases (EC 3.2.1.-) activity). For example, the polypeptide may be a polypeptide having beta-glucanase activity from within a genus *Bacillus*, *e.g.* from *Bacillus sp-62449, Bacillus akibai, Bacillus agaradhaerens, Bacillus mojavensis*. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

Catalytic Domains

In one embodiment, the present invention also relates to cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising catalytic domains having a sequence identity to amino acids 33 to 249 of SEQ ID NO: 2 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 33 to 249 of SEQ ID NO: 2. The catalytic domain preferably comprises or consists of amino acids 33 to 249 of SEQ ID NO: 2 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 62 to 245 of SEQ ID NO: 2 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 62 to 245 of SEQ ID NO: 2. The catalytic domain preferably comprises or consists of amino acids 62 to 245 of SEQ ID NO: 2 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising

said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 33 to 249 of SEQ ID NO: 3 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 33 to 249 of SEQ ID NO: 3. The catalytic domain preferably comprises or consists of amino acids 33 to 249 of SEQ ID NO: 3 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 62 to 245 of SEQ ID NO: 3 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 62 to 245 of SEQ ID NO: 3. The catalytic domain preferably comprises or consists of amino acids 62 to 245 of SEQ ID NO: 3 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 32 to 254 of SEQ ID NO: 5 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 32 to 254 of SEQ ID NO: 5. The catalytic domain preferably comprises or consists of amino acids 32 to 254 of SEQ ID NO: 5 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a

sequence identity to amino acids 60 to 249 of SEQ ID NO: 5 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 60 to 249 of SEQ ID NO: 5. The catalytic domain preferably comprises or consists of amino acids 60 to 249 of SEQ ID NO: 5 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 20 to 236 of SEQ ID NO: 7 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 20 to 236 of SEQ ID NO: 7. The catalytic domain preferably comprises or consists of amino acids 20 to 236 of SEQ ID NO: 7 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 49 to 230 of SEQ ID NO: 7 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 49 to 230 of SEQ ID NO: 7. The catalytic domain preferably comprises or consists of amino acids 49 to 230 of SEQ ID NO: 7 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity.

An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 30 to 243 of SEQ ID NO: 9 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 30 to 243 of SEQ ID NO: 9. The catalytic domain preferably comprises or consists of amino acids 30 to 243 of SEQ ID NO: 9 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to catalytic domains having a sequence identity to amino acids 55 to 239 of SEQ ID NO: 9 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In one aspect, the catalytic domains comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 55 to 239 of SEQ ID NO: 9. The catalytic domain preferably comprises or consists of amino acids 55 to 239 of SEQ ID NO: 9 or an allelic variant thereof; or is a fragment thereof having beta-glucanase activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

Binding Domains

The GH16 beta-glucanase of the invention may comprise a carbohydrate binding module (or CBM). In one embodiment a CBM is in amino acids 264-377 of SEQ ID NO: 2. An embodiment of the present invention is a composition (e.g. a cleaning or detergent composition) comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In

another embodiment a CBM is in amino acids 264-377 of SEQ ID NO: 3. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to carbohydrate binding module having a sequence identity to amino acids 264 to 377 of SEQ ID NO: 2 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In one aspect, the carbohydrate binding module comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 264 to 377 of SEQ ID NO: 2. An embodiment of the present invention is cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention also relates to carbohydrate binding module having a sequence identity to amino acids 264 to 377 of SEQ ID NO: 3 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). In one aspect, the carbohydrate binding module comprise amino acid sequences that differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from amino acids 264 to 377 of SEQ ID NO: 3. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

The carbohydrate binding module preferably comprises or consists of amino acids 264 to 377 of SEQ ID NO: 2 or an allelic variant thereof; or is a fragment thereof having carbohydrate binding activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases). The carbohydrate binding module preferably comprises or consists of amino acids

264 to 377 of SEQ ID NO: 3 or an allelic variant thereof; or is a fragment thereof having carbohydrate binding activity. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

In another embodiment, the present invention also relates to carbohydrate binding module variants of amino acids 264 to 377 of SEQ ID NO: 2 (or SEQ ID NO: 3) comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In one aspect, the number of amino acid substitutions, deletions and/or insertions introduced into the sequence of amino acids 264 to 377 of SEQ ID NO: 2 (or SEQ ID NO: 3) is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 8, 9, or 10. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

A carbohydrate binding module of the present invention may be applied in a fusion protein comprising at least one carbohydrate binding module operably linked to a catalytic domain. The catalytic domain may be from a hydrolase, isomerase, ligase, lyase, oxidoreductase, or transferase, aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellobiohydrolase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, endoglucanase, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, xylanase, or beta-xylosidase. The polynucleotide encoding the catalytic domain may be obtained from any prokaryotic, eukaryotic, or other source.

Polynucleotides

The present invention also relates to polynucleotides encoding a polypeptide, a catalytic domain, or carbohydrate binding module of the present invention, as described herein. In an embodiment, the polynucleotide encoding the polypeptide, catalytic domain, or carbohydrate binding module of the present invention has been isolated.

The techniques used to isolate or clone a polynucleotide are known in the art and include isolation from genomic DNA or cDNA, or a combination thereof. The cloning of the polynucleotides from genomic DNA can be effected, *e.g.*, by using the well known polymerase chain reaction (PCR) or antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. Other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligation activated transcription (LAT) and polynucleotide-based amplification (NASBA) may be used. The polynucleotides may be cloned from a strain of *Bacillus*, or a related organism and thus, for example, may be an allelic or species variant of the polypeptide encoding region of the polynucleotide.

Modification of a polynucleotide encoding a polypeptide of the present invention may be

necessary for synthesizing polypeptides substantially similar to the polypeptide. The term "substantially similar" to the polypeptide refers to non-naturally occurring forms of the polypeptide. These polypeptides may differ in some engineered way from the polypeptide isolated from its native source, *e.g.*, variants that differ in specific activity, thermostability, pH optimum, or the like. The variants may be constructed on the basis of the polynucleotide presented as the mature polypeptide coding sequence of sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or the cDNA sequence thereof, *e.g.*, a subsequence thereof, and/or by introduction of nucleotide substitutions that do not result in a change in the amino acid sequence of the polypeptide, but which correspond to the codon usage of the host organism intended for production of the enzyme, or by introduction of nucleotide substitutions that may give rise to a different amino acid sequence.

Compositions

The present invention also relates to cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising polypeptide(s) of the present invention. An embodiment is a cleaning or detergent composition, wherein said composition is a dishwashing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases). Preferably, the compositions are enriched in such a polypeptide. The term "enriched" indicates that the beta-glucanase activity of the composition has been increased, *e.g.*, with an enrichment factor of at least 1.1.

The compositions may comprise polypeptide(s) of the present invention as the major enzymatic component, *e.g.*, a mono-component composition. Alternatively, the compositions may comprise multiple enzymatic activities, such as one or more (*e.g.*, several) enzymes selected from the group consisting of hydrolase, isomerase, ligase, lyase, oxidoreductase, or transferase, *e.g.*, an alpha-galactosidase, alpha-glucosidase, aminopeptidase, amylase, beta-galactosidase, beta-glucosidase, beta-xylosidase, carbohydrase, carboxypeptidase, catalase, cellobiohydrolase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, endoglucanase, esterase, glucoamylase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or xylanase. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

The compositions may be prepared in accordance with methods known in the art and may be in the form of a liquid or a dry composition. The compositions may be stabilized in accordance with methods known in the art. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition

comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

Examples are given below of preferred uses of the compositions of the present invention. The dosage of the composition and other conditions under which the composition is used may be determined on the basis of methods known in the art.

Uses

The beta-glucanases of the invention may be used in applications where beta-glucan (e.g. beta-D-glucan, beta-1,3-1,4 glucan, mix-linkage beta-glucan, barley beta-glucan, oatmeal beta-glucan) needs to be degraded (e.g. under alkaline conditions). An embodiment is a cleaning or detergent composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases). Examples of where beta-glucanases could be used include detergent applications, paper and pulp productions. In one aspect, beta-glucanases of the invention may be used for cleaning dish ware, dish wash including Automatic Dish Wash (ADW) especially household automatic dish wash, Hand Dish Wash (HDW), and/or in a cleaning process such as dish wash including Automatic Dish Wash (ADW), especially household automatic dish wash, and industrial dish cleaning, dish wash including Automatic Dish Wash (ADW), and/or for at least one of the following: preventing, reducing or removing a biofilm and/or malodor from an item, and/or for anti-redeposition. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

Such beta-glucanases preferably have at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9. An embodiment of the present invention is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising said beta-glucanase polypeptide and one or more amylases (and/or one or more proteases).

Biofilm can develop when microorganisms are present on an item and stick together on the item. Some microorganisms tend to adhere to the surface of items such as textiles. Some microorganisms adhere to such surfaces and form a biofilm on the surface. The biofilm may be sticky and the adhered microorganisms and/or the biofilm may be difficult to remove. Furthermore the biofilm adhere soil due to the sticky nature of the biofilm.

The present invention concerns the use of polypeptide(s) having beta-glucanase activity for preventing, reducing or removing a biofilm from an item, wherein the polypeptide is obtained from a bacterial source and wherein the item is dishware. An embodiment is a cleaning or

detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases). In one embodiment of the invention the polypeptide having beta-glucanase activity is used for preventing, reducing or removing the stickiness of an item. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

The present invention also relates to cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase of the invention (e.g., polypeptide(s) of the present invention). The present invention also relates to said compositions comprising a beta-glucanase of the invention (e.g., polypeptide(s) of the present invention) and one or more additional enzymes. The present invention also relates to cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase of the invention (e.g., polypeptide(s) of the present invention) and one or more amylases (and/or one or more proteases), preferably said one or more amylases is one or more alpha-amylases. An embodiment is a cleaning or detergent composition wherein said composition is a dishwashing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment, the present invention relates to cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase of the invention and a suitable surfactant. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

The present invention also relates to cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising an isolated polypeptide having beta-glucanase activity selected from the group consisting of: a) a polypeptide having at least 75% sequence identity, at least 80% sequence identity, at least 81% sequence identity, at least 82% sequence identity, at least 83% sequence identity, at least 84% sequence identity, at least 85% sequence identity, at least 86% sequence identity, at least 87% sequence identity, at least 88% sequence identity, at least 89% sequence identity, at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity or even 100% sequence identity to the mature polypeptide of the sequence

selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9; b) a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or (ii) the full-length complementary strand of (i); c) a polypeptide encoded by a polynucleotide having at least 75% sequence identity, at least 80% sequence identity, at least 81% sequence identity, at least 82% sequence identity, at least 83% sequence identity, at least 84% sequence identity, at least 85% sequence identity, at least 86% sequence identity, at least 87% sequence identity, at least 88% sequence identity, at least 89% sequence identity, at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity or even 100% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8; e) a variant comprising a substitution, deletion, and/or insertion of one or more (e.g. several) amino acids of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9; and f) a fragment of a polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 60% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 75% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 81% sequence identity to the mature

polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 82% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 83% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 84% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 85% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 86% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said

composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 87% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 88% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 89% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 90% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 91% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide

having beta-glucanase activity and having at least 92% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 93% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 94% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 95% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 96% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 97% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent

compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 98% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 99% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment the compositions of the invention comprises an isolated polypeptide having beta-glucanase activity and having at least 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment, the detergent composition may be adapted for specific uses such as dish washing.

In another embodiment a composition of the present invention is a cleaning or a detergent composition. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

Alkaline Liquid detergents having high pH are widely used in cleaning, such dish wash cleaning. Liquid detergents with elevated pH are especially commonly used by consumers in North America. The high pH cleaning compositions are also used in industrial cleaning processes. Alkaline detergents include liquids having detergent properties. The pH of such detergents usually ranges in pH from 9 to 12.5. The high pH detergents typically comprise components such as surfactants, builders and bleach components and additionally they may also contain a significant amount of water and alkalis such as NaOH, TSP (Trisodium phosphate), ammonia, Sodium

carbonate, Potassium hydroxide (KOH) these alkalis are usually added in amount corresponding to 0.1 to 30 percent weight (wt). Adding enzymes to detergents is highly advantageous as the specific activities of these enzymes effectively removes specific stains from surfaces such as dishes and cutlery. However, the difficulty of maintaining acceptable enzyme stability in the high pH liquid detergents has for many years prohibited inclusion of enzymes into these detergents. In another embodiment the present invention relates high pH liquid cleaning compositions comprising an alkaline stable beta-glucanase of the present invention suitable for use in such compositions.

In another embodiment a composition of the present invention preferably contains alkaline buffer system to provide a pH of at least about 7.5, at least about 8, at least about 9, preferably pH 10 or above. Preferably the pH is from about 9 to about 13. In order to achieve the high pH it is necessary to have present an alkali metal hydroxide especially sodium or potassium hydroxide, normally in an amount of 0.1 to about 30% by weight (percentage by weight, abbreviated wt%) of the composition, and preferably 1.0 to 2.5%, or higher amounts of a suitable alkali metal silicate such as metal silicate, according to the desired pH for the product.

In another embodiment a composition of the present invention has pH 6.5 or above, preferably pH of 7.0 or above, more preferably pH 7.5 or above, and optionally comprises a bleaching agent; preferably said pH is selected in the range from about 7.5 to about 13.5, further preferably said pH is selected in the range from about 7.5 to about 12.5, most preferably said pH is selected in the range from about 8.5 to about 11.5. In a preferred embodiment, dish washing compositions with such preferred pH-ranges are solid.

In another embodiment a dish washing composition of the present invention more preferably a automatic dish washing or hand dish washing composition in form of a liquid or a gel, has a pH of 6.5 or above; preferably said pH is selected in the range from about 6.5 to 9.5, more preferably from 7.0 to about 9.0, more preferably from 7.5 to 8.5.

In another embodiment the present invention relates to a liquid cleaning composition having pH 6.5 or above, pH 6.5 or above, preferably pH 7.5 or above, comprising at least 0.001 wt % beta-glucanase, (e.g. at least 0.01 wt % beta-glucanase), wherein said beta-glucanase has an amino acid sequence which has at least 81% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. In further related embodiments beta-glucanase has an amino acid sequence which has at least 82% (or at least 83%, or 84%, or 85%, or 86%, or 87%, or 88%, or 89%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98% or 99% or 100%) sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent

composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

The cleaning or detergent compositions of the invention, especially the dish washing compositions, are formulated for hand or machine dishwashing operations, especially in household dishwashing machines, prefereably for cleaning purposes added in the main wash or with the rinse aid. It can also be used to clean the parts of the dishwasher interior during dish washing process, especially the hidden parts, like the water pipelines inside the machine, especially these in the rotatable arms, and the sieve/filter. The detergent compositions of the invention may find use in automatic dishwashing applications. An embodiment is a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

The detergent composition of the invention may be in any convenient form, e.g., a bar, a tablet, a powder, a granule, a paste or a liquid. A liquid detergent may be aqueous, typically containing up to 70% water and 0-30% organic solvent, or non-aqueous. An embodiment is a cleaning or detergent composition wherein said composition is a dishwashing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

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. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

The beta-glucanase of the invention is normally incorporated in the cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, at a level of from 0.000001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.00001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.0001% to 0.75% of enzyme protein by weight of the composition, even more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition. An embodiment is a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

Furthermore, the beta-glucanase of the invention is normally incorporated in the cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, in such amounts that their concentration in the wash water is at a level of from 0.0000001% to 1% enzyme protein, preferably at a level of from 0.000005% to 0.01% of enzyme

protein, more preferably at a level of from 0.000001% to 0.005% of enzyme protein, even more preferably at a level of from 0.00001% to 0.001% of enzyme protein in wash water. An embodiment is a cleaning or detergent composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

As is well known, the amount of enzyme will also vary according to the particular application and/or as a result of the other components included in the compositions.

A composition for use in automatic dishwash (ADW), for example, may include 0.0001%-50%, 0.001%-50%, such as 0.01%-25%, such as 0.02%-20%, such as 0.1-15% of enzyme protein by weight of the composition. An embodiment is a cleaning or detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

A preferred dish washing composition, preferably automatic dish washing composition comprises the polypetide of the invention in concentrations of 0.00001 mg enzyme protein/g composition to 100 mg enzyme protein/g composition, preferred 0.0001 mg enzyme protein/g composition to 50 mg enzyme protein/g composition, more preferred 0.001 mg enzyme protein/g composition to 20 mg enzyme protein/g composition, especially preferred 0.01 mg enzyme protein/g composition to 10 mg enzyme protein/g composition.

A preferred dish washing composition, preferably automatic dish washing composition, especially a composition formulated as unit dose product, comprises the polypeptide of the invention in amounts from 0.01 mg/job to 100 mg enzyme protein/job, preferred 0.1 mg enzyme protein/job to 20 mg/job, more preferred 0.2 to 10 mg enzyme protein/job, especially preferred 0.3 to 5 mg enzyme protein/job. For example, amounts of 0.5 mg 1 mg, 1.5 mg, 2 mg or 2.5 mg enzyme protein/job can be used. The expression mg per job (mg/job) or mg/application refers to the amount of active substance used in relation to the total weight of the composition used for a complete cleaning cycle (which is to say in the case of automatic dishwashing agents, the total amount of the cleaning agent used in a complete cleaning cycle of a dishwasher). In the case of preportioned cleaning agents (preferably automatic dishwashing agents), this information is the amount of the active substance in mg based on the total weight of the preportioned cleaning composition.

Said amounts are also applicable for each of the other invidual enzyme proteins (e.g. amylase or protease) used in the dishwashing composition of the invention.In some preferred embodiments, the detergent compositions provided herein are typically formulated such that, during use in aqueous cleaning operations, the wash water has a pH of from about 5.0 to about 13.5, or in alternative embodiments, even from about 6.0 to about 10.5, such as from about 5 to about 11, from about 5 to about 10, from about 5 to about 9, from about 5 to about 8, from about 5 to about 7, from about 6 to about 11, from about 6 to about 10, from about 6 to about 9, from about 6 to about 8, from about 6 to about 7, from about 7 to about 11, from about 7 to about 10, from about 7 to about

9, or from about 7 to about 8. Preferably, the detergent compositions provided herein are typically formulated such that, during use in aqueous cleaning operations, the wash water has a pH selected in the range from about 7.5 to about 13.5, further preferably said pH is selected in the range from about 8.5 to about 11.5, most preferably said pH is selected in the range from about 9.5 to about 10.5; further most preferably pH 7.5 or above. An embodiment is a cleaning or detergent composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

In one embodiment, the beta-glucanase in the compositions of the invention has improved stability, in particular improved storage stability in a high pH liquid cleaning composition, compared to known beta-glucanases. In a preferred embodiment, the beta-glucanase of the invention has improved stability, in particular improved storage stability, and on par or improved wash performance compared to the known beta-glucanases. An embodiment is a cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

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. An embodiment is a cleaning or detergent composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

Enzyme components weights are based on total 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 composition, 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 composition.

The enzymes of the present invention also find use in dishwashing detergent additive products. A detergent additive product comprising a beta-glucanase of the invention is suited for inclusion in a wash process when, e.g., temperature is low, such as at temperatures about 40°C or below, the pH is between 6 and 8 and the washing time short, e.g., below 30 min. A detergent additive product comprising a beta-glucanase of the invention is further ideally suited for inclusion in a alkaline wash process when, e.g., a pH selected in the range from about 7.5 to about 13.5, a temperature selected in the range from about 20°C to about 75°C, and the washing time short, e.g., below 30 min, e.g. at least 15 minutes. An embodiment is a cleaning or detergent composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases). Alternatively, a detergent additive product comprising a beta-glucanase of the invention is suited for cleaning of a household dishwasher, e.g. from built-up residues on the filter and in the sump of the machines, preferebly from residues containing beta-glucan-containig fibres. Such a machine-cleaning additive product may be suitable to clean the machine at the same time from other residues like fat or limescale.

The dishwashing detergent additive product may be a beta-glucanase of the invention and preferably an additional enzyme. In one embodiment, the additive is packaged in dosage form for addition to a cleaning process. The single dosage may comprise a pill, tablet, gelcap or other single dosage unit including powders and/or liquids. In some embodiments, filler and/or carrier material(s) are included, 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. In some embodiments filler and/or carrier materials for liquid compositions include water and/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 one particularly preferred embodiment of the dish washing composition or dishwashing detergent additives the beta-glucanase according to the invention is employed in a granular composition or liquid, the beta-glucanase may be in form of an encapsulated particle. In one embodiment, the encapsulating material is selected from the group consisting of carbohydrates, natural or synthetic gums, chitin and chitosan, cellulose and cellulose derivatives, silicates, phosphates, borates, polyvinyl alcohol, polyethylene glycol, paraffin waxes and combinations thereof.

The compositions according to the invention typically comprise one or more detergent ingredients. The term detergent compositions include articles and cleaning and treatment compositions. The term cleaning composition includes, unless otherwise indicated, tablet, granular or powder form; liquid, gel- or paste-form, Hand dishwashing agents or light duty dishwashing agents, especially those of the high-foaming type; machine dishwashing agents (or automatic dishwashing compositions), including the various tablet, granular, gel-form, liquid and rinse-aid types for household and institutional use are possible. The composition is preferably in unit dose packages, including those known in the art and those that are water soluble, water insoluble and/or water permeable. These may encompass single chamber and multichamber pouches.

In embodiments in which cleaning and/or detergent components may not be compatible with the beta-glucanase of the present invention, suitable methods may be used for keeping the cleaning and/or detergent components and the beta-glucanase separated (i.e., not in contact with each other) until combination of the two components is appropriate. Such separation methods include any suitable method known in the art (e.g., gelcaps, encapsulation, tablets, and physical separation e.g., by use of a water dissolvable pouch having one or more compartments).

As mentioned when the beta-glucanase of the invention is employed as a component of a detergent composition (e.g. a dishwashing detergent composition), it may, for example, be included in the detergent composition in the form of a non-dusting granulate, a stabilized liquid, or a protected enzyme. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are polyethyleneglycol (PEG) products with

mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591.

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)). The enzymes of the detergent compositions of the invention may also be stabilized using conventional stabilizing agents such as polyol, e.g., propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, and the composition may be formulated as described in, e.g., WO 92/19709 and WO 92/19708. The enzymes of the invention may also be stabilized by adding reversible enzyme inhibitors, e.g., of the protein type (as described in EP 544 777) or the boronic acid type. In a preferred embodiment the enzyme stabilizers are of the boronic acid type, more preferably 4-formyl phenyl boronic acid. The dishwashing composition of the invention is preferably free of boric acid and/or borate, which is to say in particular comprises boric acid and borate in amounts of less than 0.1 wt.%, preferably less than 0.01 wt.%, based on the total composition.

Other enzyme stabilizers are well known in the art, such as peptide aldehydes and protein hydrolysate, e.g. the beta-glucanase according to the invention may be stabilized using peptide aldehydes or ketones such as described in WO2005/105826 and WO2009/118375.

Protected enzymes for inclusion in a detergent composition of the invention may be prepared, as mentioned above, according to the method disclosed in EP 238 216.

The composition may be augmented with one or more agents for preventing or removing the formation of the biofilm. These agents may include, but are not limited to, dispersants, surfactants, detergents, other enzymes, anti-microbials, and biocides.

The compositions of the invention may be applied in dosing elements to be used in an auto-dosing device. The dosing elements comprising the composition of the present invention can be placed into a delivery cartridge as that described in WO 2007/052004 and WO 2007/0833141 or WO 2011/051420, WO 2011/051415, WO 2011/051416, WO 2011/051417, WO 2011/051418, WO 2011/120546 and WO 2011/131260. The dosing elements can have an elongated shape and set into an array forming a delivery cartridge which is the refill for an auto-dosing dispensing device as described in case WO 2007/051989. The delivery cartridge is to be placed in an auto-dosing delivery device, such as that described in WO 2008/053191.

Suitable disclosure of auto-dosing devices can be found in WO 2007/083139, WO 2007/051989, WO 2007/083141, WO 2007/083142 and EP2361964.

Other enzymes

In one embodiment of the dish washing composition, a beta-glucanase of the invention is combined with one or more enzymes, such as at least two enzymes, more preferred at least three, four or five enzymes. Preferably, the enzymes have different substrate specificity, e.g., proteolytic activity, amylolytic activity, lipolytic activity, hemicellulytic activity or pectolytic activity. An embodiment is a cleaning or detergent composition comprising a beta-glucanase polypeptide of the invention and one or more amylases (and/or one or more proteases).

The detergent additive as well as the detergent composition according to the invention may comprise one or more enzymes such as a protease, lipase, cutinase, an amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, xylanase, oxidase, e.g., a laccase and/or peroxidase.

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

Cellulases: Suitable cellulases include those of animal, vegetable or microbial origin. Particularly suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered variants are included. Suitable cellulases include cellulases from the genera Bacillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, e.g., the fungal cellulases produced from Humicola insolens, Myceliophthora thermophila and Fusarium oxysporum disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having color care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and WO 1999/001544.

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

Proteases: Suitable proteases include those of bacterial, fungal, plant, viral or animal origin e.g. microbial or vegetable origin. Microbial origin is preferred. Chemically modified or protein engineered variants are included. It may be an alkaline protease, such as a serine protease or a metalloprotease. A serine protease may for example be of the S1 family, such as trypsin, or the S8 family such as subtilisin. A metalloproteases protease may for example be a thermolysin from e.g. family M4 or other metalloprotease such as those from M5, M7 or M8 families.

The term "subtilases" refers to a sub-group of serine protease. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 sub-divisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin

family and the Pyrolysin family.

Examples of subtilases are those derived from Bacillus such as Bacillus lentus, B. alkalophilus, B. subtilis, B. amyloliquefaciens, Bacillus pumilus and Bacillus gibsonii described in; US7262042 and WO09/021867, and subtilisin lentus, subtilisin Novo, subtilisin Carlsberg, Bacillus licheniformis, subtilisin BPN', subtilisin 309, subtilisin 147 and subtilisin 168 described in WO89/06279 and protease PD138 described in (WO93/18140). Other useful proteases may be those described in WO92/175177, WO01/016285, WO02/026024 and WO02/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the Fusarium protease described in WO89/06270, WO94/25583 and WO05/040372, and the chymotrypsin proteases derived from Cellulomonas described in WO05/052161 and WO05/052146.

A further preferred protease is the alkaline protease from Bacillus lentus DSM 5483, as described for example in WO95/23221, and variants thereof which are described in WO92/21760, WO95/23221, EP1921147 and EP1921148.

Examples of metalloproteases are the neutral metalloprotease as described in WO07/044993 (Genencor Int.) such as those derived from Bacillus amyloliquefaciens.

Examples of useful proteases are the variants described in: WO92/19729, WO96/034946, WO98/20115, WO98/20116, WO99/011768, WO01/44452, WO03/006602, WO04/03186, WO04/041979, WO07/006305, WO11/036263, WO11/036264, especially the variants with substitutions in one or more of the following positions: 3, 4, 9, 15, 27, 36, 57, 68, 76, 87, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 106, 118, 120, 123, 128, 129, 130, 160, 167, 170, 194, 195, 199, 205, 206, 217, 218, 222, 224, 232, 235, 236, 245, 248, 252 and 274 using the BPN' numbering. More preferred the protease variants may comprise the mutations: S3T, V4I, S9R, A15T, K27R, *36D, V68A, N76D, N87S,R, *97E, A98S, S99G,D,A, S99AD, S101G,M,R S103A, V104I,Y,N, S106A, G118V,R, H120D,N, N123S, S128L, P129Q, S130A, G160D, Y167A, R170S, A194P, G195E, V199M, V205I, L217D, N218D, M222S, A232V, K235L, Q236H, Q245R, N252K, T274A (using BPN' numbering).

Suitable commercially available protease enzymes include those sold under the trade names Alcalase®, DuralaseTM, DurazymTM, Relase®, Relase® Ultra, Savinase®, Savinase® Ultra, Primase®, Polarzyme®, Kannase®, Liquanase®, Liquanase® Ultra, Ovozyme®, Coronase®, Coronase® Ultra, Neutrase®, Everlase® and Esperase® (Novozymes A/S), those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Purafect®, Purafect Prime®, PreferenzTM, Purafect MA®, Purafect Ox®, Purafect OxP®, Puramax®, Properase®, EffectenzTM, FN2®, FN3® , FN4®, Excellase®, Opticlean® and Optimase® (Danisco/DuPont), AxapemTM (Gist-Brocases N.V.), BLAP (sequence shown in Figure 29 of US5352604) and variants hereof (Henkel AG) and KAP (Bacillus alkalophilus subtilisin) from Kao.

Lipases: Suitable lipases include those of animal, vegetable or microbial origin. Particularly suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered variants are included. Examples of useful lipases include lipases from

Humicola (synonym Thermomyces), e.g., from H. lanuginosa (T. lanuginosus) as described in EP 258 068 and EP 305 216 or from H. insolens as described in WO 96/13580, a Pseudomonas lipase, e.g., from P. alcaligenes or P. pseudoalcaligenes (EP 218 272), P. cepacia (EP 331 376), P. stutzeri (GB 1,372,034), P. fluorescens, Pseudomonas sp. strain SD 705 (WO 95/06720 and WO 96/27002), P. wisconsinensis (WO 96/12012), a Bacillus lipase, e.g., from B. subtilis , B. stearothermophilus (JP 64/744992) or B. pumilus (WO 91/16422).

Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202.

Preferred commercially available lipase enzymes include LipolaseTM, Lipolase UltraTM, and Lipex™ (Novozymes A/S).

Amylases: Suitable amylases which can be used together with beta-glucanase of the invention may be an alpha-amylase or a glucoamylase and may be of bacterial or fungal origin. Chemically modified or protein engineered variants are included. Amylases include, for example, alpha-amylases obtained from Bacillus, e.g., a special strain of Bacillus licheniformis, described in more detail in GB 1,296,839. Suitable amylases include amylases having SEQ ID NO: 3 in WO 95/10603 or variants having 90% sequence identity to SEQ ID NO: 3 thereof. Preferred variants are described in WO 94/02597, WO 94/18314, WO 97/43424 and SEQ ID NO: 4 of WO 99/019467, such as variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and 444. Different suitable amylases include amylases having SEQ ID NO: 6 in WO 02/010355 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a deletion in positions 181 and 182 and a substitution in position 193. Other amylases which are suitable are hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from B. amyloliquefaciens shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of the B. licheniformis alpha-amylase shown in SEQ ID NO: 4 of WO 2006/066594 or variants having 90% sequence identity thereof. Preferred variants of this hybrid alpha-amylase are those having a substitution, a deletion or an insertion in one of more of the following positions: G48, T49, G107, H156, A181, N190, M197, I201, A209 and Q264. Most preferred variants of the hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from B. amyloliquefaciens shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 are those having the substitutions:

M197T;

H156Y+A181T+N190F+A209V+Q264S; or

G48A+T49I+G107A+H156Y+A181T+N190F+I201F+A209V+Q264S.

Further amylases which are suitable are amylases having SEQ ID NO: 6 in WO 99/019467 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a substitution, a deletion or an insertion in one or more of the following

positions: R181, G182, H183, G184, N195, I206, E212, E216 and K269. Particularly preferred amylases are those having deletion in positions R181 and G182, or positions H183 and G184. Additional amylases which can be used are those having SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873 or variants thereof having 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7 are those having a substitution, a deletion or an insertion in one or more of the following positions: 140, 181, 182, 183, 184, 195, 206, 212, 243, 260, 269, 304 and 476. More preferred variants are those having a deletion in positions 181 and 182 or positions 183 and 184. Most preferred amylase variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 are those having a deletion in positions 183 and 184 and a substitution in one or more of positions 140, 195, 206, 243, 260, 304 and 476. Other amylases which can be used are amylases having SEQ ID NO: 2 of WO 08/153815, SEQ ID NO: 10 in WO 01/66712 or variants thereof having 90% sequence identity to SEQ ID NO: 2 of WO 08/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 01/66712. Preferred variants of SEQ ID NO: 10 in WO 01/66712 are those having a substitution, a deletion or an insertion in one of more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264. Further suitable amylases are amylases having SEQ ID NO: 2 of WO 09/061380 or variants having 90% sequence identity to SEQ ID NO: 2 thereof. Preferred variants of SEQ ID NO: 2 are those having a truncation of the C-terminus and/or a substitution, a deletion or an insertion in one of more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320, Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one of more of the following positions: Q87E,R, Q98R, S125A, N128C, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:

N128C+K178L+T182G+Y305R+G475K;

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

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

S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K wherein the variants are C-terminally truncated and optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181. Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO01/66712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one of more of the following positions of SEQ ID NO: 12 in WO01/66712: R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, N484. Particular preferred amylases include variants having a deletion of D183 and G184 and having

the substitutions R118K, N195F, R320K and R458K, and a variant additionally having substitutions in one or more position selected from the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions. Other examples are amylase variants such as those described in WO2011/098531, WO2013/001078 and WO2013/001087. Commercially available amylases are DuramylTM, TermamylTM, FungamylTM, Stainzyme TM, Stainzyme PlusTM, NatalaseTM, Liquozyme X and BANTM (from Novozymes A/S), and RapidaseTM , PurastarTM/EffectenzTM, Powerase and Preferenz S100 (from Genencor International Inc./DuPont).

Peroxidases/Oxidases: Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered variants are included. Examples of useful peroxidases include peroxidases from Coprinus, e.g., from C. cinereus, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

Commercially available peroxidases include Guardzyme® (Novozymes A/S).

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

Surfactants

Typically, the cleaning or detergent compositions, wherein said cleaning or detergent composition is a dish washing composition, said composition comprises (by weight of the composition, total amount of surfactant by weight of the composition) one or more surfactants in the range of 0% to 50%, preferably from 2% to 40%, more preferably from 5% to 35%, more preferably from 7% to 30%, most preferably from 10% to 25%, even most preferably from 15% to 20%. In a preferred embodiment the detergent is a liquid or powder detergent comprising less than 40%, preferably less than 30%, more preferably less than 25%, even more preferably less than 20% by weight of surfactant. The composition may comprise from 0.1% to 15%, preferably from 0.2% to 12%, 0.5 % to 10%, most preferably from 1.0 % to 8.0 %, of one or more surfactants (total amount of surfactant by weight of the composition). Preferred surfactants are anionic surfactants, non-ionic surfactants, cationic surfactants, zwitterionic surfactants, amphoteric surfactants, and mixtures thereof.

All nonionic surfactants known to a person skilled in the art may be used as nonionic surfactants. Suitable nonionic surfactants are, for example, alkyl glycosides of the general formula $RO(G)_x$, where R corresponds to a primary straight-chain or methyl-branched, in particular methyl-branched at the 2-position, aliphatic group having 8 to 22, preferably 12 to 18

carbon atoms, and G is the symbol that denotes a glycose unit having 5 or 6 carbon atoms, preferably glucose. The degree of oligomerization x, which indicates the distribution of monoglycosides and oligoglycosides, is any number between 1 and 10; x is preferably 1.2 to 1.4.

Another class of nonionic surfactants that can preferably be used, which can be used either as the sole nonionic surfactant or in combination with other nonionic surfactants, is alkoxylated, preferably ethoxylated or ethoxylated and propoxylated fatty acid alkyl esters, preferably having 1 to 4 carbon atoms in the alkyl chain.

Nonionic surfactants of the amine oxide type, for example N-cocoalkyl-N,N-dimethylamine oxide and N-tallowalkyl-N,N-dihydroxyethylamine oxide, and of the fatty acid alkanolamide type may also be suitable. The quantity of these nonionic surfactants is preferably no more than that of the ethoxylated fatty alcohols, in particular no more than half thereof. Further suitable surfactants are polyhydroxyfatty acid amides, also known as PHFA.

Low-foaming nonionic surfactants can be used as preferred surfactants. With particular preference, the cleaning agents, preferably dishwashing agents, in particular machine dishwashing agents contain nonionic surfactants from the group of alkoxylated alcohols. Alkoxylated, advantageously ethoxylated, in particular primary alcohols having preferably 8 to 18 carbon atoms and on average 1 to 12 mol ethylene oxide (EO) per mol of alcohol, in which the alcohol residue can be linear or preferably methyl-branched at the 2-position, or can contain linear and methyl-branched residues in the mixture, such as those usually present in oxo alcohol groups, are preferably used as nonionic surfactants. However, alcohol ethoxylates having linear groups of alcohols of native origin having 12 to 18 carbon atoms, for example of coconut, palm, tallow fatty or oleyl alcohol, and an average of 2 to 8 mol EO per mol of alcohol are particularly preferred. The preferred ethoxylated alcohols include, for example, C_{12-14} alcohols having 3 EO or 4 EO, C_{9-11} alcohol having 7 EO, C_{13-15} alcohols having 3 EO, 5 EO, 7 EO, or 8 EO, C_{12-18} alcohols having 3 EO, 5 EO, or 7 EO, and mixtures thereof, such as mixtures of C_{12-14} alcohol having 3 EO and C_{12-18} alcohol having 5 EO. The degrees of ethoxylation indicated represent statistical averages that can correspond to an integer or a fractional number for a specific product. Preferred alcohol ethoxylates exhibit a restricted distribution of homologs (narrow range ethoxylates, NRE). In addition to these nonionic surfactants, fatty alcohols having more than 12 EO can also be used. Examples of these are tallow fatty alcohol having 14 EO, 25 EO, 30 EO, or 40 EO.

Nonionic surfactants that have a melting point above room temperature are particularly preferred. Nonionic surfactant(s) having a melting point above 20°C, preferably above 25°C, particularly preferably between 25 and 60°C, and in particular between 26.6 and 43.3°C, is/are particularly preferred.

Surfactants that are preferably to be used come from the groups of alkoxylated nonionic surfactants, in particular ethoxylated primary alcohols. It has been found that dishwashing compositions comprising polypeptide(s) according to the invention in combination with nonionic surfactants are surprisingly capable of reducing the built up of soils in the interior of the dish washing machine, especially on the sieve/filter.

Builders and Co-Builders

The main role of builder is to sequester divalent metal ions (such as calcium and magnesium ions) from the wash solution that would otherwise interact negatively with the surfactant system. Builders are also effective at removing metal ions and inorganic soils from the fabric surface, leading to improved removal of particulate and beverage stains. Builders are also a source of alkalinity and can buffer the pH of the wash water to a level above 7.5, e.g. 9.5 to 11. The buffering capacity is also termed reserve alkalinity, and should preferably be greater than 4 (e.g. for solid automatic dishwashing compositions).

The detergent compositions of the present invention may comprise one or more detergent builders or builder systems. Many suitable builder systems are described in the literature. The detergent composition may contain about 0-65% by weight, such as about 5% to about 50% of a detergent builder or co-builder, or a mixture thereof. In a dish washing detergent, the level of builder is typically 40-65%, particularly 50-65%. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg.

The builders include in particular silicates, carbonates and organic cobuilders, especially polycarboxylate(s) and/or aminocarboxylate(s).

Crystalline layered silicates may be used in the agents described herein. Such cleaning agents, preferably dishwashing agents, in particular machine dishwashing agents, preferably contain a weight fraction of crystalline layered silicate from 0.1 to 20 wt%, preferably from 0.2 to 15 wt%, and in particular from 0.4 to 10 wt%, in each case based on the total weight of these agents.

Other builders are the alkali carriers. Valid examples of alkali carriers include alkali metal hydroxides, alkali metal carbonates, alkali metal hydrogen carbonates, alkali metal sesquicarbonates, the described alkali silicates, alkali metal silicates and mixtures of the above-mentioned substances, wherein within the meaning of the present invention preferably the alkali carbonates, in particular sodium or potassium carbonate, sodium hydrogen carbonate or sodium sesquicarbonate may be used. However, also the corresponding potassium analogs may be useful in additon to or in complete replacement of the sodium salts. Due to the low chemical compatibility of the optional alkali metal hydroxides with the remaining ingredients of cleaning agents, in particular dishwashing agents, preferably machine dishwashing agents, compared to other builder substances, they are preferably used only in small quantities or not at all.

Builders include, but are not limited to, alkali metal silicates, alkaline earth and alkali metal carbonates, aluminosilicate builders (e.g., zeolite) and polycarboxylate compounds, ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid, the various alkali metal, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic acid and nitrilotriacetic acid, as well as polycarboxylates such as mellitic acid, succinic acid, citric acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof. Ethanole amines (MEA, DEA, and TEA) may also contribute to the buffering capacity in liquid detergents.

Any builder and/or co-builder known in the art for use in dish washing compositions, especially ADW or HDW cleaning detergents may be utilized. Non-limiting examples of builders include zeolites, carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (*e.g.*, SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as 2,2'-iminodiethan-1-ol), triethanolamine (TEA, also known as 2,2',2"-nitrilotriethan-1-ol), and (carboxymethyl)inulin (CMI), and combinations thereof.

Prefered dishwash compositions of the invention are "phosphate-free". "Phosphate-free," as used herein, means that the composition in question is essentially free of phosphates, which is to say in particular comprises phosphates in amounts of less than 0.1 wt.%, preferably less than 0.01 wt.%, based on the total composition. The expression "phosphates", as used in this context, does not include the phosphonates described hereafter.

The use of carbonate(s) and/or hydrogen carbonate(s), preferably alkali carbonate(s), particularly preferably sodium carbonate, in quantities from 2 to 50 wt%, preferably from 5 to 40 wt%, and in particular from 7.5 to 30 wt%, in each case based on the weight of the agent, preferably machine dishwashing agent, is particularly preferred. Agents that, based on the weight of the machine dishwashing agent, contain less than 20 wt%, especially less than 17 wt%, preferably less than 13 wt%, and in particular less than 9 wt% carbonate(s) and/or hydrogen carbonate(s), preferably alkali carbonate(s), particularly preferably sodium or potassium carbonate, are particularly preferred.

In particular, polycarboxylates/polycarboxylic acids, polymeric polycarboxylates, aspartic acid, polyacetals, dextrins, further organic cobuilders, and phosphonates should be mentioned as organic cobuilders. These substance classes are described hereafter.

Usable organic builder substances are, for example, the polycarboxylic acids that can be used in the form of the free acid and/or of the sodium salts thereof, wherein polycarboxylic acids shall be understood to mean those carboxylic acids that carry more than one acid function. These include, for example, citric acid, adipic acid, succinic acid, glutaric acid, malic acid, tartaric acid, maleic acid, fumaric acid, saccharic acids, nitrilotriacetic acid (NTA), provided that such use is not objectionable for ecological reasons, and mixtures thereof. In addition to the builder effect, the free acids typically also have the property of being an acidifying component and are thus also used as agents to set a lower and milder pH value. In particular, citric acid, succinic acid, glutaric acid, adipic acid, gluconic acid and arbitrary mixtures of these should be mentioned here.

The use of citric acid and/or citrates in these agents has proven to be particularly advantageous for the cleaning and rinsing power of agents described herein. Preferred are therefore cleaning agents, preferably dishwashing agents, particularly preferably machine dishwashing agents, characterized in that the agent contains citric acid or a salt of citric acid, and the weight fraction of the citric acid or of the salt of citric acid especially is more than 10 wt%, preferably more than 15 wt%, and in particular between 20 and 40 wt%.

Further preferred examples include chelators such as aminocarboxylates, aminopolycarboxylates and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2"-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-*N*,*N*'-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-*N*,*N*-diacetic acid (GLDA), *N*-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-*N*-monoacetic acid (ASMA), aspartic acid-*N*,*N*-diacetic acid (ASDA), aspartic acid-*N*-monopropionic acid (ASMP), iminodisuccinic acid (IDA), *N*-(2-sulfomethyl)-aspartic acid (SMAS), *N*-(2-sulfoethyl)-aspartic acid (SEAS), *N*-(2-sulfomethyl)-glutamic acid (SMGL), *N*-(2-sulfoethyl)-glutamic acid (SEGL), *N*-methyliminodiacetic acid (MIDA), α-alanine-*N*,*N*-diacetic acid (α-ALDA), serine-*N*,*N*-diacetic acid (SEDA), isoserine-*N*,*N*-diacetic acid (ISDA), phenylalanine-*N*,*N*-diacetic acid (PHDA), anthranilic acid-*N*,*N*-diacetic acid (ANDA), sulfanilic acid-*N*,*N*-diacetic acid (SLDA) , taurine-*N*,*N*-diacetic acid (TUDA) and sulfomethyl-*N*,*N*-diacetic acid (SMDA), *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N'*,*N''*-triacetic acid (HEDTA), diethanolglycine (DEG), and combinations and salts thereof. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053.

Aminocarboxylic acids and/or the salts thereof are another significant class of phosphate-free builders. Particularly preferred representatives of this class are methylglycine diacetic acid (MGDA) or the salts thereof, and glutamine diacetic acid (GLDA) or the salts thereof, or ethylenediamine diacetic acid (EDDS) or the salts thereof. The content of these amino carboxylic acids or of the salts thereof can amount to, for example, between 0.1 and 15

wt%, preferably between 0.5 and 10 wt%, and in particular between 0.5 and 6 wt%. Aminocarboxylic acids and the salts thereof can be used together with the above-mentioned builders, in particular also with the phosphate-free builders.

The detergent composition may also contain 0-50% by weight, such as about 5% to about 30%, of a detergent co-builder. The detergent composition may include include a co-builder alone, or in combination with a builder, for example a zeolite builder. Non-limiting examples of co-builders include homopolymers of polyacrylates or copolymers thereof, such as poly(acrylic acid) (PAA) or copoly(acrylic acid/maleic acid) (PAA/PMA).

Suitable builders moreover include polymeric polycarboxylates; for example, these are the alkali metal salts of polyacrylic acid or of polymethacrylic acid, for example those having a relative molar mass from 500 to 70,000 g/mol. Suitable polymers are in particular polyacrylates, which preferably have a molar mass from 2000 to 20,000 g/mol. Due to the superior solubility thereof, short-chain polyacrylates having molar masses from 2000 to 10,000 g/mol, and particularly preferably from 3000 to 5000 g/mol, may in turn be preferred from this group.

In a preferred embodiment the dish washing composition of the invention may comprise, if allowed according to the jurisdiction of the country where the dishwashing composition is used, phosphonates, preferable 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis(methylenephosphonic acid) (DTMPA or DTPMPA), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP).

In an alternative embodiment the dish washing composition of the invention are phosphate-free as defined above and comprise no ore only small amounts of phosphonates. In a preferred embodiment the dish washing composition contains less than 15 mg/job phosphorus, more preferred less than 10 mg/job phosphorus, most preferred less than 1 mg/job phosphorus.

Bleaches

The detergent compositions of the present invention may comprise one or more bleaching agents. In particular powdered detergents may comprise one or more bleaching agents. Suitable bleaching agents include other photobleaches, pre-formed peracids, sources of hydrogen peroxide, bleach activators, hydrogen peroxide, bleach catalysts and mixtures thereof. In general, when a bleaching agent is used, the compositions of the present invention may comprise from about 0.1% to about 50% or even from about 0.1% to about 25% bleaching agent by weight of the subject cleaning composition. Examples of suitable bleaching agents include:

(1) other photobleaches for example Vitamin K3;

(2) preformed peracids: Suitable preformed peracids include, but are not limited to, compounds selected from the group consisting of percarboxylic acids and salts, percarbonic acids

and salts, perimidic acids and salts, peroxymonosulfuric acids and salts, for example, Oxone , and mixtures thereof. Suitable percarboxylic acids include hydrophobic and hydrophilic peracids having the formula R-(C=O)O-O-M wherein R is an alkyl group, optionally branched, having, when the peracid is hydrophobic, from 6 to 14 carbon atoms, or from 8 to 12 carbon atoms and, when the peracid is hydrophilic, less than 6 carbon atoms or even less than 4 carbon atoms; and M is a counterion, for example, sodium, potassium or hydrogen;

(3) sources of hydrogen peroxide, for example, inorganic perhydrate salts, including alkali metal salts such as sodium salts of perborate (usually mono- or tetra-hydrate), percarbonate, persulphate, perphosphate, persilicate salts and mixtures thereof. In one aspect of the invention the inorganic perhydrate salts are selected from the group consisting of sodium salts of perborate, percarbonate and mixtures thereof. When employed, inorganic perhydrate salts are typically present in amounts of from 0.05 to 40 wt%, or 1 to 30 wt% of the overall composition and are typically incorporated into such compositions as a crystalline solid that may be coated. Suitable coatings include inorganic salts such as alkali metal silicate, carbonate or borate salts or mixtures thereof, or organic materials such as water-soluble or dispersible polymers, waxes, oils or fatty soaps. Useful bleaching compositions are described in U.S. Patent Nos. 5,576,282, and 6,306,812;

(4) bleach activators having R-(C=O)-L wherein R is an alkyl group, optionally branched, having, when the bleach activator is hydrophobic, from 6 to 14 carbon atoms, or from 8 to 12 carbon atoms and, when the bleach activator is hydrophilic, less than 6 carbon atoms or even less than 4 carbon atoms; and L is leaving group. Examples of suitable leaving groups are benzoic acid and derivatives thereof - especially benzene sulphonate. Suitable bleach activators include dodecanoyl oxybenzene sulphonate, decanoyl oxybenzene sulphonate, decanoyl oxybenzoic acid or salts thereof, 3,5,5-trimethyl hexanoyloxybenzene sulphonate, tetraacetyl ethylene diamine (TAED) and nonanoyloxybenzene sulphonate (NOBS). Suitable bleach activators are also disclosed in WO 98/17767. While any suitable bleach activator may be employed, in one aspect of the invention the subject cleaning composition may comprise NOBS, TAED or mixtures thereof; and

(5) bleach catalysts that are capable of accepting an oxygen atom from peroxyacid and transferring the oxygen atom to an oxidizable substrate are described in WO 2008/007319. Suitable bleach catalysts include, but are not limited to: iminium cations and polyions; iminium zwitterions; modified amines; modified amine oxides; N-sulphonyl imines; N-phosphonyl imines; N-acyl imines; thiadiazole dioxides; perfluoroimines; cyclic sugar ketones and mixtures thereof. The bleach catalyst will typically be comprised in the detergent composition at a level of from 0.0005% to 0.2%, from 0.001% to 0.1%, or even from 0.005% to 0.05% by weight.

When present, the peracid and/or bleach activator is generally present in the composition in an amount of from about 0.1 to about 60 wt%, from about 0.5 to about 40 wt% or even from about 0.6 to about 10 wt% based on the composition. One or more hydrophobic peracids or

precursors thereof may be used in combination with one or more hydrophilic peracid or precursor thereof.

The amounts of hydrogen peroxide source and peracid or bleach activator may be selected such that the molar ratio of available oxygen (from the peroxide source) to peracid is from 1:1 to 35:1, or even 2:1 to 10:1.

Adjunct materials

Dispersants - The detergent compositions of the present invention can also contain dispersants. In particular powdered detergents may comprise dispersants. Suitable water-soluble organic materials include the homo- or 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.

Soil release polymers - The dishwashing compositions of the present invention may also include one or more soil release polymers which aid the removal of soils from fabrics such as cotton and polyester based fabrics, in particular the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalte based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides. Another type of soil release polymers are amphiphilic alkoxylated grease cleaning polymers comprising a core structure and a plurality of alkoxylate groups attached to that core structure. The core structure may comprise a polyalkylenimine structure or a polyalkanolamine structure as described in detail in WO 2009/087523. Furthermore random graft co-polymers are suitable soil release polymers Suitable graft co-polymers are described in more detail in WO 2007/138054, WO 2006/108856 and WO 2006/113314. Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose deriviatives such as those described in EP 1 867 808 or WO 2003/040279. Suitable cellulosic polymers include cellulose, cellulose ethers, cellulose esters, cellulose amides and mixtures thereof. Suitable cellulosic polymers include anionically modified cellulose, nonionically modified cellulose, cationically modified cellulose, zwitterionically modified cellulose, and mixtures thereof. Suitable cellulosic polymers include methyl cellulose, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, ester carboxy methyl cellulose, and mixtures thereof.

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

Other suitable adjunct materials include, but are not limited to bactericides, binders,

carriers, dyes, enzyme stabilizers, fillers, foam regulators, hydrotropes, perfumes, pigments, sod suppressors, solvents, structurants for liquid detergents and/or structure elasticizing agents.

A typical basic formulation for a machine dishwashing composition, that can preferably be used, for example, for solid powders or preferably in tablet form, comprises the following materials:

One ore more polypeptide(s) according to the invention 0.001 to 5 wt% (enzyme protein) and

Sodium citrate	10 to 50 wt%
MGDA or GLDA, sodium salt	0-40 wt%
sodium carbonate	10 to 30 wt%
sodium disilicate	0 to 40 wt%
sodium percarbonate	5 to 20 wt%
bleach activator	1 to 4 wt%
bleach catalyst	0.001 to 1 wt%
sulfopolymer	2.5 to 15 wt%
polycarboxylate	0.5 to 15 wt%
nonionic surfactant(s)	1.5 to 15 wt%
phosphonate	0 to 10 wt%
proteases (enzyme protein)	0.0001 to 5 wt%
amylase (enzyme protein)	0.0001 to 5 wt%
Glass corrosion inhibitor	0 to 1 wt%

wherein the information in wt% in each case is based on the total composition.
Additionally the composition may contain additives as disintegrants, silver protection agents, filling agents, processing aids, pH adjusting agents, perfume, dyes etc.

A typical basic formulation for a automatic dishwashing composition, especially useful in household dishwashers, that can preferably be used, for example, in gel-form or preferably in liquid form, comprises the following materials:

One or more polypeptide according to the invention (enzyme protein) 0.001 to 5 wt% and

Sodium citrate	5-50 wt%
MGDA or GLDA, tetrasodium salt	0-20 wt%
Sulfopolymer	2.5-15 wt%
polycarboxylate	0-10 wt%
nonionic surfactant(s)	0.5-10 wt%
Phosphonate	0-10 wt%
proteases (enzyme protein)	0.0001 to 5 wt%
amylase (enzyme protein)	0.0001 to 5 wt%

wherein the information in wt% in each case is based on the total composition.

Additionally the composition may contain additives as rheology modifiers, filling agents, processing aids, pH adjusting agents, perfume, dyes etc.

The soils and stains that are important for detergent formulators are composed of many different substances, and a range of different enzymes, all with different substrate specificities have been developed for use in detergents both in relation to laundry and hard surface cleaning, such as dishwashing. These enzymes are considered to provide an enzyme detergency benefit, since they specifically improve stain removal in the cleaning process they are applied in as compared to the same process without enzymes. Stain removing enzymes that are known in the art include enzymes such as carbohydrases, amylases, proteases, lipases, cellulases, hemicellulases, xylanases, cutinases, and pectinase.

In a preferred aspect of the present invention the beta-glucanase of the invention may be combined with at least two enzymes. These additional enzymes are described in details in the section “other enzymes”, more preferred at least three, four or five enzymes. Preferably, the enzymes have different substrate specificity, e.g., carbolytic activity, proteolytic activity, amylolytic activity, lipolytic activity, hemicellulytic activity or pectolytic activity. The enzyme combination may for example be a beta-glucanase of the invention with another stain removing enzyme, e.g., a beta-glucanase of the invention and a protease, a beta-glucanase of the invention and a serine protease, a beta-glucanase of the invention and an amylase, a beta-glucanase of the invention and a cellulase, beta-glucanase of the invention and a lipase, a beta-glucanase of the invention and a cutinase, a beta-glucanase of the invention and a pectinase or a beta-glucanase of the invention and an anti-redeposition enzyme. More preferably, the beta-glucanase of the invention is combined with at least two other stain removing enzymes, e.g., a beta-glucanase of the invention, a lipase and an amylase; or a beta-glucanase of the invention, a protease and an amylase; or a beta-glucanase of the invention, a protease and a lipase; or a beta-glucanase of the invention, a protease and a pectinase; or a beta-glucanase of the invention, a protease and a cellulase; or a beta-glucanase of the invention, a protease and a hemicellulase; or a beta-glucanase of the invention, a protease and a cutinase; or a beta-glucanase of the invention, an amylase and a pectinase; or a beta-glucanase of the invention, an amylase and a cutinase; or a beta-glucanase of the invention, an amylase and a cellulase; or a beta-glucanase of the invention, an amylase and a hemicellulase; or a beta-glucanase of the invention, a lipase and a pectinase; or a beta-glucanase of the invention, a lipase and a cutinase; or a beta-glucanase of the invention, a lipase and a cellulase; or a beta-glucanase of the invention, a lipase and a hemicellulase. Even more preferably, a beta-glucanase of the invention may be combined with at least three other stain removing enzymes, e.g., a beta-glucanase of the invention, a protease, a lipase and an amylase; or a beta-glucanase of the invention, a protease, an amylase and a pectinase; or a beta-glucanase of the invention, a protease, an amylase and a cutinase; or a beta-glucanase of the invention, a protease, an amylase and a cellulase; or a beta-glucanase of the invention, a

protease, an amylase and a hemicellulase; or a beta-glucanase of the invention, an amylase, a lipase and a pectinase; or a beta-glucanase of the invention, an amylase, a lipase and a cutinase; or a beta-glucanase of the invention, an amylase, a lipase and a cellulase; or a beta-glucanase of the invention, an amylase, a lipase and a hemicellulase; or a beta-glucanase of the invention, a protease, a lipase and a pectinase; or a beta-glucanase of the invention, a protease, a lipase and a cutinase; or a beta-glucanase of the invention, a protease, a lipase and a cellulase; or a beta-glucanase of the invention, a protease, a lipase and a hemicellulase. A beta-glucanase according to the present invention may be combined with any of the enzymes selected from the non-exhaustive list comprising: carbohydrases, such as an amylase, a hemicellulase, a pectinase, a cellulase, a xanthanase or a pullulanase, a peptidase, a protease or a lipase.

In a preferred embodiment, a beta-glucanase of the invention is combined with a serine protease, e.g., an S8 family protease such as Savinase®.

In another embodiment of the present invention, a beta-glucanase of the invention may be combined with one or more metalloproteases, such as an M4 metalloprotease, including Neutrase® or Thermolysin. Such combinations may further comprise combinations of the other detergent enzymes as outlined above.

The cleaning process is a dishwashing process.The cleaning process can for example be carried out in a machine washing process or in a manual washing process. The washing solution can for example be an aqueous washing solution containing a detergent composition.

The last few years there has been an increasing interest in replacing components in detergents, which is derived from petrochemicals with renewable biological components such as enzymes and polypeptides without compromising the wash performance. When the components of detergent compositions change new enzyme activities or new enzymes having alternative and/or improved properties compared to the common used detergent enzymes such as proteases, lipases and amylases is needed to achieve a similar or improved wash performance when compared to the traditional detergent compositions.

Typical detergent compositions includes various components in addition to the enzymes, these components have different effects, some components like the surfactants lower the surface tension in the detergent, which allows the stain being cleaned to be lifted and dispersed and then washed away, other components like bleach systems removes discolor often by oxidation and many bleaches also have strong bactericidal properties, and are used for disinfecting and sterilizing. Yet other components like builder and chelator softens, e.g., the wash water by removing the metal ions from the liquid.

In a particular embodiment, the invention concerns the use of a composition comprising a beta-glucanase of the invention, wherein said composition further comprises at least one or more of the following a surfactant, a builder, a chelator or chelating agent, bleach system or bleach component in dish wash.

In a preferred embodiment of the invention the amount of a surfactant, a builder, a chelator

or chelating agent, bleach system and/or bleach component are reduced compared to amount of surfactant, builder, chelator or chelating agent, bleach system and/or bleach component used without the added beta-glucanase of the invention. Preferably the at least one component which is a surfactant, a builder, a chelator or chelating agent, bleach system and/or bleach component is present in an amount that is 1% less, such as 2% less, such as 3% less, such as 4% less, such as 5% less, such as 6% less, such as 7% less, such as 8% less, such as 9% less, such as 10% less, such as 15% less, such as 20% less, such as 25% less, such as 30% less, such as 35% less, such as 40% less, such as 45% less, such as 50% less than the amount of the component in the system without the addition of beta-glucanase of the invention, such as a conventional amount of such component. In one aspect, the beta-glucanase of the invention is used in detergent compositions wherein said composition is free of at least one component which is a surfactant, a builder, a chelator or chelating agent, bleach system or bleach component and/or polymer.

Detergent compositions

In one embodiment, the invention is directed to detergent compositions comprising an enzyme of the present invention in combination with one or more additional cleaning composition components. The choice of additional components is within the skill of the artisan and includes conventional ingredients, including the exemplary non-limiting components set forth below.

In one embodiment, the invention is directed to an ADW (Automatic Dish Wash) compositions comprising an enzyme of the present invention in combination with one or more additional ADW composition components. The choice of additional components is within the skill of the artisan and includes conventional ingredients, including the exemplary non-limiting components set forth below.

Surfactants

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

When included therein the detergent will usually contain from about 1% to about 40% by weight of an anionic surfactant, such as from about 5% to about 30%, including from about 5% to about 15%, or from about 15% to about 20%, or from about 20% to about 25% of an anionic surfactant. Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, linear alkylbenzenesulfonates (LAS), isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-

2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS), primary alcohol sulfates (PAS), alcohol ethersulfates (AES or AEOS or FES, also known as alcohol ethoxysulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenyl/tetradecenyl succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or salt of fatty acids (soap), and combinations thereof.

When included therein the detergent will usually contain from about from about 1% to about 40% by weigh of a cationic surfactant, for example from about 0.5% to about 30%, in particular from about 1% to about 20%, from about 3% to about 10%, such as from about 3% to about 5%, from about 8% to about 12% or from about 10% to about 12%. Non-limiting examples of cationic surfactants include alkyldimethylethanolamine quat (ADMEAQ), cetyltrimethylammonium bromide (CTAB), dimethyldistearylammonium chloride (DSDMAC), and alkylbenzyldimethylammonium, alkyl quaternary ammonium compounds, alkoxylated quaternary ammonium (AQA) compounds, ester quats, and combinations thereof.

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

When included therein the detergent will usually contain from about 0 % to about 40% by weight of a semipolar surfactant. Non-limiting examples of semipolar surfactants include amine oxides (AO) such as alkyldimethylamineoxide, *N*-(coco alkyl)-*N,N*-dimethylamine oxide and *N*-(tallow-alkyl)-*N,N*-bis(2-hydroxyethyl)amine oxide, and combinations thereof.

When included therein the detergent will usually contain from about 0 % to about 40% by weight of a zwitterionic surfactant. Non-limiting examples of zwitterionic surfactants include betaines such as alkyldimethylbetaines, sulfobetaines, and combinations thereof.

Hydrotropes

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

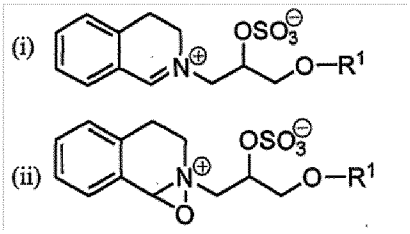
The detergent may contain 0-10% by weight, for example 0-5% by weight, such as about 0.5 to about 5%, or about 3% to about 5%, of a hydrotrope. Any hydrotrope known in the art for use in detergents may be utilized. Non-limiting examples of hydrotropes include sodium benzenesulfonate, sodium p-toluene sulfonate (STS), sodium xylene sulfonate (SXS), sodium cumene sulfonate (SCS), sodium cymene sulfonate, amine oxides, alcohols and polyglycolethers, sodium hydroxynaphthoate, sodium hydroxynaphthalene sulfonate, sodium ethylhexyl sulfate, and combinations thereof.

Bleaching Systems

The detergent may contain 0-30% by weight, such as about 1% to about 20%, of a bleaching system. Any bleaching system known in the art for use in dish wash, especially automatic dish washing (ADW) cleaning detergents may be utilized. Suitable bleaching system components include bleaching catalysts, photobleaches, bleach activators, sources of hydrogen peroxide such as sodium percarbonate, sodium perborates and hydrogen peroxide—urea (1:1), preformed peracids and mixtures thereof. Suitable preformed peracids include, but are not limited to, peroxycarboxylic acids and salts, diperoxydicarboxylic acids, perimidic acids and salts, peroxymonosulfuric acids and salts, for example, Oxone (R), and mixtures thereof. Non-limiting examples of bleaching systems include peroxide-based bleaching systems, which may comprise, for example, an inorganic salt, including alkali metal salts such as sodium salts of perborate (usually mono- or tetra-hydrate), percarbonate, persulfate, perphosphate, persilicate salts, in combination with a peracid-forming bleach activator. The term bleach activator is meant herein as a compound which reacts with hydrogen peroxide to form a peracid via perhydrolysis. The peracid

thus formed constitutes the activated bleach. Suitable bleach activators to be used herein include those belonging to the class of esters, amides, imides or anhydrides. Suitable examples are tetraacetylethylenediamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene-1-sulfonate (ISONOBS), 4-(dodecanoyloxy)benzene-1-sulfonate (LOBS), 4-(decanoyloxy)benzene-1-sulfonate, 4-(decanoyloxy)benzoate (DOBS or DOBA), 4-(nonanoyloxy)benzene-1-sulfonate (NOBS), and/or those disclosed in WO98/17767.
It is also possible to use combinations of conventional bleach activators. These bleach activators are preferably used in quantities of up to 10 wt%, in particular 0.1 wt% to 8 wt%, particularly 2 to 8 wt%, and particularly preferably 2 to 6 wt%, based in each case on the total weight of the bleach activator-containing agent.

A particular family of bleach activators of interest was disclosed in EP624154 and particulary preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that it is environmentally friendly Furthermore acetyl triethyl citrate and triacetin have good hydrolytical stability in the product upon storage and are efficient bleach activators. Finally ATC is multifunctional, as the citrate released in the perhydrolysis reaction may function as a builder. Alternatively, the bleaching system may comprise peroxyacids of, for example, the amide, imide, or sulfone type. The bleaching system may also comprise peracids such as 6-(phthalimido)peroxyhexanoic acid (PAP). The bleaching system may also include a bleach catalyst. In some embodiments the bleach component may be an organic catalyst selected from the group consisting of organic catalysts having the following formulae:



(iii) and mixtures thereof;

wherein each R^1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R^1 is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R^1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, dodecyl, tetradecyl, hexadecyl, octadecyl, isononyl, isodecyl, isotridecyl and isopentadecyl. Other exemplary bleaching systems are described, e.g. in WO2007/087258, WO2007/087244, WO2007/087259, EP1867708 (Vitamin K) and WO2007/087242. Suitable photobleaches may for example be sulfonated zinc or aluminium phthalocyanines.

Preferably the bleach component comprises a source of peracid in addition to bleach catalyst, particularly organic bleach catalyst.

In a preferred embodiment the dishwashing compositions, in particular machine dishwashing compositions, especially solid automatic dishwashing compositions can furthermore contain bleach catalysts. The usable bleach catalysts include, but are not limited to, the group of

the bleach-boosting transition metal salts and transition metal complexes, preferably the Mn, Fe, Co, Ru or Mo complexes, particularly preferably from the group of the manganese and/or cobalt salts and/or complexes, in particular the cobalt (ammine) complexes, the cobalt (acetate) complexes, the cobalt (carbonyl) complexes, the chlorides of cobalt or manganese, manganese sulfate and the complexes of manganese with 1,4,7-trimethyl-1,4,7-triazacyclononane (Mn_3-TACN) or 1,2,4,7-tetramethyl-1,4,7-triazacyclononane (Mn_4-TACN).

Cleaning compositions, preferably dishwashing compositions, in particular machine dishwashing compositions that contain 0.001 to 1 wt%, preferably 0.01 to 0.1 wt% bleach catalyst, preferably an Mn complex, in particular a complex of manganese with 1,4,7-trimethyl-1,4,7-triazacyclononane (Mn_3-TACN) or 1,2,4,7-tetramethyl-1,4,7-triazacyclononane (Mn_4-TACN) are preferred.

The source of peracid may be selected from (a) pre-formed peracid; (b) percarbonate, perborate or persulfate salt (hydrogen peroxide source) preferably in combination with a bleach activator; and (c) perhydrolase enzyme and an ester for forming peracid in situ in the presence of water in a dish wash treatment step.

Polymers

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

In a preferred embodiment the composition of the invention also comprises one or more copolymeric polycarboxylates, in particular those of acrylic acid with methacrylic acid, and of acrylic acid or methacrylic acid with maleic acid.

The (co)polymeric polycarboxylates can be used either as a powder or as an aqueous solution. The content of (co)polymeric polycarboxylates in the cleaning agents, preferably dishwashing

agents, in particular machine dishwashing agents, is preferably 0.5 to 20 wt%, and in particular 3 to 10 wt%.

To improve water solubility, the polymers can also contain allyl sulfonic acids, such as allyloxybenzene sulfonic acid and methallyl sulfonic acid, as a monomer. Further preferred copolymers are those that contain acrolein and acrylic acid/acrylic acid salts or acrolein and vinylacetate as monomers.

Moreover, all compounds that are able to form complexes with alkaline earth ions can be used as builders.

In a most preferred embodiment of the invention the dishwash detergent and cleaning composition of the invention additionally comprises a copolymer that contains at least one sulfonic acid containing monomer, a so-called sulfo polymer.

The amount by weight of the sulfo polymer in the total weight of the detergent or cleaning agent produced according to the invention is preferably 0.1 to 20% by weight, in particular 0.5 to 18% by weight, particularly preferably 1.0 to 15% by weight, in particular 4 to 14% by weight, particularly 6 to 12% by weight.

The aqueous solutions of the at least one sulfo polymer typically contain 20 to 70% by weight, in particular 30 to 50% by weight, preferably approx. 35 to 40% by weight sulfo polymer(s).

A polysulfonate copolymer, optionally a hydrophobically modified polysulfonate copolymer, is preferably used as the sulfo polymer. The copolymers may contain two, three, four or more different monomer units.

Preferred polysulfonate copolymers contain at least one monomer from the group of unsaturated carboxylic acids in addition to monomer(s) containing sulfonic acid groups.

Unsaturated carboxylic acids of the formula $R^1(R^2)C=C(R^3)COOH$, in which R^1 to R^3 independently of one another stand for -H, $-CH_3$, a linear or branched saturated alkyl radical with 2 to 12 carbon atoms, a linear or branched mono- or polyunsaturated alkenyl radical with 2 to 12 carbon atoms, $-NH_2$, -OH or -COOH-substituted alkyl or alkenyl radicals as defined above, or standing for -COOH or $-COOR^4$, where R^4 is a saturated or unsaturated linear or branched hydrocarbon radical with 1 to 12 carbon atoms are particularly preferably used as unsaturated carboxylic acid(s).

Particularly preferred unsaturated carboxylic acids include acrylic acid, methacrylic acid, ethacrylic acid, α-chloroacrylic acid, α-cyanoacrylic acid, crotonic acid, α-phenylacrylic acid, maleic acid, maleic anhydride, fumaric acid, itaconic acid, citraconic acid, methylene malonic acid, sorbic acid, cinnamic acid or mixtures thereof. The unsaturated dicarboxylic acids may of course also be used.

Preferred monomers containing sulfonic acid groups are those of the formula

$R^5(R^6)C=C(R^7)-X-SO_3H$,

where R^5 to R^7 independently of one another stand for -H, $-CH_3$, a linear or branched saturated alkyl radical with 2 to 12 carbon atoms, a linear or branched mono- or polyunsaturated alkenyl radical with 2 to 12 carbon atoms, $-NH_2$, -OH or -COOH-substituted alkyl or alkenyl radicals or -COOH or $-COOR^4$, where R^4 is a saturated or unsaturated linear or branched hydrocarbon radical with 1 to 12 carbon atoms, and X stands for a spacer group, which is optionally present and is selected from $-(CH_2)_n-$ where n = 0 to 4, $-COO-(CH_2)_k-$ where k = 1 to 6, $-C(O)-NH-C(CH_3)_2-$, $-C(O)-NH-C(CH_3)_2-CH_2-$ and $-C(O)-NH-CH(CH_3)-CH_2-$.

Among these monomers, the preferred ones are those of the formulas

$$H_2C=CH-X-SO_3H$$

$$H_2C=C(CH_3)-X-SO_3H$$

$$HO_3S-X-(R^6)C=C(R^7)-X-SO_3H,$$

where R^6 and R^7, independently of one another, are selected from -H, $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$ and $-CH(CH_3)_2$, and X stands for a spacer group, which is optionally present and is selected from $-(CH_2)_n-$ where n = 0 to 4, $-COO-(CH_2)_k-$ where k = 1 to 6, $-C(O)-NH-C(CH_3)_2-$, $-C(O)-NH-C(CH_3)_2-CH_2-$ and $-C(O)-NH-CH(CH_3)-CH_2-$.

Particularly preferred monomers that contain sulfonic acid groups include 1-acrylamido-1-propanesulfonic acid, 2-acrylamido-2-propanesulfonic acid, 2-acrylamido-2-methyl-1-propanesulfonic acid, 2-methacrylamido-2-methyl-1-propanesulfonic acid, 3-methacrylamido-2-hydroxypropanesulfonic acid, allylsulfonic acid, methallylsulfonic acid, allyloxybenzenesulfonic acid, methallyloxybenzenesulfonic acid, 2-hydroxy-3-(2-propenyloxy)propanesulfonic acid, 2-methyl-2-propene-1-sulfonic acid, styrenesulfonic acid, vinylsulfonic acid, 3-sulfopropyl acrylate, 3-sulfopropyl methacrylate, sulfomethacrylamide, sulfomethyl methacrylamide as well as mixtures of the aforementioned acids or their water-soluble salts.

The sulfonic acid groups in the polymers may be present entirely or partially in neutralized form, i.e., in some or all of the sulfonic acid groups, the acidic hydrogen atom in the sulfonic acid group may be replaced by metal ions, preferably alkali metal ions and in particular sodium ions. The use of copolymers containing partially or fully neutralized sulfonic acid groups is preferred according to the invention.

The monomer distribution in the copolymers preferred for use according to the invention is preferably 5% to 95% by weight in copolymers that contain only monomers containing carboxylic acid groups and monomers containing sulfonic acid groups, particularly preferably the amount of the monomer containing sulfonic acid groups is 50% to 90% by weight and the amount of the monomer containing carboxylic acid groups is 10% to 50% by weight and the monomers here are preferably selected from those listed above.

The molecular weight of the sulfo copolymers preferred for use according to the invention may be varied to adjust the properties of the polymers to the desired intended purpose. Preferred cleaning compositions are characterized in that the copolymers have molecular weights of 2000 to 200,000 $gmol^{-1}$, preferably 4000 to 25,000 $gmol^{-1}$ and in particular 5000 to 15,000 $gmol^{-1}$.

In another preferred embodiment, the copolymers also comprise at least one nonionic, preferably hydrophobic, monomer in addition to the monomer that contains carboxyl groups and the monomer that contains sulfonic acid groups. The clear rinsing performance of automatic dishwasher detergents according to the invention has been improved by using these polymers in particular.

Anionic copolymers comprising monomers that contain carboxylic acid groups, monomers that contain sulfonic acid groups and nonionic monomers, in particular hydrophobic monomers, are therefore preferred according to the invention.

Preferably monomers of the general formula $R^1(R^2)C=C(R^3)-X-R^4$, in which R^1 to R^3 independently of one another stand for -H, $-CH_3$ or $-C_2H_5$, X stands for a spacer group that is optionally present and is selected from $-CH_2-$, -C(O)O- and -C(O)-NH-, and R^4 stands for a linear or branched saturated alkyl radical with 2 to 22 carbon atoms or for an unsaturated, preferably aromatic radical with 6 to 22 carbon atoms, are preferably used as the nonionic monomers.

Particularly preferred nonionic monomers include butene, isobutene, pentene, 3-methylbutene, 2-methylbutene, cyclopentene, hexene, 1-hexene, 2-methyl-1-pentene, 3-methyl-1-pentene, cyclohexene, methyl cyclopentene, cycloheptene, methyl cyclohexene, 2,4,4-trimethyl-1-pentene, 2,4,4-trimethyl-2-pentene, 2,3-dimethyl-1-hexene, 2,4-dimethyl-1-hexene, 2,5-dimethyl-1-hexene, 3,5-dimethyl-1-hexene, 4,4-dimethyl-1-hexane, ethyl cyclohexyne, 1-octene, α-olefins with 10 or more carbon atoms such as, for example, 1-decene, 1-dodecene, 1-hexadecene, 1-octadecene and C_{22} α-olefin, 2-styrene, α-methylstyrene, 3-methylstyrene, 4-propylstyrene, 4-cyclohexylstyrene, 4-dodecylstyrene, 2-ethyl-4-benzylstyrene, 1-vinylnaphthalene, 2-vinylnaphthalene, acrylic acid methyl ester, acrylic acid ethyl ester, acrylic acid propyl ester, acrylic acid butyl ester, acrylic acid pentyl ester, acrylic acid hexyl ester, methacrylic acid methyl ester, N-(methyl)acrylamide, acrylic acid 2-ethylhexyl ester, methacrylic acid 2-ethylhexyl ester, N-(2-ethylhexyl)acrylamide, acrylic acid octyl ester, methacrylic acid octyl ester, N-(octyl)acrylamide, acrylic acid lauryl ester, methacrylic acid lauryl ester, N-(lauryl)acrylamide, acrylic acid stearyl ester, methacrylic acid stearyl ester, N-(stearyl)acrylamide, acrylic acid behenyl ester, methacrylic acid behenyl ester and N-(behenyl)acrylamide or mixtures thereof.

The monomer distribution of the hydrophobically modified copolymers preferred for use according to the invention preferably amounts to 5% to 80% by weight, with respect to the monomers that contain sulfonic acid groups, the hydrophobic monomer and the monomer that contains carboxylic acid groups; the amount of the monomer that contains sulfonic acid groups and of the hydrophobic monomer is particularly preferably 5% to 30% by weight each, and the amount of the monomer that contains carboxylic acid groups is 60% to 80% by weight; the monomers here are preferably selected from those listed above.

Surprisingly, it has been found that polypeptide(s) of the invention in combination with a copolymer that comprises monomers that contain sulfonic acid groups (Sulfopolymer) in a dish washing composition, preferably an automatic dish washing composition has several advantages.

Firstly, the compositions do not only clean the dishes surprisingly better, show less filming on glasses, show less limescale accumulation, exhibit excellent shine after rinsing and show less deposits on the dish ware. These compositions also reduce the built up of mixed dirt in the interior of the dishwashing machine, especially the sieve.

Furthermore, the compositions contain specific enzyme stabilizing agents. It has been found that these combinations comprising polypeptide(s) of the invention in combination with a copolymer that comprises monomers that contain sulfonic acid groups (Sulfopolymer) show a better cleaning performance on enzyme related soil. This is due without being bound to the theory due to a better stabilization of the enzymes in the composition. This can be observed especially in dish washing composition that are in form of a liquid or a gel.

Adjunct materials

Any detergent components known in the art for use in dish wash, especially ADW cleaning detergents may also be utilized. Other optional detergent components include anti-corrosion agents, anti-soil redeposition agents, bactericides, binders, corrosion inhibitors and glass corrosion inhibitors, disintegrants/disintegration agents, dyes, enzyme stabilizers (including boric acid, borates, CMC, and/or polyols such as propylene glycol), fillers/processing aids, foam boosters, foam (suds) regulators, perfumes, soil-suspending agents, suds suppressors, tarnish inhibitors, either alone or in combination. Any ingredient known in the art for use in laundry/ADW/hard surface cleaning detergents may be utilized. The choice of such ingredients is well within the skill of the artisan.

Soil release polymers

The detergent compositions of the present invention may also include one or more soil release polymers which aid the removal of soils from fabrics such as cotton and polyester based fabrics, in particular the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalte based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides. Another type of soil release polymers are amphiphilic alkoxylated grease cleaning polymers comprising a core structure and a plurality of alkoxylate groups attached to that core structure. The core structure

may comprise a polyalkylenimine structure or a polyalkanolamine structure as described in detail in WO 2009/087523 (hereby incorporated by reference). Furthermore random graft co-polymers are suitable soil release polymers. Suitable graft co-polymers are described in more detail in WO 2007/138054, WO 2006/108856 and WO 2006/113314 (hereby incorporated by reference). Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose deriviatives such as those described in EP 1867808 or WO 2003/040279 (both are hereby incorporated by reference). Suitable cellulosic polymers include cellulose, cellulose ethers, cellulose esters, cellulose amides and mixtures thereof. Suitable cellulosic polymers include anionically modified cellulose, nonionically modified cellulose, cationically modified cellulose, zwitterionically modified cellulose, and mixtures thereof. Suitable cellulosic polymers include methyl cellulose, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, ester carboxy methyl cellulose, and mixtures thereof.

Anti-redeposition agents

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

Rheology Modifiers

The detergent compositions of the present invention may also include one or more rheology modifiers, structurants or thickeners, as distinct from viscosity reducing agents. The rheology modifiers are selected from the group consisting of non-polymeric crystalline, hydroxy-functional materials, polymeric rheology modifiers which impart shear thinning characteristics to the aqueous liquid matrix of a liquid detergent composition. The rheology and viscosity of the detergent can be modified and adjusted by methods known in the art, for example as shown in EP 2169040.

Other suitable adjunct materials include, but are not limited to, bactericides, binders, carriers, dyes, enzyme stabilizers, fillers, foam regulators, hydrotropes, perfumes, pigments, sud suppressors, solvents, and structurants for liquid detergents and/or structure elasticizing agents.

Washing method

The detergent compositions of the present invention are ideally suited for dish washing processes. The solution preferably has a pH of from about 5.5 to about 8, further preferably pH selected in the range from about 7.5 to about 13.5, or in the range from about 7.5 to about 12.5,

or in the range from about 8.5 to about 11.5, or in the range from about 9.5 to about 10.5, or pH 7.5 or above.

A preferred embodiment concerns a method of cleaning, the method comprising the steps of: contacting an object with a high pH cleaning composition (e.g. pH 7.5 or above) comprising a beta-glucanase of the invention under conditions suitable for cleaning the object. In a preferred embodiment the cleaning composition is used in a a dish wash process.

Still another embodiment relates to a method for removing stains from fabric or dishware which comprises contacting the dishware with a cleaning composition (e.g. pH 6.0 or above), preferably a high pH cleaning composition (e.g. pH 7.5 or above) comprising a beta-glucanase of the invention under conditions suitable for cleaning the object.

In another embodiment the cleaning composition, preferably the high pH cleaning composition of the present invention is suited for use in liquid dish wash applications. Accordingly, the present invention includes a method for washing a hard surface such as dishware. The method comprises the steps of contacting the dishware to be cleaned with a solution comprising the dishwashing composition, preferably high pH cleaning composition according to the invention. The hard surface may comprise any dishware such as crockery, cutlery, ceramics, plastics such as melamine, metals, china, glass, acrylics.. The solution preferably has a pH of 6.5, e.g. 7.5 or above, *e.g.* from about 9 to about 13.5.

The compositions may be employed at concentrations of from about 100 ppm, preferably 500 ppm to about 15,000 ppm in solution. The water temperatures typically range from about 5°C to about 90°C, including about 10°C, about 15°C, about 20°C, about 25°C, about 30°C, about 35°C, about 40°C, about 45°C, about 50°C, about 55°C, about 60°C, about 65°C, about 70°C and about 75°C,

In particular embodiments, the washing method is conducted at a pH of from about 5.0 to about 11.5, or in alternative embodiments, even from about 6 to about 10.5, such as about 5 to about 11, about 5 to about 10, about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5.5 to about 11, about 5.5 to about 10, about 5.5 to about 9, about 5.5 to about 8, about 5.5. to about 7, about 6 to about 11, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 6.5 to about 11, about 6.5 to about 10, about 6.5 to about 9, about 6.5 to about 8, about 6.5 to about 7, about 7 to about 11, about 7 to about 10, about 7 to about 9, or about 7 to about 8, preferably about 5.5 to about 9, and more preferably about 6 to about 8. In preferred embodiments the washing method is conducted at a pH selected in the range from about 7.5 to about 13.5, or in the range from about 7.5 to about 12.5, or in the range from about 8.5 to about 11.5, or in the range from about 9.5 to about 10.5, or pH 7.5 or above.

In some preferred embodiments, the high pH cleaning compositions provided herein are typically formulated such that, during use in aqueous cleaning operations, the wash water has a pH of from about 9 to about 13.5, or in alternative embodiments, or from about 10 to about 13.5 even from about 11 to about 13.5. In some preferred embodiments the liquid laundry products

are formulated to have a pH from about 12 to about 13.5. Techniques for controlling pH at recommended usage levels include the use of buffers, acids, alkalis, etc., and are well known to those skilled in the art. In the context of the present invention alkalis are used to adjust pH to about 9 to 13.5 preferably about 10 to 13.5.

In particular embodiments, the washing method is conducted at a degree of hardness of from about 0°dH to about 30°dH, such as about 1°dH, about 2°dH, about 3°dH, about 4°dH, about 5°dH, about 6°dH, about 7°dH, about 8°dH, about 9°dH, about 10°dH, about 11°dH, about 12°dH, about 13°dH, about 14°dH, about 15°dH, about 16°dH, about 17°dH, about 18°dH, about 19°dH, about 20°dH, about 21°dH, about 22°dH, about 23°dH, about 24°dH, about 25°dH, about 26°dH, about 27°dH, about 28°dH, about 29°dH,about 30°dH. Under typical European wash conditions, the degree of hardness is about 15°dH, under typical US wash conditions about 6°dH, and under typical Asian wash conditions, about 3°dH.

The present invention relates to a method of cleaning a dishware or with a detergent composition comprising a beta-glucanase of the invention.

A preferred embodiment concerns a method of cleaning, said method comprising the steps of: contacting an object with a cleaning composition comprising a beta-glucanase of the invention under conditions suitable for cleaning said object. In a preferred embodiment the cleaning composition is a detergent composition and the process is a dish wash process.

Low temperature uses

One embodiment of the invention concerns a method of doing dish wash or industrial dish cleaning comprising contacting a surface to be cleaned with a beta-glucanase of the invention, and wherein said dish wash, industrial or institutional dish cleaning is performed at a temperature of about 40ºC or below. One embodiment of the invention relates to the use of a beta-glucanase in dish wash or a cleaning process wherein the temperature in, dish wash, industrial dish cleaning is about 40ºC or below

In another embodiment, the invention concerns the use of a beta-glucanase according to the invention in a beta-glucan removing process, wherein the temperature in the beta-glucan removing process is about 40ºC or below.

In each of the above-identified methods and uses, the wash temperature is about 40ºC or below, such as about 39ºC or below, such as about 38ºC or below, such as about 37ºC or below, such as about 36ºC or below, such as about 35ºC or below, such as about 34ºC or below, such as about 33ºC or below, such as about 32ºC or below, such as about 31ºC or below, such as about 30ºC or below, such as about 29ºC or below, such as about 28ºC or below, such as about 27ºC or below, such as about 26ºC or below, such as about 25ºC or below, such as about 24ºC or below, such as about 23ºC or below, such as about 22ºC or below, such as about 21ºC or below, such as about 20ºC or below, such as about 19ºC or below, such as about 18ºC or below, such as about 17ºC or below, such as about 16ºC or below, such as about 15ºC or below, such as about 14ºC or below, such as about 13ºC or below, such as about 12ºC or below, such as

about 11°C or below, such as about 10°C or below, such as about 9°C or below, such as about 8°C or below, such as about 7°C or below, such as about 6°C or below, such as about 5°C or below, such as about 4°C or below, such as about 3°C or below, such as about 2°C or below, such as about 1°C or below.

In another preferred embodiment, the wash temperature is in the range of about 5-40°C, such as about 5-30°C, about 5-20°C, about 5-10°C, about 10-40°C, about 10-30°C, about 10-20°C, about 15-40°C, about 15-30°C, about 15-20°C, about 20-40°C, about 20-30°C, about 25-40°C, about 25-30°C, or about 30-40°C. In particular preferred embodiments the wash temperature is about 20°C, about 30°C, or about 40°C.

High temperature uses

One embodiment of the invention concerns a method of doing dish wash or industrial dish cleaning comprising contacting a surface to be cleaned with a beta-glucanase of the invention, and wherein said dish wash, industrial or institutional dish cleaning is performed at a temperature of about 75°C or below. One embodiment of the invention relates to the use of a beta-glucanase in dish wash or a industrial dish cleaning process wherein the temperature in dish wash, industrial dish cleaning is about 70°C or below.

In another embodiment, the invention concerns the use of a beta-glucanase according to the invention in a beta-glucan removing process, wherein the temperature in the beta-glucan removing process is about 65°C or below.

In each of the above-identified methods and uses, the wash temperature is about 60°C or below, such as about 59°C or below, such as about 58°C or below, such as about 57°C or below, such as about 56°C or below, such as about 55°C or below, such as about 54°C or below, such as about 53°C or below, such as about 52°C or below, such as about 51°C or below, such as about 50°C or below, such as about 49°C or below, such as about 48°C or below, such as about 47°C or below, such as about 46°C or below, such as about 45°C or below, such as about 44°C or below, such as about 43°C or below, such as about 42°C or below, such as about 41°C or below.

In another preferred embodiment, the wash temperature is in the range of about 41-90°C, such as about 41-80°C, about 41-85°C, about 41-80°C, about 41-75°C, about 41-70°C, about 41-65°C, about 41-60°C.

Methods for reducing or preventing soil redeposition using polypeptide(s) or detergent composition comprising polypeptide(s) of the present invention

An embodiment of the invention is a method for reducing or preventing soil redeposition using a detergent composition comprising polypeptide(s) of the invention.

In one embodiment, the detergent composition further comprises one or more detergent components selected from the group comprising surfactants, builders, hydrotopes, bleaching systems, polymers, adjunct materials, dispersants, soil release polymers, or any mixture thereof.

The detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition may be in the form of a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, the compartment(s) containing one or more different phases, a regular or compact powder, a granulate, a paste, a gel, or a regular, compact or concentrated liquid, two or more liquids and/or gels in a multichamber-bottle and may be used for dish wash.

In another embodiment, the detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition) comprises one or more additional enzymes selected from the group comprising proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases and mannanases, or any mixture thereof.

In a further embodiment, the detergent composition wherein said cleaning or detergent composition is a dish washing composition, said composition comprises one or more detergent components selected from the group comprising surfactants, builders, hydrotopes, bleaching systems, polymers, adjunct materials, dispersants and soil release polymers, or any mixture thereof and one or more additional enzymes selected from the group comprising proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases and mannanases, or any mixture thereof.

The method may comprise the following steps:

(a) providing a wash liquor by dissolving/mixing the detergent composition in water;
(b) washing the objects/ in the wash liquor;
(c) draining the wash liquor and optionally repeating the wash cycle; and
(d) rinsing and optionally drying the objects.

In a preferred embodiment the method may comprise the following steps:

(1) providing water and rinsing the objects
(2) optionally, drainig the water and providing fresh water
(3) dosing the detergent composition into the water to form a wash liquor
(4) agitating the wash liquor, thereby washing the objects, optionally heating the liquor
(5) draining the wash liquor
(6) optionally providing fresh water, rinsing the objects, and draining the liquid
(7) optionally providing fresh water, rinsing the objects, and during this step dosing an optional additional agent into the liquor, e.g. a rinse-aid, optionally heating the liqur, and afterwards draining the liquor.
(8) optionally letting remaining liquid evaporate from the objects.

A preferred embodiment of the invention is a method for reducing soil redeposition using a detergent composition wherein said cleaning or detergent composition is a dish washing

composition, said composition comprising: a polypeptide having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity

A preferred embodiment of the invention is a method for reducing soil redeposition using a detergent wherein said cleaning or detergent composition is a dish washing composition, said composition composition comprising: a polypeptide having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity; wherein said cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases.

The dishwashing compositions of the invention further relate to the following paragraphs:

1. A polypeptide having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose.

2. The polypeptide of paragraph 1, having at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9.

3. The polypeptide of paragraph 1 or 2, which is encoded by a polynucleotide that hybridizes under low stringency conditions, low-medium stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions, or very high stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected

from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8; or (ii) the full-length complement of (i).

4. The polypeptide of any of paragraphs 1-3, which is encoded by a polynucleotide having at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8.

5. The polypeptide of any of paragraphs 1-4, comprising or consisting of: i) the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9; or ii) the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9.

6. The polypeptide of paragraph 5, wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 214 of SEQ ID NO: 9.

7. The polypeptide of any of paragraphs 1-4, which is a variant of the mature polypeptide of the sequence selected from the group consisting of: i) SEQ ID NO: 2, ii) SEQ ID NO: 3, iii) SEQ ID NO: 5, iv) SEQ ID NO: 7, v) SEQ ID NO: 9; wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions.

8. The polypeptide of paragraph 1, which is a fragment of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein the fragment has beta-glucanase activity.

9. The polypeptide of any of paragraphs 1-8, wherein said polypeptide is capable of having beta-glucanase activity in an aqueous solution with a pH selected in the range from about 7.5 to about 13.5, wherein said aqueous solution optionally comprises a bleaching agent, preferably said pH is selected in the range from about 7.5 to about 12.5, further preferably said pH is selected in the range from about 8.5 to about 11.5, most preferably said pH is selected in the range from about 9.5 to about 10.5.

10. The polypeptide of any of paragraphs 1-9, wherein said polypeptide is capable of having beta-glucanase activity in an aqueous solution at a temperature selected in the range from about 20°C to about 75°C, wherein said aqueous solution optionally comprises a bleaching agent, preferably said temperature is selected in the range from about 40°C to about 60°C.

11. The polypeptide of any of paragraphs 9-10, wherein said polypeptide is capable of having beta-glucanase activity for at least 15 minutes, preferably for 30 minutes.

12. The polypeptide of any of paragraphs 1-11, wherein said beta-glucanase activity comprises alkaline beta-glucanase activity, wherein said alkaline beta-glucanase activity is beta-glucanase activity at pH 7.5 or above.

13. The polypeptide of any of paragraphs 1-12, wherein said beta-glucanase activity comprises licheninase EC 3.2.1.73 activity, preferably said beta-glucanase activity is licheninase EC 3.2.1.73 activity.

14. A composition comprising one or more polypeptide(s) of any of paragraphs 1-13, said composition is a dish washing composition.

15. The composition of paragraph 14, further comprising one or more detergent components.

16. The composition of paragraph 15, wherein the detergent component is selected from the group consisting of: surfactants, hydrotropes, builders, co-builders, chelators, bleach components, polymers, fabric hueing agents, fabric conditioners, foam boosters, suds suppressors, dispersants, dye transfer inhibitors, fluorescent whitening agents, perfume, optical brighteners, bactericides, fungicides, soil suspending agents, soil release polymers, anti-redeposition agents, enzyme inhibitors, enzyme stabilizers, enzyme activators, antioxidants, and solubilizers.

17. The composition of any of paragraphs 14-16, further comprising one or more additional enzymes, preferably said one or more additional enzymes is:

i) one or more amylases, further preferably said one or more amylases is one or more alpha-amylases; or

ii) one or more proteases; or

iii) one or more amylases as in (i) and one or more proteases.

18. The composition of any of paragraphs 14-17, further comprising an enzyme selected from the group consisting of: DNases, perhydrolases, amylases, proteases, peroxidases, cellulases, betaglucanases, xyloglucanases, hemicellulases, xanthanases, xanthan lyases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo-beta-mannanases, exo-beta-mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof.

19. The composition of any of paragraphs 14-18, wherein said composition has pH of 7.5 or above and optionally, comprises a bleaching agent; preferably said pH is selected in the range from about 7.5 to about 13.5, further preferably said pH is selected in the range from about 7.5 to about 12.5, most preferably said pH is selected in the range from about 8.5 to about 11.5, further most preferably said pH is selected in the range from about 9.5 to about 10.5.

20. The composition of any of paragraphs 14-19, wherein said composition has improved stability and/or performance under alkaline conditions, preferably said alkaline conditions have pH 7.5 or above.

21. The composition of any of paragraphs 14-20, wherein said composition is a cleaning or detergent composition, said cleaning or detergent composition is a dish washing composition.

22. Use of one or more polypeptide(s) of any of paragraphs 1-13 or the composition of any of paragraphs 14-21 for degrading a beta-glucan, preferably said beta-glucan is a beta-D-glucan, further preferably said beta-glucan is a beta-1,3-1,4 glucan, most preferably said beta-glucan is a mix-linkage beta-glucan, further most preferably said beta-glucan is a barley beta-glucan or oatmeal beta-glucan (e.g., from cooked oats and/or from cooked and burned-in oats and/or from uncooked oats); optionally said use is carried out under alkaline conditions having pH 7.5 or above.

23. Use of one or more polypeptide(s) of any of paragraphs 1-13 or the composition of any of paragraphs 14-21 for washing or cleaning a textile and/or a hard surface such as dish wash including Automatic Dish Wash (ADW), preferably said washing or cleaning is washing or cleaning of cooked oats and/or cooked and burned-in oats and/or uncooked oats; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

24. Use of one or more polypeptide(s) of any of paragraphs 1-13 or the composition of any of paragraphs 14-21 in a cleaning process such as laundry or hard surface cleaning including dish wash including Automatic Dish Wash (ADW) and industrial cleaning; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

25. Use of one or more polypeptide(s) of any of paragraphs 1-13 or the composition of any of paragraphs 14-21 for laundering and/or hard surface cleaning including dish wash including Automatic Dish Wash (ADW), wherein said polypeptide or said composition has an enzyme detergency benefit; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

26. Use of one or more polypeptide(s) of any of paragraphs 1-13 or the composition of any of paragraphs 14-21 for at least one of the following: preventing, reducing or removing a biofilm from an item, preferably a malodor is reduced or removed from said item; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

27. A process of degrading a beta-glucan comprising applying one or more polypeptide(s) of any of paragraphs 1-13 or a composition of any of paragraphs 14-21 to said beta-glucan, preferably said beta-glucan is a beta-D-glucan, further preferably said beta-glucan is a beta-1,3-1,4 glucan, most preferably said beta-glucan is a mix-linkage beta-glucan, further most preferably said beta-glucan is a barley beta-glucan or oatmeal beta-glucan (e.g., from cooked oats and/or from cooked and burned-in oats and/or from uncooked oats); optionally, said process is carried out under alkaline conditions having pH 7.5 or above.

28. The process of paragraph 27, wherein said beta-glucan is on the surface of a textile or hard surface, such as dish wash, preferably said beta-glucan is from cooked oats and/or from cooked and burned-in oats and/or from uncooked oats.

29. A fermentation broth formulation or cell culture composition comprising the polypeptide of any of paragraphs 1-13.

30. A polynucleotide encoding the polypeptide of any of paragraphs 1-13.

31. A nucleic acid construct or expression vector capable of expressing a polynucleotide of paragraph 30, preferably said nucleic acid construct or said expression vector comprising the polynucleotide of paragraph 30 operably linked to one or more control sequences that direct the production of the polypeptide in an expression host.

32. A recombinant host cell comprising the polynucleotide of paragraph 30, preferably said polynucleotide is operably linked to one or more control sequences that direct the production of the polypeptide, further preferably said recombinant host cell is an isolated recombinant host cell, further most preferably said recombinant host cell is a heterologous host cell (e.g., a host cell that is not a Bacillus agaradhaerens host cell or a host cell that is not a Bacillus sp-62449 host cell or a host cell that is not a Bacillus akibai host cell or a host cell that is not a Bacillus mojavensis host cell).

33. A composition comprising at least one of the following: i) a polynucleotide of paragraph 30; or ii) a nucleic acid construct of paragraph 31; or iii) an expression vector of paragraph 31.

34. A method for producing the polypeptide of any of paragraphs 1-13, comprising cultivating a cell, which in its wild-type form produces the polypeptide, under conditions conducive for production of the polypeptide.

35. The method of paragraph 34, further comprising recovering the polypeptide.

36. A method for producing a polypeptide having beta-glucanase activity, comprising cultivating the host cell of paragraph 32 under conditions conducive for production of the polypeptide.

37. The method of paragraph 36, further comprising recovering the polypeptide.

38. A transgenic plant, plant part or plant cell transformed with a polynucleotide encoding the polypeptide of any of paragraphs 1-13.

39. A method for producing a polypeptide having beta-glucanase activity, comprising cultivating the transgenic plant or plant cell of paragraph 38 under conditions conducive for production of the polypeptide.

40. The method of paragraph 39, further comprising recovering the polypeptide.

41. A polypeptide having beta-glucanase activity, wherein said polypeptide is selected from the group consisting of:

(a) a polypeptide having at least 89% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO:

3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide having at least 89% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8.

42. The polypeptide of paragraph 41, having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9.

43. The polypeptide of any of paragraphs 41-42, wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9.

44. The polypeptide of any of paragraphs 41-43, wherein said polypeptide is capable of:

i) having beta-glucanase activity for at least 15 minutes in an aqueous solution with a pH selected in the range from about 7.5 to about 13.5, wherein said aqueous solution optionally comprises a bleaching agent, preferably said pH is selected in the range from about 7.5 to about 12.5, further preferably said pH is selected in the range from about 8.5 to about 11.5, most preferably said pH is selected in the range from about 9.5 to about 10.5; and/or

ii) having beta-glucanase activity for at least 15 minutes in an aqueous solution at a temperature selected in the range from about 20°C to about 75°C, wherein said aqueous solution optionally comprises a bleaching agent.

45. The polypeptide of any of paragraphs 41-44, wherein said beta-glucanase activity comprises licheninase EC 3.2.1.73 activity.

46. The polypeptide of paragraph 45, wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity.

47. A composition comprising one or more polypeptide(s) of any of paragraphs 41-46, said composition is a dish washing composition.

48. The composition of paragraph 47, further comprising:

i) one or more detergent components; and/or

ii) one or more additional enzymes, preferably said one or more additional

enzymes is:

a) one or more amylases, further preferably said one or more amylases is one or more alpha-amylases; or

b) one or more proteases; or

c) one or more amylases as in (a) and one or more proteases.

49. The composition of any of paragraphs 47-48, wherein said composition has pH of 7.5 or above and optionally comprises a bleaching agent; preferably said pH is selected in the range from about 7.5 to about 13.5, further preferably said pH is selected in the range from about 7.5 to about 12.5, most preferably said pH is selected in the range from about 8.5 to about 11.5, further most preferably said pH is selected in the range from about 9.5 to about 10.5.

50. The composition of any of paragraphs 47-49, wherein said composition is a cleaning or a detergent composition, said cleaning or detergent composition is a dish washing composition.

51. Use of one or more polypeptide(s) of any of paragraphs 41-46 or the composition of any of paragraphs 47-50 in a cleaning process such as laundry or hard surface cleaning including dish wash; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

52. A fermentation broth formulation or cell culture composition comprising the polypeptide of any of paragraphs 41-46.

53. A polynucleotide encoding the polypeptide of any of paragraphs 41-46.

54. A nucleic acid construct or expression vector capable of expressing a polynucleotide of paragraph 53, preferably said nucleic acid construct or said expression vector comprising the polynucleotide of paragraph 53 operably linked to one or more control sequences that direct the production of the polypeptide in an expression host.

55. A recombinant host cell comprising the polynucleotide of paragraph 53, preferably said polynucleotide is operably linked to one or more control sequences that direct the production of the polypeptide, further preferably said recombinant host cell is an isolated recombinant host cell, further most preferably said recombinant host cell is a heterologous host cell (e.g., a host cell that is not a Bacillus agaradhaerens host cell or a host cell that is not a Bacillus sp-62449 host cell or a host cell that is not a Bacillus akibai host cell or a host cell that is not a Bacillus mojavensis host cell).

56. A cleaning or detergent composition comprising one or more polypeptide(s) having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity; and

(i) one or more amylases; and/or

(ii) one or more proteases,

preferably said polypeptide having beta-glucanase activity and said one or more amylases and/or one or more proteases have a synergistic effect; further preferably said synergistic effect is a REM synergistic effect, further most preferably said REM synergistic effect is of more than 6.5 at about 40°C for about 30 minutes at pH of about 7.5, further most preferably said REM synergistic effect is of more than 6.1 at about 40°C for about 30 minutes at pH of about 10, further most preferably said REM synergistic effect is of more than 6.2 at about 40°C for about 30 minutes at pH of about 10, further most preferably said beta-glucanase activity is not an endo-cellulase activity on β-1,4 linkages between D-glucose units of cellulose;

said cleaning or detergent composition is a dish washing composition.

57. The cleaning or detergent composition of paragraph 56, wherein said polypeptide has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9.

58. The cleaning or detergent composition of paragraph 57 or 58, wherein said

polypeptide is encoded by a polynucleotide that hybridizes under low stringency conditions, low-medium stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions, or very high stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8; or (ii) the full-length complement of (i).

59. The cleaning or detergent composition of any of paragraphs 56-58, wherein said polypeptide is encoded by a polynucleotide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8.

60. The cleaning or detergent composition of any of paragraphs 56-59, wherein said polypeptide comprises or consists of: i) the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9; or ii) the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9.

61. The cleaning or detergent composition of paragraph 60, wherein the mature polypeptide is selected from the group consisting of: i) amino acids 1 to 351 of SEQ ID NO: 2, ii) amino acids 1 to 351 of SEQ ID NO: 3, iii) amino acids 1 to 245 of SEQ ID NO: 5, iv) amino acids 1 to 222 of SEQ ID NO: 7, v) amino acids 1 to 214 of SEQ ID NO: 9.

62. The cleaning or detergent composition of any of paragraphs 56-59, wherein said polypeptide is a variant of the mature polypeptide of the sequence selected from the group consisting of: i) SEQ ID NO: 2, ii) SEQ ID NO: 3, iii) SEQ ID NO: 5, iv) SEQ ID NO: 7, v) SEQ ID NO: 9; wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions.

63. The cleaning or detergent composition of paragraph 56, wherein said polypeptide is a fragment of the sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, wherein the fragment has beta-glucanase activity.

64. The cleaning or detergent composition of any of paragraphs 56-63, wherein said polypeptide is capable of having beta-glucanase activity in an aqueous solution with a pH in the range from about 7.5 to about 13.5, wherein said aqueous solution optionally comprises a

bleaching agent, preferably said pH is in the range from about 7.5 to about 12.5, further preferably said pH is in the range from about 8.5 to about 11.5, most preferably said pH is in the range from about 9.5 to about 10.5.

65. The cleaning or detergent composition of any of paragraphs 56-64, wherein said polypeptide is capable of showing beta-glucanase activity in an aqueous solution at a temperature selected in the range from about 20°C to about 75°C, and/or in the range from about 40°C to about 60°C, wherein said aqueous solution optionally comprises a bleaching agent.

66. The cleaning or detergent composition of any of paragraphs 64-65, wherein said polypeptide is capable of having beta-glucanase activity for at least 15 minutes, preferably for at least 30 minutes.

67. The cleaning or detergent composition of any of paragraphs 56-66, wherein said beta-glucanase activity comprises alkaline beta-glucanase activity, wherein said alkaline beta-glucanase activity is beta-glucanase activity at pH 7.5 or above.

68. The cleaning or detergent composition of any of paragraphs 56-67, wherein said beta-glucanase activity comprises licheninase EC 3.2.1.73 activity, preferably said beta-glucanase activity is licheninase EC 3.2.1.73 activity.

69. The cleaning or detergent composition of any of paragraphs 56-68, wherein said amylase is an alpha-amylase.

70. The cleaning or detergent composition of any of paragraphs 56-69, further comprising one or more detergent components.

71. The cleaning or detergent composition of paragraph 70, wherein the detergent component is selected from the group consisting of: surfactants, hydrotropes, builders, co-builders, chelators, bleach components, polymers, fabric hueing agents, fabric conditioners, foam boosters, suds suppressors, dispersants, dye transfer inhibitors, fluorescent whitening agents, perfume, optical brighteners, bactericides, fungicides, soil suspending agents, soil release polymers, anti-redeposition agents, enzyme inhibitors, enzyme stabilizers, enzyme activators, antioxidants, and solubilizers.

72. The cleaning or detergent composition of any of paragraphs 56-71, further comprising one or more additional enzymes.

73. The cleaning or detergent composition of any of paragraphs 56-72, further comprising an enzyme selected from the group consisting of: DNases, perhydrolases, amylases, proteases, peroxidases, cellulases, betaglucanases, xyloglucanases, hemicellulases, xanthanases, xanthan lyases, lipases, acyl transferases, phospholipases, esterases, laccases, catalases, aryl esterases, amylases, alpha-amylases, glucoamylases, cutinases, pectinases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, carrageenases, pullulanases, tannases, arabinosidases, hyaluronidases, chondroitinases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, other endo-beta-mannanases, exo-beta-mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases, and combinations thereof.

74. The cleaning or detergent composition of any of paragraphs 56-73, wherein said composition has pH of 7.5 or above and optionally, comprises a bleaching agent; preferably said pH is selected in the range from about 7.5 to about 13.5, further preferably said pH is selected in the range from about 7.5 to about 12.5, most preferably said pH is selected in the range from about 8.5 to about 11.5, further most preferably said pH is selected in the range from about 9.5 to about 10.5.

75. The cleaning or detergent composition of any of paragraphs 69-74, wherein said alpha-amylase is selected from the group consisting of:

(a) a polypeptide having at least 90% sequence identity to SEQ ID NO: 13 (corresponding to SEQ ID NO: 2 of WO 95/10603);

(b) a polypeptide having at least 90% sequence identity to SEQ ID NO: 13 (corresponding to SEQ ID NO: 2 in WO 95/10603) wherein the polypeptide comprises a substitution in one or more of positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and/or 444;

(c) a polypeptide having at least 90% sequence identity to SEQ ID NO: 14 (corresponding to SEQ ID NO: 6 in WO 02/010355);

(d) a polypeptide having at least 90% sequence identity to the hybrid polypeptide of SEQ ID NO: 15 (comprising residues 1-33 of SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 of WO 2006/066594);

(e) a polypeptide having at least 90% sequence identity to the hybrid polypeptide of SEQ ID NO: 15 (comprising residues 1-33 of SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 of WO 2006/066594), wherein the hybrid polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 48, 49, 107, 156, 181, 190, 197, 201, 209 and/or 264;

(f) a polypeptide having at least 90% sequence identity to SEQ ID NO: 16 (corresponding to SEQ ID NO: 6 of WO 02/019467);

(g) a polypeptide having at least 90% sequence identity to SEQ ID NO: 16 (corresponding to SEQ ID NO: 6 of WO 02/019467), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 181, 182, 183, 184, 195, 206, 212, 216 and/or 269;

(h) a polypeptide having at least 90% sequence identity to SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19 (corresponding to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873)

(i) a polypeptide having at least 90% sequence identity to SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19 (corresponding to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 140, 183, 184 195, 206, 243, 260, 304 and/or 476;

(j) a polypeptide having at least 90% sequence identity to SEQ ID NO: 20 (corresponding to SEQ ID NO: 2 of WO 08/153815);

(k) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21 (corresponding to SEQ ID NO: 10 of WO 01/66712);

(l) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21 (corresponding to SEQ ID NO: 10 of WO 01/66712), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 176, 177, 178, 179, 190, 201, 207, 211 and/or 264;

(m) a polypeptide having at least 90% sequence identity to SEQ ID NO: 22 (corresponding to SEQ ID NO: 2 of WO 09/061380);

(n) a polypeptide having at least 90% sequence identity to SEQ ID NO: 22 (corresponding to SEQ ID NO: 2 of WO 09/061380), wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 87, 98, 125, 128, 131, 165, 178, 180, 181, 182, 183, 201, 202, 225, 243, 272, 282, 305, 309, 319, 320, 359, 444 and/or 475;

(o) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21, wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 28, 118, 174; 181, 182, 183, 184, 186, 189, 195, 202, 298, 299, 302, 303, 306, 310, 314; 320, 324, 345, 396, 400, 439, 444, 445, 446, 449, 458, 471 and/or 484; and

(p) a polypeptide having at least 90% sequence identity to SEQ ID NO: 12;

(q) a variant of SEQ ID NO:23 having alterations G182* + D183*;

(r) a variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T;

(s) a variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K;

(t) a variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K

(u) a variant of SEQ ID NO: 27 having alteration M202L;

(v) a variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K;

(w) a variant of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K;

(x) a variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K;

(y) a variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K.

76. The cleaning or detergent composition of any of paragraphs 56-75, wherein said protease is selected from the group consisting of:

1) a polypeptide having protease activity, which has at least 60% sequence identity (e.g., at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to SEQ ID NO: 34;

2) a polypeptide having protease activity, which has at least 60% sequence identity (e.g., at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to SEQ ID NO: 35;

3) a polypeptide having protease activity, which has at least 60% sequence identity (e.g., at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to SEQ ID NO: 36.

77. The cleaning or detergent composition of any of paragraphs 56-76, wherein said composition has improved stability and/or performance under alkaline conditions, preferably said alkaline conditions have pH 7.5 or above.

78. The cleaning or detergent composition of any of paragraphs 56-77, wherein said composition is in form selected from a group consisting of: a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

79. The cleaning or detergent composition of any of paragraphs 56-78, having an enzyme detergency benefit in cleaning or detergent applications.

80. The cleaning or detergent composition of any of paragraphs 56-79 having improved stability and/or performance, preferably said improved stability and/or performance is under alkaline conditions having pH 7.5 or above.

81. A method for removing a stain from a surface which comprises contacting the surface with a composition according to any of paragraphs 56-80.

82. Use of the cleaning or detergent composition of any of paragraphs 56-80 for degrading a beta-glucan, preferably said beta-glucan is a beta-D-glucan, further preferably said beta-glucan is a beta-1,3-1,4 glucan, most preferably said beta-glucan is a mix-linkage beta-glucan, further most preferably said beta-glucan is a barley beta-glucan or oatmeal beta-glucan (e.g., from cooked oats and/or from cooked and burned-in oats and/or from uncooked oats); optionally said use is carried out under alkaline conditions having pH 7.5 or above.

83. Use of the cleaning or detergent composition of any of paragraphs 56-80 for washing or cleaning a textile and/or a hard surface such as dish wash including Automatic Dish Wash (ADW), preferably said washing or cleaning is washing or cleaning of cooked oats and/or cooked and burned-in oats and/or uncooked oats; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

84. Use of the cleaning or detergent composition of any of paragraphs 56-80 in a cleaning process such as laundry or hard surface cleaning including dish wash including Automatic Dish Wash (ADW) and industrial cleaning; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

85. Use of the cleaning or detergent composition of any of paragraphs 56-80 for

laundering and/or hard surface cleaning including dish wash including Automatic Dish Wash (ADW), wherein said composition has an enzyme detergency benefit; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

86. Use of the cleaning or detergent composition of any of paragraphs 56-80 for at least one of the following: preventing, reducing or removing a biofilm from an item, preferably a malodor is reduced or removed from said item; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

87. A process of degrading a beta-glucan comprising applying the cleaning or detergent composition of any of paragraphs 56-80 to said beta-glucan, preferably said beta-glucan is a beta-D-glucan, further preferably said beta-glucan is a beta-1,3-1,4 glucan, most preferably said beta-glucan is a mix-linkage beta-glucan, further most preferably said beta-glucan is a barley beta-glucan or oatmeal beta-glucan (e.g., from cooked oats and/or from cooked and burned-in oats and/or from uncooked oats); optionally, said process is carried out under alkaline conditions having pH 7.5 or above.

88. The process of paragraph 87, wherein said beta-glucan is on the surface of a textile or hard surface, such as dish wash.

89. A method for reducing or preventing soil redeposition using a polypeptide or detergent composition of any of preceding paragraphs, preferably said detergent composition is a dish washing composition.

90. The method of paragraph 89, wherein the detergent composition also comprises one or more further enzymes.

91. The method of any of paragraphs 89-90, wherein the further enzymes are selected from the group comprising proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases and mannanases, or any mixture thereof.

92. The method of any of paragraphs 89-91, wherein the detergent composition also comprises one or more detergent components.

93. The method of any of paragraphs 89-92, wherein the detergent components are selected from the group comprising surfactants, builders, hydrotopes, bleaching systems, polymers, fabric hueing agents, adjunct materials, dispersants, dye transfer inhibiting agents,

fluorescent whitening agents and soil release polymers, or any mixture thereof.

94. The method of any of paragraphs 89-93, wherein the detergent composition is in the form of a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granulate, a paste, a gel, or a regular, compact or concentrated liquid.

95. The method of any of paragraphs 89-94, for dish wash or laundering.

96. Use of one or more polypeptide(s) or cleaning or detergent composition of any of preceding paragraphs for one or more of the following:

a) reducing or preventing soil redeposition, preferably said use is in a cleaning process or during a cleaning process, further preferably said cleaning or detergent composition is a dish wash composition, further preferably said cleaning process is a dish washing process;

b) removal of cereal containing soil, especially dried-on cereal containing soil, preferably oat flakes containing soil, especially dried-on oat flakes containing soil and/or cooked oats containing soil, and/or cooked and burned-in oats containing soil, and/or uncooked oats containing soil, further preferably said use is in a cleaning process or during a cleaning process, further most preferably said cleaning process is a dish washing process;

c) facilitating removal of starch-containing soil in the presence of one or more amylases (e.g., according to any of the preceding paragraphs) and/or for enhancing amylase related cleaning performance, preferably said use is in a cleaning process or during a cleaning process, further preferably said cleaning process is t a dish washing process

d) facilitating removal of protein-containing soil in the presence of one or more proteases (e.g., according to any of the preceding paragraphs) and/or for enhancing protease related cleaning performance, preferably said use is in a cleaning process or during a cleaning process, further preferably said cleaning process is a dish washing process.

97. The cleaning or detergent composition of any of preceding paragraphs, wherein said composition has pH of 6 or above, preferably of 7 or above, more preferably of 7.5 or above and optionally comprises a bleaching agent; preferably said pH is in the range from about 7.5 to about 13.5, further preferably said pH is in the range from about 7.5 to about 12.5, most preferably said pH is in the range from about 8.5 to about 11.5, further most preferably said pH is in the range from about 9.5 to about 10.5; preferably said cleaning or detergent composition is a dish washing composition.

98. The cleaning or detergent composition of any of preceding paragraphs, further comprising a copolymer that contains at least one sulfonic acid containing monomer, preferably in an amount from 0.1 to 20% by weight, in particular 0.5 to 18% by weight, particularly preferably 1.0 to 15% by weight, in particular 4 to 14% by weight, particularly 6 to 12% by weight, preferably said cleaning or detergent composition is a dish washing composition.

99. The cleaning or detergent composition of any of preceding paragraphs, wherein said composition comprises said polypetide in concentrations of 0.00001 mg enzyme protein/g composition to 100 mg enzyme protein/g composition, preferred 0.0001 mg enzyme protein/g composition to 50 mg enzyme protein/g composition, more preferred 0.001 mg enzyme protein/g composition to 20 mg enzyme protein/g composition, especially preferred 0.01 mg enzyme protein/g composition to 10 mg enzyme protein/g composition; preferably said cleaning or detergent composition is a dish washing composition.

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

Examples

Detergent compositions used in the example sections as described herein included the following:

Table A: Model detergent A:

Compound	Content of compound (% w/w)	Active component (% w/w)
LAS	12.0	97
AEOS, SLES	17.6	28
Soy fatty acid	2.8	90
Coco fatty acid	2.8	99
AEO	11.0	100
Sodium hydroxide	1.8	99
Ethanol / Propan-2-ol	3.0	90/10
MPG	6.0	98
Glycerol	1.7	99.5
TEA	3.3	100
Sodium formate	1.0	95
Sodium citrate	2.0	100
DTMPA (as Na7-salt)	0.5	42
PCA (as Na-salt)	0.5	40

Phenoxy ethanol	0.5	99
Ion exchanged water	33.6	---
Water hardness was adjusted to 15°dH by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ (Ca^{2+}:Mg^{2+}:HCO_{3-} = 4:1:7.5) to the test system.		

Table B: Model detergent X:		
Compound	Content of compound (% w/w)	Active component (% w/w)
LAS	16.5	91
AEO*	2	99.5
Sodium carbonate	20	100
Sodium (di)silicate	12	82.5
Zeolite A	15	80
Sodium sulfate	33.5	100
PCA	1	100
* Model detergent X was mixed without AEO. AEO was added separately before wash. Water hardness was adjusted to 12°dH by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ (Ca^{2+}:Mg^{2+}:HCO_{3-} = 2:1:4.5) to the test system.		

Table C: Model detergent Z without bleach:		
Compound	Content of compound (% w/w)	% active component (% w/w)
LAS	7.0	85.3
Soap	1.1	93
AEO*	1.5	99.5
Soda ash	20.1	99.5
Hydrous sodium silicate	10.0	80.1
Zeolite A	5.0	80
Sodium citrate	2.0	100
HEDP-Na4	0.2	84
Polyacrylate	1.1	92
Sodium sulfate	52.0	100
* Model detergent Z without bleach was mixed without AEO. AEO was added separately before wash. Water hardness was adjusted to 15°dH by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ (Ca^{2+}:Mg^{2+}:HCO_{3-} = 4:1:7.5) to the test system. pH was used as is (10.6) or adjusted to 11.3 with 4 M NaOH.		

Table D: Model detergent Z with bleach:		
Compound	Content of compound (% w/w)	% active component (% w/w)

LAS	7.0	85.3
Soap	1.1	93
AEO*	1.5	99.5
Soda ash	20.1	99.5
Hydrous sodium silicate	10.0	80.1
Zeolite A	5.0	80
Sodium citrate	2.0	100
HEDP-Na4	0.2	84
Polyacrylate	1.1	92
Sodium percarbonate	9.3	86
TEAD	1.1	91.8
Sodium sulfate	41.6	100
* Model detergent Z with bleach was mixed without AEO. AEO was added separately before wash. Water hardness was adjusted to 15°dH by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ (Ca^{2+}:Mg^{2+}:HCO_{3-} = 4:1:7.5) to the test system. pH was either as is (10.5) or adjusted to 11.1 with 4 M NaOH.		

Table E: ADW model detergent A:		
Compound	Content of compound (% w/w)	Active component (% w/w)
MGDA (Trilon M Granules SG)	20	59
Sodium citrate	20	100
Sodium carbonate	20	100
Sodium percarbonate	10	88
Sodium Silicate	5	80
Sodium sulfate	12	100
Acusol 588G	5	92
TAED	3	92
Surfac 23-6.5 (liq)	5	100
Water hardness was adjusted to 21°dH by addition of $CaCl_2$, $MgCl_2$, and $NaHCO_3$ (Ca^{2+}:Mg^{2+}:HCO_{3-} = 4:1:10) to the test system.		

Example 1: Determination of beta-glucanase (Lichenase) activity:

An AZCL-Barley beta-glucan (azurine dye covalently cross-linked beta-glucan) assay was used for detection of endo-glucancase activity (Lichenase activity).

AZCL-Barley beta-glucan (75 mg) was suspended in 15 mL detergent (Model detergents A, X, Z with and without bleach and pH adjusted, ADW Model A). To 1 mL of this solution in Eppendorf tubes was added 10 µL enzyme (0.33 mg enzyme protein/Liter), incubated for 15 min at 40°C

while shaking at 1250 rpm in a pre-heated thermo mixer and spun down for 2 min at 13200 rpm, diluted 5 times with a 5% Triton-X-100 including 10 µM $CaCl_2$ and 250 µL of the solution was transferred to a micro-titer plate and the sample absorbance was measured at 590 nm.

Example 2: Cloning, expression and purification of GH16 endo- β-1,3-1,4-glucanase from the genus *Bacillus:*

The beta-glucanases were derived from bacterial strains obtain either from the German collection of Microorganisms and Cell Cultures (DSMZ) or by isolation from environmental samples by classical microbiological techniques according to Table 1.

Table 1: Source and Source country of GH16 endo- β-1,3-1,4-glucanase from the genus Bacillus:

Strain name	Source	Source Country
Bacillus sp-62449	Environmental sample	United States
Bacillus akibai	Soil	Greece
Bacillus agaradhaerens	Soil	United States
Bacillus mojavensis	DSMZ (DSM9205)	United States

Chromosomal DNA from pure cultures of the individual strains was purified and subjected to full genome sequencing using Illumina technology. The assembled genome sequence and subsequent analysis of the 16S ribosomal subunit gene sequences confirmed the identity of the strains.

The individual genes encoding β-1,3-1,4-glucanases were amplified by PCR and fused with regulatory elements and homology regions for recombination into the B. subtilis genome.

The linear integration construct was a SOE-PCR fusion product (Horton, R.M., Hunt, H.D., Ho, S.N., Pullen, J.K. and Pease, L.R. (1989) Engineering hybrid genes without the use of restriction enzymes, gene splicing by overlap extension Gene 77: 61-68) made by fusion of the gene between two Bacillus subtilis chromosomal regions along with strong promoters and a chloramphenicol resistance marker. The SOE PCR method is also described in patent application WO 2003095658.

The gene was expressed under the control of a triple promoter system (as described in WO 99/43835), consisting of the promoters from *Bacillus licheniformis* alpha-amylase gene (amyL), *Bacillus amyloliquefaciens* alpha-amylase gene (amyQ), and the *Bacillus thuringiensis* cryIIIA promoter including stabilizing sequence.

The gene was expressed with a *Bacillus clausii* secretion signal (encoding the following amino acid sequence: MKKPLGKIVASTALLISVAFSSSIASA (SEQ ID NO: 10) replacing the native secretion signal. Furthermore the expression construct results in the addition of a N-terminal poly histidine affinity purification tag consisting of the sequence HHHHHHPR (SEQ ID

NO: 11) to the expressed mature protein.

The SOE-PCR product was transformed into *Bacillus subtilis* and integrated in the chromosome by homologous recombination into the pectate lyase locus. Subsequently, a recombinant *Bacillus subtilis* clone containing the integrated expression construct was grown in rich liquid culture. The culture broth was centrifuged (20000 x g, 20 min) and the supernatant was carefully decanted from the precipitate and used for purification of the enzyme.

Purification of recombinant enzymes by nickel affinity chromatography

The pH of the cleared supernatant was adjusted to pH 8, filtrated through a 0.2µM filter, and the supernatant applied to a 5 ml HisTrap™ excel column. Prior to loading, the column had been equilibrated in 5 column volumes (CV) of 50 mM Tris/HCl pH 8. In order to remove unbound material, the column was washed with 8 CV of 50 mM Tris/HCl pH 8, and elution of the target was obtained with 50 mM HEPES pH 7 + 10mM imidazole. The eluted protein was desalted on a HiPrep™ 26/10 desalting column, equilibrated using 3 CV of 50 mM HEPES pH 7 + 100 mM NaCl. This buffer was also used for elution of the target, and the flow rate was 10 ml/min. Relevant fractions were selected and pooled based on the chromatogram and SDS-PAGE analysis.

Example 3: AZCL-assay with beta-glucanase enzymes:

In this example enzymatic activity were measured on AZCL-Barely beta-glucan substrate under various pH's, temperature and detergent thus modeling various laundry conditions. Measurements of enzymatic activity were carried out as described in example 1, but without the 5 times dilution with 5% Triton-X-100 including 10 µM $CaCl_2$. Comparisons were made with beta-glucanase from *Bacillus amyloliquefaciens* and beta-glucanase from *Bacillus subtilis* in Model detergent A, Model detergent X, Model detergent Z with bleach, Model detergent Z without bleach, Model detergent Z with bleach pH-adjusted and Model Z without bleach pH-adjusted detergent compositions.

Table 2: Beta-glucanase activity measured under various pH's, temperatures and laundry detergents using the AZCL-Barley beta-glucan assay (Absorbance):

Enzyme	pH 7.7 Model A		pH 10.1 Model X		pH 10.5 Model Z with bleach		pH 10.6 Model Z without bleach		pH 11.1 Model Z with bleach pH-adjusted		pH 11.3 Model Z without bleach pH-adjusted	
	40°C	60°C	40°C	60°C	40°C	60°C	40°C	60°C	40°C	60° C	40°C	60° C
B.amyloliquefaciens beta-	2.44	0.71	2.83	0.83	0.05	0.04	0.10	0.01	0.01	0.03	0.07	0.01

glucanase / lichenase												
B.subtilis beta-glucanase / lichenase	2.45	0.62	3.41	0.30	0.05	0.01	0.08	0.01	0.00	0.04	0.07	0.02
B.akibai Beta-glucanase / lichenase	0.18	0.10	3.41	1.55	0.03	0.37	0.05	0.27	0.03	0.15	0.04	0.05
B.agaradhaerens beta-glucanase / lichenase	0.36	0.70	3.41	2.50	0.58	0.16	0.47	0.04	0.17	0.03	0.01	0.02
B.sp-62449 beta-glucanase / lichenase	1.22	1.15	3.25	0.08	0.22	0.10	0.30	0.11	0.05	0.04	0.04	0.01
B.mojavensis beta-glucanase / lichenase	1.65	0.20	3.41	2.36	0.17	0.11	0.18	0.01	0.03	0.03	0.01	0.02
For details of the model detergent compositions see Tables A-D above.												

Example 4: AZCL-assay of enzyme activity on AZCL-beta-barley substrate in automated dish wash model detergent:

Measurements of enzymatic activity were carried out as described in example 1. In this example enzymatic activities of novel beta-glucanases were compared to enzymatic activities of beta-glucanases from *Bacillus amyloliquefaciens* and *Bacillus subtilis* in the automated dish wash detergent ADW model A. The obtained data are shown in Table 3 below:

Table 3: Beta-glucanase activity measured under various temperatures in ADW Model A

detergent using the AZCL-Barley beta-glucan assay (Absorbance), pH of the ADW model detergent A was 10.2:		
Enzyme	ADW model detergent A	
	40°C	60°C
Blank	0.07	0.11
Bacillus amyloliquefaciens beta-glucanase (lichenase)	0.46	0.34
Bacillus subtilis beta-glucanase (lichenase)	0.42	0.21
Bacillus akibai beta-glucanase (lichenase)	0.15	2.07
Bacillus agaradhaerens beta-glucanase (lichenase)	0.85	1.77
Bacillus mojavensis beta-glucanase (lichenase)	0.85	1.06
Bacillus sp-62449 beta-glucanase (lichenase)	1.60	0.49

Example 5: Beta-glucanase stability measured by TSA:

In this example stability of novel beta-glucanases were compared to stabilities of beta-glucanases from *Bacillus amyloliquefaciens* and *Bacillus subtilis*. Thermal shift assays (TSA) were performed with enzyme samples diluted to 0.3 mg/ml in assay buffers: 0.1 M succinic acid, 0.1 M HEPES, 0.1 M CHES, 0.1 M CAPS, 0.15 M KCl, 1 mM CaCl2, 0.01 % Triton X100, pH adjusted to 5, 7.5 and 10 respectively. SYPRO Orange dye (Life Technologies S6650) diluted 101x in mQ water. 10 µl diluted enzyme sample + 10 µl assay buffer + 10 µl dye were mixed in wells of TSA assay plates (LightCycler 480 Multiwell plate 96, white (Roche) and covered with optic seal (LightCycler 480 Sealing foil, Roche). Protein melting analysis was conducted at 25-99 °C at 200 °C/h in a Roche Lightcycler 480 II machine running Roche LightCycler 480 software (release 1.5.0 SP4). All samples were analyzed in duplicate. The reported readout is Tm, defined as the midpoint value of the protein melting curves. The obtained data are shown in Table 4 below.

Table 4: Stability measured by TSA:		
Enzyme	Buffer pH	TSA
Bacillus akibai beta-glucanase (lichenase)	5	70.9
	7.5	71.8
	10	71.6
Bacillus agaradhaerens beta-glucanase (lichenase)	5	58.2
	7.5	64.0
	10	58.6
Bacillus mojavensis beta-glucanase (lichenase)	5	72.8

	7.5	71.2
	10	72.2
Bacillus sp-62449 beta-glucanase (lichenase)	5	43.2
	7.5	53.9
	10	49.4
Bacillus amyloliquefaciens beta-glucanase (lichenase)	5	72.8
	7.5	70.1
	10	73.2
Bacillus subtilis beta-glucanase (lichenase)	5	64.2
	7.5	64.7
	10	64.8

Example 6: Beta-glucanase substrate specificity:

The substrate specificities of beta-glucanases were further tested using various AZCL-assays from Megazymes (AZCL-Barely beta-glucan, AZCL-HE-cellulose, AZCL-pachyman and AZCL-curdlan (azurine dye covalently cross-linked beta-glucan). The AZCL-substrate (75 mg) was suspended in 15 mL model detergent X. To 1 mL of this solution in Eppendorf tubes was added 10 µL enzyme (0.33 mg enzyme protein/Liter), incubated for 15 min at 40°C while shaking at 1250 rpm in a pre-heated thermo mixer and spun down for 2 min at 13200 rpm, diluted 5 times with a 5% Triton-X-100 including 10 µM $CaCl_2$ and 250 µL of the solution was transferred to a micro-titer plate and the sample absorbance was measured at 590 nm.

In this example substrate specificity of all 6 beta-glucanases (i.e. from *Bacillus akibai, Bacillus agaradhaerens, Bacillus mojavensis, Bacillus sp-62449, Bacillus amyloliquefaciens* and *Bacillus subtilis*) were tested on AZCL–Barley beta-glucan, AZCL-HE-Cellulose AZCL-pachyman and AZCL-curdlan substrates. The obtained results have further confirmed that all 6 tested beta-glucanases have activity on AZCL–Barley beta-glucan substrate only (i.e. positive reaction on AZCL–Barley beta-glucan as a substrate and negative reactions on AZCL-HE-Cellulose AZCL-pachyman and AZCL-curdlan as substrates, Table 5 below). The data shows that tested beta-glucanases only showed activity on beta-glucans containing both beta-1,3 and beta-1,4 linkages and not beta-glucans consisting of pure beta-1,4-glucans or beta-1,3 glucans only or a mixture of beta-1,3- and beta-1,6 linkages. Based on the above results, beta-glucanases of the present invention can be further distinguished from endo-cellulases within beta-glucanase definition as used herein, said endo-cellulases having activity on β-1,4 linkages between D-glucose units of cellulose. Based on the above it is concluded that beta-glucanases of the present invention have licheninase (EC 3.2.1.73) enzymatic activity.

Table 5: Substrate specificity of 6 beta-glucanases measured by AZCL-substrates:

Substrate	Reaction	Substrate for the assay of:	Polymer description
AZCL–Barley	Yes	Lichenase, endo-glucanase	β-1,4;β-1,3 linkages between D-

beta-glucan		and cellulase	glucose units
AZCL-HE-cellulose	No	Endo-cellulase	β-1,4 linkages between D-glucose units
AZCL-curdlan	No	Endo-1,3-beta-D-glucanase	β-1,3 linkages between D-glucose
AZCL-pachyman	No	Endo-1,3-beta-D-glucanase	β-1,3 linkages between D-glucose units (branched with β-1,6 glucose units average on every 4)

Example 7: Synergistic effect of beta-glucanases (lichenases) of the invention when combined with an alpha-amylase:

I. Wascator bottle wash method description:

A Wascator bottle wash method was used to detect the performance of the enzymes. In a Wascator washing machine (FOM 71 Lab) bottles (60 mL, DSE PP 70X35 Aseptisk, material No.: 216-2620, from VWR) with 25 mL detergent solution including enzyme(s) and four stains (035KC Chocolate porridge oat from Equest, 2 cm in diameter) were added. Two kg ballast (tea towels, cotton) was included in the washing machine. Washed in 25 L water for 30 min at 40°C in liquid and powder model detergents for laundry (model detergent A1 and model detergent X1,, respectively) and in ADW model detergent (ADW model detergent A1). After wash the stains were rinsed with tap water twice (3 L) and dried ON at rt (room temperature) in drying cabinet (Electrolux, Intuition, EDD2400). The remission was measured on a spectrophotometer (Macbeth Color-Eye 7000 Remissions) at 460 nm.

II. Results:

In this example the results of combining the individual lichenases with an alpha-amylase (Stainzyme) (SEQ ID NO: 12) were studied in order to investigate a potential synergistic effect between the two enzymes in various detergents with various pHs using the Wascator bottle wash method. Comparisons were made with lichenase from *Bacillus amyloliquefaciens* and lichenase from *Bacillus subtilis* in Model detergent A1, Model detergent X1 and ADW model detergent A1 using 0.01 mg enzyme protein per liter of lichenase and 0.05 mg enzyme protein per liter of Stainzyme at 40°C. The detailed conditions used in this example are described in Tables F-K and the results are shown in Tables 6-8 below.

Table F: Experimental condition:	
Detergent	Model detergent A1 (see Table G below)
Detergent dosage	3.33 g/L
Test solution volume	25 mL
pH	As is
Wash time	30 minutes

Temperature	40°C
Water hardness	15°dH
Amylase concentration in test	0.05 mg/L
Beta-glucanase (Lichenase) concentration in test	0.01 mg/L
Test material	O35 KC Chocolate porridge oats

Table G: Model detergent A1:

Compound	Content of compound (% w/w)	Active component (% w/w)
LAS	12.0	97
AEOS, SLES	17.6	28
Soy fatty acid	2.8	90
Coco fatty acid	2.8	99
AEO	11.0	100
Sodium hydroxide	1.8	99
Ethanol / Propan-2-ol	3.0	90/10
MPG	6.0	98
Glycerol	1.7	99.5
TEA	3.3	100
Sodium formate	1.0	95
Sodium citrate	2.0	100
DTMPA (as Na_7-salt)	0.5	42
PCA (as Na-salt)	0.5	40
Phenoxy ethanol	0.5	99
Ion exchanged water	33.6	---
Water hardness was adjusted to 15°dH by addition of CaCl2, MgCl2, and NaHCO3 (Ca2+:Mg2+:HCO3- = 4:1:7.5) to the test system.		

Table H: Experimental condition:

Detergent	Model detergent X1 (see Table I below)
Detergent dosage	1.75 g/L
Test solution volume	25 mL
pH	As is
Wash time	30 minutes
Temperature	40°C
Water hardness	12°dH
Amylase concentration in test	0.05 mg/L
Beta-glucanase (Lichenase) concentration in test	0.01 mg/L
Test material	O35 KC Chocolate porridge oats

Table I: Model detergent X1:		
Compound	Content of compound (% w/w)	Active component (% w/w)
LAS	16.5	91
AEO*	2	99.5
Sodium carbonate	20	100
Sodium (di)silicate	12	82.5
Zeolite A	15	80
Sodium sulfate	33.5	100
PCA	1	100

* Model detergent X1 is mixed without AEO. AEO is added separately before wash.

Water hardness was adjusted to 12°dH by addition of CaCl2, MgCl2, and NaHCO3 (Ca2+:Mg2+:HCO3-= 2:1:4.5) to the test system.

Table J: Experimental condition:	
Detergent	ADW model detergent A1 (see Table K below)
Detergent dosage	3.77 g/L
Test solution volume	25 mL
pH	As is
Wash time	30 minutes
Temperature	40°C
Water hardness	15°dH
Amylase concentration in test	0.05 mg/L
Beta-glucanase (Lichenase) concentration in test	0.01 mg/L
Test material	O35 KC Chocolate porridge oats

Table K: ADW model detergent A1:		
Compound	Content of compound (% w/w)	Active component (% w/w)
MGDA (Trilon M Granules SG)	20	59
Sodium citrate	20	100
Sodium carbonate	20	100
Sodium percarbonate	10	88
Sodium Silicate	5	80
Sodium sulfate	12	100

Acusol 588G	5	92
TAED	3	92
Surfac 23-6.5 (liq)	5	100
Water hardness was adjusted to 21°dH by addition of CaCl2, MgCl2, and NaHCO3 (Ca2+:Mg2+:HCO3-= 4:1:10) to the test system.		

Abbreviations as used herein:

REM = Measured value

ΔREM = REM - Blank

REM combined = Measured value

ΔREM combined = REM combined - Blank

ΔREM theoretic = ΔREM (Amylase) + ΔREM (Lichenase)

REM Synergistic effect = ΔREM combined – ΔREM theoretic

Table 6: Wascator bottle wash in Model detergent A1 at 40°C, 30 min (pH 7.7):

	Enzymes solo		Beta-glucanase (Lichenase) in combination with the amylase (Stainzyme)			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergistic effect
B. agaradhaerens beta-glucanase (lichenase)	66.0	0.4	80.1	14.5	6.7	7.8
B. akibai beta-glucanase (lichenase)	65.3	-0.2	79.1	13.6	6.1	7.5
B. mojavensis beta-glucanase (lichenase)	65.8	0.2	79.3	13.7	6.5	7.2
B. SP-62449 beta-glucanase (lichenase)	64.9	-0.7	80.0	14.4	5.6	8.8
B.amyloliquefaciens beta-glucanase (lichenase)	67.3	1.8	79.5	13.9	8.1	5.9
B. subtilis beta-glucanase (lichenase)	67.3	1.7	80.1	14.5	8.0	6.5
Stainzyme	71.8	6.3	---	---	---	---
Blank	65.5	0.0	---	---	---	---

Table 7: Wascator bottle wash in Model detergent X1 at 40°C, 30 min (pH 10.1):

	Enzymes solo	Beta-glucanase (Lichenase) in combination with the amylase Stainzyme

	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergistic effect
B. agaradhaerens beta-glucanase (lichenase)	61.4	-0.4	74.5	12.7	4.4	8.2
B. akibai beta-glucanase (lichenase)	62.2	0.3	74.9	13.1	5.2	7.9
B. mojavensis beta-glucanase (lichenase)	61.8	-0.1	74.3	12.4	4.8	7.6
B. SP-62449 beta-glucanase (lichenase)	61.9	0.1	73.0	11.1	5.0	6.1
B.amyloliquefaciens beta-glucanase (lichenase)	59.9	-1.9	72.0	10.2	2.9	7.3
B. subtilis beta-glucanase (lichenase)	60.8	-1.0	71.8	10.0	3.8	6.1
Stainzyme	66.7	4.9	---	---	---	---
Blank	61.8	0.0	---	---	---	---

Table 8. Wascator bottle wash in ADW Model detergent A1 at 40°C, 30 min (pH 10.2):

	Enzymes solo		Beta-glucanase (Lichenase) in combination with the amylase Stainzyme			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergistic effect
B. agaradhaerens beta-glucanase (lichenase)	60.5	-2.1	75.1	12.5	6.1	6.4
B. akibai beta-glucanase (lichenase)	60.7	-1.9	73.9	11.3	6.3	5.0
B. mojavensis beta-glucanase (lichenase)	63.0	0.3	73.3	10.7	8.5	2.1
B. SP-62449 beta-glucanase (lichenase)	60.8	-1.8	74.5	11.9	6.4	5.5
B. amyloliquefaciens beta-glucanase (lichenase)	61.6	-1.0	71.3	8.6	7.2	1.4
B. subtilis beta-glucanase (lichenase)	58.1	-4.5	72.5	9.9	3.7	6.2
Stainzyme	70.8	8.2	---	---	---	---
Blank	62.6	0.0	---	---	---	---

Example 8: Determination of the pH optimum

Subsequently, the pH optimum of all 6 beta-glucanases was determined on 0,4% w/v AZCL-glucan(barley) substrate in Britton Robinson buffer (100mM phosphoric acid, 100mM acetic acid, 100mM boric acid, 0.01% Trinton X-100, 100 mM KCl, 2mM CaCl2) adjusted to pH 2-12 with NaOH. An enzyme dilution expected to be in the high end of the linear assay range was selected for all pH values under investigation. The pH optimum was investigated in the pH 2-10 range, and for a few samples both lower and higher pH values were included to positively identify the optimum. The results are shown in this Table 9.

Table 9. pH optimum of beta-glucanases (lichenases):

Organism	Mw, kDa	pI	A595/mg	pH optimum	pH10/pHopt
Bacillus amyloliquefaciens	24	5.2	765	6	0.01
Bacillus subtilis	24	6.1	242	6	0.11
Bacillus sp-62449	40	4.4	763	8	0.73
Bacillus akibai	29	5.2	5	6-9	0.9
Bacillus agaradhaerens	27	4.5	106	9	0.68
Bacillus mojavensis	25	7.4	313	8	0.23

Based on the above a number of observations were made:

The beta-glucanase from *Bacillus amyloliquefaciens* and *Bacillus subtilis* was found to have a pH optimum of 6.0, and relative to this activity only between 1-11% percent activity at pH 10.0. The new bacterial beta-glucanases were found to have pH optimum ranging from pH 6-9, but with a significantly higher relative activity at pH 10 ranging from 23-90% compared to the enzymes from *Bacillus subitilis* and *Bacillus amyloliquefaciens.* The GH16 beta-glucanase from *B. akibai* had a very broad pH optimum.

Example 9: Synergistic effect of lichenases combined with alpha-amylases:

I. Wascator bottle wash method description:

A Wascator bottle wash method was used to detect the performance of the enzymes. In a Wascator washing machine (FOM 71 Lab) was added bottles (60 mL, DSE PP 70X35 Aseptisk, material #: 216-2620, from VWR) with 25 mL detergent solution including enzyme(s) and four stains (035KC Chocolate porridge oat from Warwick Equest Ltd, Unit 55, Consett Business Park, Consett,County Durham,DH8 6BN, United Kingdom, 2 cm in diameter). Two kg ballast (tea towels, cotton) was included in the washing machine. Washed in 25 L water for 20 or 30 min at 40°C in liquid and powder model detergents for laundry (model detergent A and model detergent X, respectively) and in ADW model detergent (ADW model detergent A). After wash the stains were rinsed with tap water twice (3 L) and dried overnight at room temperature in drying cabinet (Electrolux, Intuition, EDD2400). The remission was measured on a spectrophotometer (Macbeth Color-Eye 7000 Remissions) at 460 nm.

II. Results:

In this example the results of combining the individual mature lichenases of Bacillus agaradhaerens Lichenase (SEQ ID NO: 39, His-tagged, recombinant), Bacillus akibai Lichenase (SEQ ID NO: 38, His-tagged, recombinant), Bacillus mojavensis Lichenase (SEQ ID NO: 40, His-tagged, recombinant), Bacillus sp-62449 Lichenase (SEQ ID NO: 37, His-tagged, recombinant), Bacillus amyloliquefaciens Lichenase (SEQ ID NO: 32) and Bacillus subtillis Lichenase (SEQ ID NO: 33)with different amylases as outlined below were studied in order to investigate a potential synergy effect between the two enzymes in various detergents with various pHs using the Wascator bottle wash method. Comparisons were made with lichenase from *Bacillus amyloliquefaciens* and lichenase from *Bacillus subtilis* in Model detergent A, Model detergent X and ADW model detergent A using lichenase concentration of 0.01 mg enzyme protein per liter and amylase concentration of 0.05 mg enzyme protein per liter at 40°C. The detailed conditions are described in Tables 10-15 and the results are shown in Tables 16-47 below.

Table 10: Experimental condition

Detergent	Model detergent A (see Table 11)
Detergent dosage	3.33 g/L
Test solution volume	25 mL
pH	As is
Wash time	20 or 30 minutes
Temperature	40°C
Water hardness	15°dH
Amylase concentration in test	0.05 mg/L
Lichenase concentration in test	0.01 mg/L
Test material	O35 KC Chocolate porridge oats

Table 11: Model detergent A

Compound	Content of compound (% w/w)	Active component (% w/w)
LAS	12.0	97
AEOS, SLES	17.6	28
Soy fatty acid	2.8	90

Coco fatty acid	2.8	99
AEO	11.0	100
Sodium hydroxide	1.8	99
Ethanol / Propan-2-ol	3.0	90/10
MPG	6.0	98
Glycerol	1.7	99.5
TEA	3.3	100
Sodium formate	1.0	95
Sodium citrate	2.0	100
DTMPA (as Na_7-salt)	0.5	42
PCA (as Na-salt)	0.5	40
Phenoxy ethanol	0.5	99
Ion exchanged water	33.6	---

Water hardness was adjusted to 15°dH by addition of CaCl2, MgCl2, and NaHCO3 (Ca^{2+}:Mg^{2+}:$HCO3^-$ = 4:1:7.5) to the test system.

Table 12: Experimental condition

Detergent	Model detergent X (see Table 13)
Detergent dosage	1.75 g/L
Test solution volume	25 mL
pH	As is
Wash time	20 or 30 minutes
Temperature	40°C
Water hardness	12°dH
Amylase concentration in test	0.05 mg/L
Lichenase concentration in test	0.01 mg/L
Test material	O35 KC Chocolate porridge oats

5

Table 13: Model detergent X

Compound	**Content of compound (% w/w)**	**Active component (% w/w)**

LAS	16.5	91
AEO*	2	99.5
Sodium carbonate	20	100
Sodium (di)silicate	12	82.5
Zeolite A	15	80
Sodium sulfate	33.5	100
PCA	1	100

*Model detergent X is mixed without AEO. AEO is added separately before wash.

Water hardness was adjusted to 12°dH by addition of CaCl2, MgCl2, and NaHCO3 (Ca^{2+}:Mg^{2+}:$HCO3^-$ = 2:1:4.5) to the test system.

Table 14: Experimental condition

Detergent	ADW model detergent A (see Table 15)
Detergent dosage	3.77 g/L
Test solution volume	25 mL
pH	As is
Wash time	20 or 30 minutes
Temperature	40°C
Water hardness	21°dH
Amylase concentration in test	0.05 mg/L
Lichenase concentration in test	0.01 mg/L
Test material	O35 KC Chocolate porridge oats

Table 15: ADW model detergent A

Compound	**Content of compound (% w/w)**	**Active component (% w/w)**

MGDA (Trilon M Granules SG)	20	59
Sodium citrate	20	100
Sodium carbonate	20	100
Sodium percarbonate	10	88
Sodium Silicate	5	80
Sodium sulfate	12	100
Acusol 588G	5	92
TAED	3	92
Surfac 23-6.5 (liq)	5	100

Water hardness was adjusted to 21°dH by addition of CaCl2, MgCl2, and NaHCO3 (Ca^{2+}:Mg^{2+}:$HCO3^-$ = 4:1:10) to the test system.

Abbreviations

REM = Measured value

ΔREM = REM - Blank

REM combined = Measured value

ΔREM combined = REM combined - Blank

ΔREM theoretic = ΔREM (Amylase) + ΔREM (Lichenase)

REM Synergy effect = ΔREM combined – ΔREM theoretic

Table 16. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase having SEQ ID NO: 12			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	65.1	-0.4	80.1	14.6	5.9	8.7
Bacillus Akibai Lichenase	66.3	0.9	79.1	13.6	7.2	6.4
Bacillus Mojavensis Lichenase	65.8	0.3	79.3	13.8	6.7	7.1
Bacillus SP-62449 Lichenase	64.9	-0,6	78.7	13.2	5.8	7.5
Bacillus amyloliquefaciens lichenase	66.1	0.7	79.5	14.0	7.0	7.0
Bacillus Subtillis Lichenase	67.3	1.8	80.1	14.6	8.2	6.4
Amylase having SEQ ID NO: 12	71.8	6.3	---	---	---	---
Blank	65.5	0.0	---	---	---	---

Table 17. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase ,which is the variant of SEQ ID NO:23 having alterations G182* + D183*			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	63.9	0.4	76.2	12.7	6.1	6.6
Bacillus Akibai Lichenase	63.5	0.1	75.3	11.9	5.8	6.1
Bacillus Mojavensis Lichenase	65.0	1.6	74.5	11.1	7.3	3.8
Bacillus SP-62449 Lichenase	64.6	1.1	75.0	11.6	6.9	4.7
Bacillus amyloliquefaciens lichenase	65.7	2.3	75.6	12.2	8.0	4.2
Amylase, which is the variant of SEQ ID NO:23 having alterations G182* + D183*	69.2	5.7	---	---	---	---
Blank	63.4	0.0	---	---	---	---

Table 18. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	63.9	0.4	77.5	14.1	8.6	5.5
Bacillus Akibai Lichenase	63.5	0.1	78.1	14.7	8.3	6.4
Bacillus Mojavensis Lichenase	65.0	1.6	77.9	14.5	9.7	4.7
Bacillus SP-62449 Lichenase	64.6	1.1	77.1	13.6	9.3	4.3
Amylase, which is the variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T	71.6	8.1	---	---	---	---
Blank	63.4	0.0	---	---	---	---

Table 19. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	65.1	-0.4	75.9	10.4	6.4	4.0
Bacillus Akibai Lichenase	66.3	0.9	75.8	10.4	7.7	2.7
Bacillus Mojavensis Lichenase	65.8	0.3	76.9	11.4	7.1	4.3
Bacillus SP-62449 Lichenase	64.9	-0.6	75.9	10.4	6.2	4.2
Bacillus amyloliquefaciens lichenase	66.1	0.7	76.7	11.2	7.5	3.7
Bacillus Subtillis Lichenase	67.3	1.8	76.9	11.4	8.6	2.8
Amylase, which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F +	72.3	6.8	---	---	---	---

N299Y + R320K + M323T + A339S + E345R + R458K						
Blank	65.5	0.0	---	---	---	---

Table 20. Wascator bottle wash in Model detergent A at 40°C, 20 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase,which is the variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	64.0	-0.8	77.7	13.0	10.6	2.3
Bacillus Akibai Lichenase	64.7	-0.1	77.6	12.8	11.3	1.5
Bacillus SP-62449 Lichenase	64.0	-0.8	77.4	12.6	10.6	2.0
Amylase, which is the variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K	76.2	11.4	---	---	---	---
Blank	64.8	0.0	---	---	---	---

Table 21. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 27 having alteration M202L			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	65.1	-0.4	72.2	6.7	3.7	3.0
Bacillus Mojavensis Lichenase	65.8	0.3	73.4	7.9	4.5	3.5
Bacillus SP-62449 Lichenase	64.9	-0.6	71.5	6.1	3.6	2.5
Bacillus amyloliquefaciens lichenase	66.1	0.7	72.1	6.6	4.8	1.8
Amylase, which is the variant of SEQ ID NO: 27 having alteration M202L	69.6	4.2	---	---	---	---
Blank	65.5	0.0	---	---	---	---

Table 22. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase , which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	65.1	-0.4	79.2	13.7	6.0	7.7

Bacillus Akibai Lichenase	66.3	0.9	75.9	10.4	7.3	3.1
Bacillus Mojavensis Lichenase	65.8	0.3	79.0	13.5	6.8	6.7
Bacillus SP-62449 Lichenase	64.9	-0.6	78.9	13.5	5.8	7.6
Bacillus amyloliquefaciens lichenase	66.1	0.7	77.9	12.5	7.1	5.4
Bacillus Subtillis Lichenase	67.3	1.8	78.2	12.7	8.2	4.5
Amylase, which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K	71.9	6.4	---	---	---	---
Blank	65.5	0.0	---	---	---	---

Table 23. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	65.1	-0.4	77.4	11.9	7.9	4.0
Bacillus Akibai	66.3	0.9	77.9	12.4	9.2	3.2

Lichenase						
Bacillus Mojavensis Lichenase	65.8	0.3	79.1	13.6	8.7	5.0
Bacillus SP-62449 Lichenase	64.9	-0.6	79.6	14.1	7.8	6.3
Bacillus amyloliquefaciens lichenase	66.1	0.7	77.7	12.3	9.0	3.3
Bacillus Subtillis Lichenase	67.3	1.8	77.2	11.8	10.2	1.6
Amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K	73.8	8.4	---	---	---	---
Blank	65.5	0.0	---	---	---	---

Table 24. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	65.1	-0.4	80.6	15.1	5.9	9.2

Bacillus Akibai Lichenase	66.3	0.9	79.4	13.9	7.2	6.8
Bacillus Mojavensis Lichenase	65.8	0.3	79.4	14.0	6.6	7.3
Bacillus SP-62449 Lichenase	64.9	-0.6	80.2	14.7	5.7	9.0
Bacillus amyloliquefaciens lichenase	66.1	0.7	79.5	14.1	7.0	7.1
Bacillus Subtillis Lichenase	67.3	1.8	80.2	14.7	8.1	6.6
Amylase, which is the variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K	71.8	6.3	---	---	---	---
Blank	65.5	0.0	---	---	---	---

Table 25. Wascator bottle wash in Model detergent A at 40°C, 30 min (pH 7.7)

	Enzymes solo	Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V +

			T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	65.5	0.8	76.2	11.4	6.8	4.7
Bacillus Akibai Lichenase	66.1	1.3	76.7	12.0	7.3	4.6
Bacillus Mojavensis Lichenase	65.8	1.0	77.5	12.7	7.0	5.7
Bacillus SP-62449 Lichenase	64.6	-0.2	76.6	11.8	5.8	6.0
Bacillus Subtillis Lichenase	67.4	2.7	76.1	11.4	8.7	2.7
Amylase, which is the variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K	70.8	6.0	---	---	---	---
Blank	64.8	0.0	---	---	---	---

Table 26. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase having SEQ ID NO: 12			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	62.0	0.2	74.5	12.7	5.0	7.6
Bacillus Akibai Lichenase	62.2	0.3	74.9	13.1	5.2	7.9
Bacillus Mojavensis Lichenase	61.8	-0.1	74.3	12.4	4.8	7.6
Bacillus SP-62449 Lichenase	61.9	0.1	73.0	11.1	5.0	6.1
amylase having SEQ ID NO: 12	66.7	4.9	---	---	---	---
Blank	61.8	0.0	---	---	---	---

Table 27. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase , which is the variant of SEQ ID NO:23 having alterations G182* + D183*			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	59.4	-0.1	72.8	13.3	6.4	6.8
Bacillus Akibai Lichenase	59.8	0.3	73.1	13.6	6.8	6.8

Bacillus Mojavensis Lichenase	59.5	-0.1	73.2	13.6	6.5	7.2
Bacillus SP-62449 Lichenase	60.9	1.3	72.1	12.6	7.9	4.7
Bacillus amyloliquefaciens lichenase	59.9	0.4	69.6	10.0	6.9	3.1
Amylase, which is the variant of SEQ ID NO:23 having alterations G182* + D183*	66.1	6.5	---	---	---	---
Blank	59.5	0.0	---	---	---	---

Table 28. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	59.4	-0.1	70.4	10.9	5.0	5.8
Bacillus Akibai Lichenase	59.8	0.3	70.1	10.5	5.4	5.1
Bacillus Mojavensis Lichenase	59.5	-0.1	70.5	10.9	5.1	5.9
Bacillus SP-62449	60.9	1.3	69.9	10.4	6.5	3.9

Lichenase						
Bacillus amyloliquefaciens lichenase	59.9	0.4	68.4	8.9	5.5	3.4
Amylase, which is the variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T	64.7	5.1	---	---	---	---
Blank	59.5	0.0	---	---	---	---

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Table 29. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	62.5	1.6	74.9	13.9	7.8	6.1
Bacillus Akibai Lichenase	61.6	0.7	73.6	12.6	6.9	5.7
Bacillus Mojavensis Lichenase	61.7	0.7	71.4	10.4	6.9	3.5

Bacillus SP-62449 Lichenase	59.2	-1.8	73.1	12.1	4.5	7.6
Bacillus amyloliquefaciens lichenase	61.2	0.2	68.9	7.9	6.4	1.5
Bacillus Subtillis Lichenase	60.8	-0.2	71.5	10.5	6.1	4.4
Amylase, which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K	67.2	6.2	---	---	---	---
Blank	61.0	0.0	---	---	---	---

Table 30. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	62.3	0.4	73.7	11.7	6.4	5.3

Bacillus Akibai Lichenase	61.6	-0.4	72.4	10.4	5.7	4.7
Bacillus Mojavensis Lichenase	61.4	-0.6	73.0	11.1	5.5	5.6
Bacillus SP-62449 Lichenase	61.0	-1.0	72.0	10.0	5.1	4.9
Bacillus amyloliquefaciens lichenase	62.1	0.1	71.5	9.5	6.2	3.3
Bacillus Subtillis Lichenase	62.2	0.2	72.8	10.8	6.3	4.6
amylase ,which is the variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K	68.0	6.1	---	---	---	---
Blank	62.0	0.0	---	---	---	---

Table 31. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase , which is the variant of SEQ ID NO: 27 having alteration M202L			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	62.3	0.4	72.0	10.1	5.4	4.7

Bacillus Akibai Lichenase	61.6	-0.4	71.3	9.3	4.6	4.7
Bacillus Mojavensis Lichenase	61.4	-0.6	71.6	9.6	4.4	5.2
Bacillus SP-62449 Lichenase	61.0	-1.0	70.6	8.6	4.0	4.6
Bacillus amyloliquefaciens lichenase	62.1	0.1	68.5	6.6	5.1	1.4
Bacillus Subtillis Lichenase	62.2	0.2	71.2	9.2	5.2	4.0
Amylase , which is the variant of SEQ ID NO: 27 having alteration M202L	67.0	5.0	---	---	---	---
Blank	62.0	0.0	---	---	---	---

Table 32. Wascator bottle wash in Model detergent X at 40°C, 20 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase , which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus Akibai Lichenase	61.8	-0.4	63.3	1.1	-0.3	1.4
Bacillus Mojavensis Lichenase	60.4	-1.8	65.9	3.7	-1.7	5.3

Bacillus SP-62449 Lichenase	62.1	-0.1	64.2	2.0	0.0	2.0
amylase , which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K	62.3	0.1	---	---	---	---
Blank	62.2	0.0	---	---	---	---

Table 33. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	62.0	0.2	66.4	4.5	2.1	2.4
Bacillus Akibai Lichenase	62.2	0.3	66.4	4.6	2.3	2.3
Bacillus Mojavensis Lichenase	61.8	-0.1	68.5	6.7	1.9	4.8
Bacillus SP-62449 Lichenase	61.9	0.1	66.9	5.1	2.1	3.0
Amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F	63.8	2.0	---	---	---	---

+ I206Y + Y243F + E260G + G304R + G476K						
Blank	61.8	0.0	---	---	---	---

Table 34. Wascator bottle wash in Model detergent X at 40°C, 20 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	60.1	-0.3	65.8	5.5	3.1	2.4
Bacillus Akibai Lichenase	58.9	-1.4	63.1	2.8	-0.1	2.9
Bacillus Mojavensis Lichenase	59.2	-1.1	62.3	1.9	0.2	1.7
Bacillus SP-62449 Lichenase	59.8	-0.6	62.6	2.3	0.8	1.5
Bacillus amyloliquefaciens lichenase	59.7	-0.7	64.3	4.0	3.1	0.9
Bacillus Subtillis Lichenase	59.9	-0.5	61.9	1.6	0.9	0.7

Amylase, which is the variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K	61.7	1.3	---	---	---	---
Blank	60.4	0.0	---	---	---	---

Table 35. Wascator bottle wash in Model detergent X at 40°C, 30 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	62.3	0.4	76.1	14.2	6.2	7.9
Bacillus Akibai Lichenase	61.6	-0.4	75.1	13.2	5.5	7.7
Bacillus Mojavensis Lichenase	61.4	-0.6	74.2	12.2	5.3	7.0
Bacillus SP-62449	61.0	-1.0	74.0	12.1	4.9	7.2

Lichenase						
Bacillus amyloliquefaciens lichenase	62.1	0.1	73.3	11.3	6.0	5.3
Bacillus Subtillis Lichenase	62.2	0.2	73.9	11.9	6.1	5.8
Amylase, which is the variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K	67.8	5.9	---	---	---	---
Blank	62.0	0.0	---	---	---	---

Table 36. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase having SEQ ID NO: 12			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	60.5	-2.1	75.1	12.5	5.4	7.1
Bacillus Akibai Lichenase	60.7	-1.9	73.9	11.3	5.6	5.7

Bacillus Mojavensis Lichenase	63.0	0.3	73.3	10.7	7.8	2.8
Bacillus SP-62449 Lichenase	60.8	-1.8	74.5	11.9	5.7	6.2
Bacillus amyloliquefaciens lichenase	61.6	-1.0	70.4	7.8	6.5	1.2
amylase having SEQ ID NO: 12	70.1	7.5	---	---	---	---
Blank	62.6	0.0	---	---	---	---

Table 37. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase , which is the variant of SEQ ID NO:23 having alterations G182* + D183*			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	60.9	1.3	71.8	12.1	8.0	4.2
Bacillus Akibai Lichenase	60.9	1.2	71.5	11.8	7.9	3.9
Bacillus Mojavensis Lichenase	61.3	1.6	71.3	11.6	8.3	3.3
Bacillus SP-62449 Lichenase	60.9	1.2	71.7	12.0	7.9	4.1
Bacillus amyloliquefaciens lichenase	60.9	1.3	68.5	8.8	8.0	0.9
Bacillus Subtillis	60.3	0.6	68.4	8.8	7.3	1.5

Lichenase						
amylase, which is the variant of SEQ ID NO:23 having alterations G182* + D183*	66.4	6.7	---	---	---	---
Blank	59.7	0.0	---	---	---	---

Table 38. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	60.9	1.3	73.3	13.7	8.0	5.6
Bacillus Akibai Lichenase	60.9	1.2	71.7	12.1	8.0	4.0
Bacillus Mojavensis Lichenase	61.3	1.6	72.2	12.5	8.4	4.2
Bacillus SP-62449 Lichenase	60.9	1.2	72.5	12.8	8.0	4.8
Bacillus amyloliquefaciens lichenase	60.9	1.3	68.9	9.2	8.1	1.2
Bacillus Subtillis Lichenase	60.3	0.6	68.6	8.9	7.4	1.5
Amylase, which is the variant of SEQ ID NO:24	66.5	6.8	---	---	---	---

having alterations H183* + G184* + I405L + A421H + A422P + A428T						
Blank	59.7	0.0	---	---	---	---

Table 39. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	60.5	-2.1	73.1	10.9	2.3	8.2
Bacillus Akibai Lichenase	60.7	-1.9	73.2	10.6	2.5	8.1
Bacillus Mojavensis Lichenase	63.0	0.3	74.0	11.4	4.7	6.6
Bacillus SP-62449 Lichenase	60.8	-1.8	75.1	12.4	2.6	9.9
Bacillus amyloliquefaciens lichenase	61.6	-1.0	70.8	8.2	3.4	4.8
Amylase, which is the variant of SEQ ID NO:24 having alterations M9L + R118K +	67.0	4.4	---	---	---	---

G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K						
Blank	62.6	0.0	---	---	---	---

Table 40. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase , which is the variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus Mojavensis Lichenase	62.4	1.0	69.8	8.4	7.1	1.3
Bacillus SP-62449 Lichenase	60.8	-0.6	69.8	8.4	5.5	2.9
amylase, which is the variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K	67.5	6.1	---	---	---	---
Blank	61.4	0.0	---	---	---	---

Table 41. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase , which is the variant of SEQ ID NO: 27 having alteration M202L			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	62.2	0.8	69.4	8.0	5.3	2.8
Bacillus Akibai Lichenase	62.0	0.6	69.5	8.1	5.1	3.0
Bacillus Mojavensis Lichenase	62.4	1.0	68.9	7.5	5.5	2.0
Bacillus SP-62449 Lichenase	60.8	-0.6	69.3	7.9	3.9	4.0
Amylase , which is the variant of SEQ ID NO: 27 having alteration M202L	65.9	4.5	---	---	---	---
Blank	61.4	0.0	---	---	---	---

Table 42. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase , which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus Akibai Lichenase	62.0	0.7	67.5	6.2	3.9	2.3
Bacillus SP-62449 Lichenase	61.2	-0.1	68.4	7.1	3.1	4.1
Bacillus amyloliquefaciens lichenase	62.3	1.0	67.4	6.1	4.2	2.0
Bacillus Subtillis Lichenase	61.9	0.6	66.5	5.2	3.8	1.3
Amylase, which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K	64.5	3.2	---	---	---	---
Blank	61.3	0.0	---	---	---	---

Table 43. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus Akibai Lichenase	60.0	-1.8	65.7	3.9	1.3	2.6
Bacillus Mojavensis Lichenase	62.1	0.4	66.9	5.2	3.5	1.7
Bacillus amyloliquefaciens lichenase	62.0	0.3	65.9	4.2	3.4	0.8
Bacillus Subtillis Lichenase	61.6	-0.2	65.7	3.9	2.9	1.0
Amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K	64.8	3.1	---	---	---	---
Blank	61.7	0.0	---	---	---	---

Table 44. Wascator bottle wash in ADW Model detergent A at 40°C, 20 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus Akibai Lichenase	59.4	-0.8	61.6	1.4	-0.5	1.9
Bacillus amyloliquefaciens lichenase	60.5	0.4	61.8	1.6	0.7	1.0
Bacillus Subtillis Lichenase	60.1	-0.1	61.5	1.3	0.3	1.0
Amylase, which is the variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K	60.5	0.3	---	---	---	---
Blank	60.2	0.0	---	---	---	---

Table 45. Wascator bottle wash in ADW Model detergent A at 40°C, 30 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase, which is the variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	61.4	-0.4	72.9	11.1	7.0	4.1
Bacillus Akibai Lichenase	60.0	-1.8	74.1	12.4	5.7	6.7
Bacillus Mojavensis Lichenase	62.1	0.4	73.2	11.5	7.8	3.7
Bacillus SP-62449 Lichenase	61.4	-0.3	75.1	13.4	7.1	6.3
Bacillus amyloliquefaciens lichenase	62.0	0.3	72.6	10.8	7.7	3.1
Bacillus Subtillis Lichenase	61.6	-0.2	71.1	9.3	7.3	2.1
amylase, which is the variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K	69.2	7.4	---	---	---	---

+ M323T + A339S + E345R + R458K						
Blank	61.7	0.0	---	---	---	---

Table 46. Wascator bottle wash in ADW Model detergent A at 40°C, 20 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase, which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	60.2	-0.9	63.9	2.9	1.0	1.9
Bacillus Akibai Lichenase	60.4	-0.6	65.5	4.5	1.2	3.3
Bacillus Mojavensis Lichenase	60.9	-0.2	65.0	4.0	1.7	2.3
Bacillus amyloliquefaciens lichenase	60.9	-0.1	63.9	2.9	1.7	1.1
Bacillus Subtillis Lichenase	60.7	-0.4	63.5	2.5	1.5	1.0
amylase,which the variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K	62.9	1.9	---	---	---	---
Blank	61.0	0.0	---	---	---	---

Table 47. Wascator bottle wash in ADW Model detergent A at 40°C, 20 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	60.2	-0.9	65.0	4.0	1.8	2.2
Bacillus amyloliquefaciens lichenase	60.9	-0.1	62.8	1.8	2.5	-0.7
amylase of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K	63.7	2.6	---	---	---	---
Blank	61.0	0.0	---	---	---	---

Example 10: Synergistic effect of lichenases combined with proteases:

I. Wascator bottle wash method description:

A Wascator bottle wash method was used to detect the performance of the enzymes. In a Wascator washing machine (FOM 71 Lab) was added bottles (60 mL, DSE PP 70X35 Aseptisk, material #: 216-2620, from VWR) with 25 mL detergent solution including enzyme(s) and four stains (C-H097- Cocoa/oatflakes, from Center for Testmaterials BV, P.O. Box 120, 3133 KT Vlaardingen, the Netherlands, 2 cm in diameter). Two kg ballast (tea towels, cotton) was included in the washing machine. Washed in 25 L water for 15 min at 40°C in model detergent for laundry (model X) and in ADW model detergent A for automated dish wash. After wash the stains were rinsed with tap water twice (3 L) and dried overnight at room temperature in drying cabinet (Electrolux, Intuition, EDD2400). The remission was measured on a spectrophotometer (Macbeth Color-Eye 7000 Remissions) at 460 nm.

II. Results:

In this example the results of combining the individual mature lichenases of Bacillus agaradhaerens Lichenase (SEQ ID NO: 39, His-tagged, recombinant), Bacillus akibai Lichenase (SEQ ID NO: 38, His-tagged, recombinant), Bacillus mojavensis Lichenase (SEQ ID NO: 40, His-tagged, recombinant), Bacillus sp-62449 Lichenase (SEQ ID NO: 37, His-tagged, recombinant), Bacillus amyloliquefaciens Lichenase (SEQ ID NO: 32) and Bacillus subtillis Lichenase (SEQ ID NO: 33) with a protease (Savinase, SEQ ID NO: 34) was studied in order to investigate a potential synergy effect between the two enzyme classes in various detergents using the Wascator bottle wash method is shown. Comparisons were made with lichenase from *Bacillus amyloliquefaciens* and lichenase from *Bacillus subtilis* in Model detergent X and ADW model detergent A using lichenase concentration of 0.01 mg enzyme protein per liter and protease concentration of 0.23 mg enzyme protein per liter at 40°C. The detailed conditions are described in Table 48 and 49 and the results are shown in Table 50 and 51.

Table 48: Experimental condition

Detergent	Model detergent X (see Table 13)
Detergent dosage	1.75 g/L
Test solution volume	25 mL
pH	As is
Wash time	15 minutes
Temperature	40°C
Water hardness	12°dH
Protease concentration in test	0.23 mg/L
Lichenase concentration in test	0.01 mg/L
Test material	C-H097 Cocoa/oatflakes

Table 49: Experimental condition

Detergent	ADW model detergent A (see Table 15)
Detergent dosage	3.77 g/L
Test solution volume	25 mL

pH	As is
Wash time	15 minutes
Temperature	40°C
Water hardness	21°dH
Protease concentration in test	0.23 mg/L
Lichenase concentration in test	0.01 mg/L
Test material	C-H097 Cocoa/oatflakes

Table 50. Wascator bottle wash in Model detergent X at 40°C, 15 min (pH 10.1)

	Enzymes solo		Lichenase in combination with the protease Savinase (SEQ ID NO: 34)			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	40.0	6.1	54.5	20.6	10.6	10.0
Bacillus Akibai Lichenase	37.6	3.8	45.4	11.5	8.2	3.3
Bacillus Mojavensis Lichenase	37.6	3.7	50.9	17.0	8.2	8.7
Bacillus SP-62449 Lichenase	37.7	3.8	48.4	14.5	8.3	6.2
Bacillus amyloliquefaciens lichenase	34.6	0.7	42.8	8.9	5.2	3.6
Bacillus Subtillis Lichenase	35.8	1.9	42.8	8.9	6.4	2.5
Savinase (SEQ ID NO: 34)	38.4	4.5	---	---	---	---

Blank	33.9	0.0	---	---	---	---

Table 51. Wascator bottle wash in ADW Model detergent A at 40°C, 15 min (pH 10.2)

	Enzymes solo		Lichenase in combination with the protease Savinase (SEQ ID NO: 34)			
	REM	ΔREM	REM combined	ΔREM combined	ΔREM theoretic	REM Synergy effect
Bacillus agaradhaerens lichenase	40.0	4.6	53.0	17.6	10.3	7.4
Bacillus Akibai Lichenase	36.8	1.4	52.2	16.8	7.1	9.7
Bacillus Mojavensis Lichenase	39.0	3.6	51.1	15.7	9.3	6.4
Bacillus SP-62449 Lichenase	42.7	7.3	59.6	24.2	12.9	11.3
Bacillus amyloliquefaciens lichenase	36.6	1.2	47.2	11.8	6.8	5.0
Bacillus Subtillis Lichenase	37.1	1.7	48.3	12.9	7.4	5.5
Savinase (SEQ ID NO: 34)	41.1	5.7	---	---	---	---
Blank	35.4	0.0	---	---	---	---

Example 11: Automated dish wash cleaning of cooked oats with lichenases

I. <u>Automated dish washing machine</u>

Automated dish washing machines (Miele, G 1223, GSL-2) were used to show lichenase performance on cooked oats.

II. Results:

Full scale dish wash performance on cooked oats was tested in ADW model detergent A under the experimental conditions given in Table 52.

Table 52. Experimental conditions:

	ADW Model detergent A (See Table 15)
Detergent dosage	3.77 g/L
Lichenase concentration	0 or 0.3 mg enzyme protein/L
Amylase concentration	0.5 mg enzyme protein/L
Water hardness	As is
Protease concentration	SEQ ID NO: 35: 3.7 mg enzyme protein/L SEQ ID NO: 36: 5.9 mg enzyme protein/L
Test solution volume	5.4 L
Miele machine	G 1223, GSL-2, program: 45°C/3'/8'/55
Soiling (Oat:Milk:Sugar)	150 g: 300 mL: 50 g
Soiling per plate	35 g
Ballast	50 g IKW ballast slurry

The soiling was prepared by mixing grinded oats (150g AXA Finvalsede Havregryn in an immersion blender "chopper), milk (300mL) and suger (50g) in a beaker. The mixture was heated to boiling point and cooked for 2 minutes. The soiling was added on porcelain plates (35 g) and dried overnight at 40°C in an oven (Heraeus Instruments, Typ UT6200). The plates were cooled to room temperature, weighted, and washed in Miele dish washing machines (G 1223, GSL-2) for 8 min (main wash) at 45°C with 50 g IKW ballast slurry in ADW Model detergent A, amylase (which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K; 0.5 mg enzyme protein/L) and proteases (SEQ ID NO: 35; 3.7 mg enzyme protein/L, SEQ ID NO: 36; 5.9 mg enzyme protein/L) or ADW Model detergent A, amylase (which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K; 0.5 mg enzyme protein/L), proteases (SEQ ID NO: 35; 3.7 mg enzyme

protein/L , SEQ ID NO: 36; 5.9 mg enzyme protein/L) and lichenase (Bacillus agaradhaerens (SEQ ID NO: 39, His-tagged, recombinant; 0.3 mg enzyme protein/L).

An effect of the lichenase on cooked oats is clearly visual seen as well as weighted. The measured numbers are shown in Table 53 as well as the calculated number for soiling left on the plates after wash.

Calculations:

Weight of soiling left on plates before wash = Weight of plate and soiling before wash - Weight of plate with no soiling before wash.

Weight of soiling left on plates after wash = Weight of plate and soiling after wash - Weight of plate with no soiling before wash.

Table 53. Wash performance on cooked oats:

	Weight of plate and soiling before wash (g)*	Weight of plate and soiling after wash (g)*	Weight of plate with no soiling before wash (g)*	Weight of soiling left on plates before wash (g)*	Weight of soiling left on plates after wash (g)*
No lichenase	530.6	515.9	514.5	16.1	1.4
With lichenase	549.0	533.0	532.8	16.2	0.2

*Average of 4 replicates.

Example 12: Automated dish wash cleaning of cooked and burned-in oats with lichenases

I. <u>Automated dish washing machine</u>

Automated dish washing machines (Miele, G 1223, GSL-2) were used to show lichenase performance on cooked and burned-in oats.

II. Results:

Full scale dish wash performance on cooked and burned-in oats was tested in ADW model detergent A under the experimental conditions given in Table 54.

Table 54. Experimental conditions:

	ADW Model detergent A (See Table 15)

Detergent dosage	3.77 g/L
Lichenase concentration	0 or 0.3 mg enzyme protein/L
Amylase concentration	0.5 mg enzyme protein/L
Water hardness	As is
Protease concentration	SEQ ID NO: 35: 3.7 mg enzyme protein/L SEQ ID NO: 36: 5.9 mg enzyme protein/L
Test solution volume	5.4 L
Miele machine	G 1223, GSL-2, program: 45°C/3'/8'/55
Soiling (Oat:Milk:Sugar)	150 g: 300 mL: 50 g
Soiling per plate	15 g
Ballast	50 g IKW ballast slurry

The soiling was prepared by mixing grinded oats (150g AXA Finvalsede Havregryn in an immersion blender "chopper), milk (300mL) and suger (50g) in a beaker. The mixture was heated to boiling point and cooked for 2 minutes. The soiling was added on steel plates (15 g) and dried in an oven (Heraeus Instruments, Typ UT6200) for 40 minutes at 140°C. The plates were cooled down, weighted, and washed in Miele dish washing machines (G 1223, GSL-2) for 8 min (main wash) at 45°C with 50 g IKW ballast slurry in ADW Model detergent A, amylase (which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K, 0.5 mg enzyme protein/L) and proteases (SEQ ID NO: 35; 3.7 mg enzyme protein/L, SEQ ID NO: 36; 5.9 mg enzyme protein/L) or ADW Model detergent A, amylase (which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K; 0.5 mg enzyme protein/L), proteases (SEQ ID NO: 35; 3.7 mg enzyme protein/L , SEQ ID NO: 36; 5.9 mg enzyme protein/L) and lichenase (Bacillus agaradhaerens, SEQ ID NO: 39, His-tagged, recombinant;; 0.3 mg enzyme protein/L). After wash the plates were dried at room temperature and weighted.

Calculations:

Weight of soiling left on plates before wash = Weight of plate and soiling before wash - Weight of plate with no soiling before wash.

Weight of soiling left on plates after wash = Weight of plate and soiling after wash - Weight of plate with no soiling before wash.

A clear effect of the lichenase is seen on cooked and burned-in oats and the measured numbers are shown in Table 55 as well as the calculated number for soiling left on the plates after wash.

Table 55. Wash performance on cooked and burned-in oats:

	Weight of plate and soiling before wash (g)*	Weight of plate and soiling after wash (g)*	Weight of plate with no soiling before wash (g)*	Weight of soiling left on plates before wash (g)*	Weight of soiling left on plates after wash (g)*
No lichenase	210.1	205.8	203.6	6.5	2.2
With lichenase	205.9	200.5	199.4	6.5	1.2

*Average of 6 replicates.

Example 13: Automated dish wash cleaning of uncooked oats with lichenases

I. <u>Automated dish washing machine</u>

Automated dish washing machines (Miele, G 1223, GSL-2) were used to show lichenase performance on uncooked oats.

II. Results:

Full scale dish wash performance on uncooked oats was tested in ADW model detergent A under the experimental conditions given in Table 56.

Table 56. Experimental conditions:

	ADW Model detergent A (see Table 15)
Detergent dosage	3.77 g/L
Lichenase concentration	0 or 0.3 mg enzyme protein/L
Amylase concentration	0.5 mg enzyme protein/L
Water hardness	As is
Protease concentration	SEQ ID NO: 35: 3.7 mg enzyme protein/L SEQ ID NO: 36: 5.9 mg enzyme protein/L
Test solution volume	5.4 L

Miele machine	G 1223, GSL-2, program: 45°C/3'/8'/55
Soiling (Oat:Milk:Sugar)	150 g: 300 mL: 50 g
Soiling per plate	35 g
Ballast	50 g IKW ballast slurry

The soiling was prepared by mixing grinded oats (150g AXA Finvalsede Havregryn in an immersion blender "chopper), milk (300mL) and suger (50g) in a beaker. The soiling was added on porcelain plates (35 g) and dried overnight at 40°C in an oven (Heraeus Instruments, Typ UT6200). The plates were cooled to room temperature, weighted, and washed in Miele dish washing machines (G 1223, GSL-2) for 8 min (main wash) at 45°C with 50 g IKW ballast slurry in ADW Model detergent A, amylase (which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K, 0.5 mg enzyme protein/L) and proteases (SEQ ID NO: 35; 3.7 mg enzyme protein/L, SEQ ID NO: 36; 5.9 mg enzyme protein/L) or ADW Model detergent A, amylase (which is the variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K; 0.5 mg enzyme protein/L), proteases (SEQ ID NO: 35; 3.7 mg enzyme protein/L, SEQ ID NO: 36; 5.9 mg enzyme protein/L) and lichenase (Bacillus agaradhaerens; SEQ ID NO: 39, His-tagged, recombinant; 0.3 mg enzyme protein/L). After wash the plates were dried at room temperature and weighted.

An effect of the lichenase on uncooked oats is clearly visual seen as well as weighted. The measured numbers are shown in Table 57 as well as the calculated number for soiling left on the plates after wash.

Calculations:

Weight of soiling left on plates before wash = Weight of plate and soiling before wash - Weight of plate with no soiling before wash.

Weight of soiling left on plates after wash = Weight of plate and soiling after wash - Weight of plate with no soiling before wash.

Table 57. Wash performance on uncooked oats:

	Weight of plate and soiling before wash (g)*	Weight of plate and soiling after wash (g)*	Weight of plate with no soiling before wash (g)*	Weight of soiling left on plates before wash (g)*	Weight of soiling left on plates after wash (g)*
No lichenase	530.5	515.2	514.5	16.0	0.7
With lichenase	548.8	532.8	532.8	16.0	0.0

*Average of 4 replicates.

Example 14: Wash performance and anti-redeposition effect of lichenases

I. Mini Terg-O-tometer (MiniTOM) wash assay

The Mini Tergo-To-Meter (MiniTOM) is a medium scale model wash system that can be applied to test 16 different wash conditions simultaneously. A MiniTOM is basically a large temperature controlled water bath with up to 16 open metal beakers (300 mL) submerged into it. Each beaker constitutes one small top loader style washing machine and during an experiment, each of them will contain a solution of a specific detergent/enzyme system and the soiled and unsoiled fabrics its performance is tested on. Mechanical stress is achieved by a rotating stirring arm, which stirs the liquid within each beaker. Because the MiniTOM beakers have no lid, it is possible to withdraw samples during a MiniTOM experiment and assay for information on-line during wash.

The MiniTOM model wash system is mainly used in medium scale testing of detergents and enzymes at US or LA/AP wash conditions. In a MiniTOM experiment, factors such as the ballast to soil ratio and the fabric to wash liquor ratio can be varied. Therefore, the MiniTOM provides the link between small scale experiments, such as AMSA and mini-wash, and the more time consuming full scale experiments in top loader washing machines.

II. Results:

MiniTergotometer (MiniTOM) anti-redeposition by the lichenase, Bacillus agaradhaerens (SEQ ID NO: 7), was tested in model detergent A under the experimental conditions given in Table 58.

Table 58: Experimental conditions:

	Model A (See table Table 11)
Detergent dosage	3.33 g/L
Lichenase concentration	0 or 0.3 mg enzyme protein/L
Amylase concentration	0.2 mg enzyme protein/L
Water hardness	15°dH (Ca^{2+}:Mg^{2+}:HCO_3^- = 4:1:7.5)

Test solution volume	100 ml
Wash time	20 minutes
Rotation	120 rpm
pH	as is
Temperature	20°C
Test material	Textile sample C-H097 (Cocoa/oatflakes) was obtained from Center for Testmaterials BV, P.O. Box 120, 3133 KT Vlaardingen, the Netherlands. Swatches with no initial soiling: Prewashed Knitted cotton was obtained from Warwick Equest Ltd, Unit 55, Consett Business Park, Consett,County Durham,DH8 6BN, United Kingdom.

The anti-redeposition (and wash performance) of the lichenase, Bacillus agaradhaserens (SEQ ID NO: 7), was tested as described below.

The wash solutions were prepared by adjusting the water hardness to 15°dH (Ca^{2+}:Mg^{2+}:HCO_3^- = 4:1:7.5) by addition of $CaCl_2$, $MgCl_2$ and $NAHCO_3$, adding the desired amount of detergent (3.33 g/L of Model detergent A) and adjusting the temperature to 40°C in the buckets. The detergent was dissolved during magnet stirring for 10 minutes (wash solution was used within 30 to 60 min after preparation). The temperature and rotation in the water bath in the MiniTOM were set to 40°C and 120 rpm, respectively. When the temperature was adjusted according to settings (tolerance is +/- 0.5°C), 100 mL of the wash solution was added to the MiniTOM beakers (300mL). Swatches (1 knitted cotton swatches (circular, 2 cm in diameter) and 12 C-H097 (circular, 2 cm in diameter), lichenase (Bacillus agaradhaerens (SEQ ID NO: 39, His-tagged, recombinant;), 0 or 0.3 mg enzyme protein/L) and amylase (SEQ ID NO: 12, 0.2 mg enzyme protein/L) were added to the beakers and washed for 20 minutes. Swatches were rinsed in cold tap water for 5 minutes. The swatches were sorted and dried between filter paper in a drying cupboard without heat overnight.

The anti-redeposition (and wash performance) was measured as the brightness of the color of the textile washed expressed in remission values (REM). Remission measurements were made using a Macbeth 7000 Color Eye spectrophotometer. Each of the dry swatches was measured. As there is a risk of interference from the back-ground, the swatches were placed on top of 2 layers of fabric during the measurement of the remission. The remission was measured at 460 nm. The UV filter was not included. An average result for remission for the swatches was calculated.

The anti-redeposition effect due to the presence of the lichenase is shown in Table 59. In the beakers without the lichenase present, the released soil from the soiled swatch (C-H097) is redeposit to the swatch with no initial soiling on. When the lichenase is present in the wash liquor, an anti-redeposition effect is clearly seen.

Table 59. Anti-redeposition effect and wash performance of lichenases:

	REM before wash	REM after wash without Lichenase	REM after wash with Lichenase
Swatch with no initial soiling (Anti-redeposition)	92.1	65.3	88.2
Swatch with soiling (C-H097) (Wash performance)	18.8	38.1	42.2

Example 15:

Cleaning performance on oat flakes:

500 g oat flakes, 167 g sugar and 1 l semi-skimmed milk (1.5 % fat) are intensely mixed. The mixture is let unstirred for at least 2 hours at room temperature. Afterwards, 15 g (+/-0.2g) of this preparation is spread evenly on a plate (china) in form of a circle using a metal ring (radius 11 cm) and left to dry over night at 40°C.

Cleaning performance is tested in an automatic dishwashing machine Miele GSL, 21 °dH, 45 °C, 8 min holding time, and 55°C rinse temperature, with soiled dish ware/cutlery placed inside (according to IKW method, Söfwjournal, 142, (06) 2016, S. 33-48) with additional 4 plates as prepared above placed therein. Pasta and starch-mix cleaning performance was measured according to IKW. The results, also for oatflakes, are documented as arithmetic averages, evaluation according to IKW. Higher values indicated a better cleaning performance, differences above 1.0 are considered to be significant.

Cleaning Performance:

A two component liquid automatic dishwashing product (15 ml of each composition A and B, Table 60, 61) was dosed at the same time into the dosing chamber of the dishwashing machine.

Table 60:

Enzymphase (EP)	A

Amylase (wt.% enzyme protein)	0.02
Protease (wt.% enzyme protein)	0.20
Glycerol	8.0
Copolymer comprising sulfonic acid group containing monomer	7.5
MGDA Na4	10.00
Nonionic surfactant(s)	2.8
Polypeptide according to invention* (Mature polypeptide according to SEQID No: 7)	s. below
Misc (perfume, colorant, stabilizers for enzymes and UV, glass corrosion inhibitors, thickener, water)	Ad 100
pH-Wert (not diluted, 25°C)	7.5

Table 61:

Alkaline Phase (AP)	B
HEDP	2.5
MGDA (Tetranatriumsalz)	3.5
KOH	3.2
Sodium Carbonate	8.5
Kationic copolymer	0.5
Sodium citrat x $2H_2O$	14.0
Misc (perfume, colorant, stabilizers for enzymes and UV, glass corrosion inhibitors, thickener, water)	Ad 100
pH-Wert (not diluted, 25°C) adjusted (KOH/Citric Acid)	10.5

Table 62

Cleaning performance	Oat flakes	Starch Mix
No Licheninase, prepared directly before testing	6.5	7.3
1.5 mg Licheninase* in A, prepared directly before testing	8.0	8.3

* (Mature polypeptide according to SEQID No: 7)

Table 63

Cleaning performance	Spaghetti
No Licheninase, prepared directly before testing	5.8
1 mg Licheninase* in A, prepared directly before testing	7.5

* (Mature polypeptide according to SEQID No: 7)

Table 64

Liquid automatic dishwashing product	Cleaning performance on Oat flakes

No Licheninase, storage conditions: 4 weeks at T=22 °C	5.9
1 mg Licheninase* in A, prepared directly before testing	7.5
1 mg Licheninase* in A, storage conditions: 4 weeks at T=22 °C	7.3
1 mg Licheninase* in A, storage conditions: 4 weeks at T=30 °C	7.6
1 mg Licheninase* in A, storage conditions: 4 weeks at T=40°C	7.1

* (Mature polypeptide according to SEQID No: 7)

Surprisingly, it has been found that the cleaning performance of a dishwash composition, preferably an automatic dishwash composition is enhanced on pasta (spaghetti) and/or starch-containing soils (Table 62, 63). Therefore the licheninases of the invention facilitate the removal of starch-containing soil in the presence of one or more amylases and enhance amylase related cleaning performance.

The cleaning performance of the dishwash composition on Oatflakes is not significantly altered after 4 weeks storage at different temperatures (Table 64). Comparable results were found for automatic dishwash compositions containing 1.5 or 2.0 mg active enzyme protein/job Licheninase, storage conditions: 8 weeks at T=40°C or 2 weeks at T=50°C.

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

Claims

1. A cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a polypeptide having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 89% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 89% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity.

2. The cleaning or detergent composition of claim 1, wherein said polypeptide having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9.

3. The cleaning or detergent composition of any of claims 1-2, wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9.

4. The cleaning or detergent composition of any of claims 1-3, wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity.

5. The cleaning or detergent composition of any of claims 1-5, wherein said composition has pH of 6.5 or above, preferably of 7.0 or above, more preferably of 7.5 or above and optionally

comprises a bleaching agent; preferably said pH is in the range from about 7.5 to about 13.5, further preferably said pH is in the range from about 7.5 to about 12.5, most preferably said pH is in the range from about 8.5 to about 11.5, further most preferably said pH is in the range from about 9.5 to about 10.5.

6. The dish washing composition of any of claims 1-5, further comprising:

i) one or more detergent components; and/or

ii) one or more additional enzymes.

7. The dish washing composition of any of claims 1-6, further comprising a copolymer that contains at least one sulfonic acid containing monomer, preferably in an amount from 0.1 to 20% by weight, in particular 0.5 to 18% by weight, particularly preferably 1.0 to 15% by weight, in particular 4 to 14% by weight, particularly 6 to 12% by weight.

8. The cleaning or detergent composition of any of claims 1-7, wherein said composition comprises said polypetide in concentrations of 0.00001 mg enzyme protein/g composition to 100 mg enzyme protein/g composition, preferred 0.0001 mg enzyme protein/g composition to 50 mg enzyme protein/g composition, more preferred 0.001 mg enzyme protein/g composition to 20 mg enzyme protein/g composition, especially preferred 0.01 mg enzyme protein/g composition to 10 mg enzyme protein/g composition.

9. A cleaning or detergent composition, wherein said cleaning or detergent composition is a dish washing composition, said composition comprising a polypeptide having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 70% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 70% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

wherein said cleaning or detergent composition further comprising:

(i) one or more amylases; and/or

(ii) one or more proteases.

10. The cleaning or detergent composition of claim 9, wherein said polypeptide has at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, preferably and/or wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9 preferably and/or wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity.

11. The cleaning or detergent composition of any of claims 1-10, wherein said amylase is an alpha-amylase.

12. The cleaning or detergent composition of any of claims 1-11, further comprising:

i) one or more detergent components; and/or

ii) one or more additional enzymes.

13. The cleaning or detergent composition of any of claims 1-12, wherein said alpha-amylase is selected from the group consisting of:

(a) a polypeptide having at least 90% sequence identity to SEQ ID NO: 13;

(b) a polypeptide having at least 90% sequence identity to SEQ ID NO: 13, wherein the polypeptide comprises a substitution in one or more of positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and/or 444;

(c) a polypeptide having at least 90% sequence identity to SEQ ID NO: 14;

(d) a polypeptide having at least 90% sequence identity to the hybrid polypeptide of SEQ ID NO: 15;

(e) a polypeptide having at least 90% sequence identity to the hybrid polypeptide of SEQ ID NO: 15, wherein the hybrid polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 48, 49, 107, 156, 181, 190, 197, 201, 209 and/or 264;

(f) a polypeptide having at least 90% sequence identity to SEQ ID NO: 16;

(g) a polypeptide having at least 90% sequence identity to SEQ ID NO: 16, wherein

the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 181, 182, 183, 184, 195, 206, 212, 216 and/or 269;

(h) a polypeptide having at least 90% sequence identity to SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19;

(i) a polypeptide having at least 90% sequence identity to SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19, wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 140, 183, 184 195, 206, 243, 260, 304 and/or 476;

(j) a polypeptide having at least 90% sequence identity to SEQ ID NO: 20;

(k) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21;

(l) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21, wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 176, 177, 178, 179, 190, 201, 207, 211 and/or 264;

(m) a polypeptide having at least 90% sequence identity to SEQ ID NO: 22;

(n) a polypeptide having at least 90% sequence identity to SEQ ID NO: 22, wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 87, 98, 125, 128, 131, 165, 178, 180, 181, 182, 183, 201, 202, 225, 243, 272, 282, 305, 309, 319, 320, 359, 444 and/or 475;

(o) a polypeptide having at least 90% sequence identity to SEQ ID NO: 21, wherein the polypeptide comprises a substitution, a deletion or an insertion in one of more of positions: 28, 118, 174; 181, 182, 183, 184, 186, 189, 195, 202, 298, 299, 302, 303, 306, 310, 314; 320, 324, 345, 396, 400, 439, 444, 445, 446, 449, 458, 471 and/or 484; and

(p) a polypeptide having at least 90% sequence identity to SEQ ID NO: 12

(q) a variant of SEQ ID NO:23 having alterations G182* + D183*;

(r) a variant of SEQ ID NO:24 having alterations H183* + G184* + I405L + A421H + A422P + A428T;

(s) a variant of SEQ ID NO:24 having alterations M9L + R118K + G149A + G182T + G186A + D183* + G184* + N195F + M202L + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K;

(t) a variant of SEQ ID NO: 24 having alterations R178* + G179* + E187P + I203Y + R458N + T459S + D460T + G476K

(u) a variant of SEQ ID NO: 27 having alteration M202L;

(v) a variant of SEQ ID NO: 28 having alterations R180* + S181* + S243Q + G475K;

(w) a variant of SEQ ID NO: 29 having alterations D183* + G184* + W140Y + N195F + I206Y + Y243F + E260G + G304R + G476K;

(x) a variant of SEQ ID NO: 30 having alterations H1* + N54S + V56T + K72R + G109A + F113Q + R116Q + W167F + Q172G + A174S + G184T + N195F + V206L + K391A + P473R + G476K;

(y) a variant of SEQ ID NO: 31 having alterations M9L + R118K + G149A + G182T +

G186A + D183* + G184* + N195F + T246V + T257I + Y295F + N299Y + R320K + M323T + A339S + E345R + R458K.

14. The cleaning or detergent composition of any of claims 1-13, wherein said protease is selected from the group consisting of:

a) a polypeptide having at least 60% sequence identity to SEQ ID NO: 34, wherein said polypeptide has protease activity;

b) a polypeptide having at least 60% sequence identity to SEQ ID NO: 35, wherein said polypeptide has protease activity; and

c) a polypeptide having at least 60% sequence identity to SEQ ID NO: 36, wherein said polypeptide has protease activity.

15. The composition of any of preceding claims 1-14, wherein said composition or said polypeptide having beta-glucanase activity comprised in said composition, has improved stability and/or wash performance under alkaline conditions, preferably said alkaline conditions have pH 7.5 or above.

16. Use of a cleaning or detergent composition of any of claims 1-15 in a cleaning process, wherein said cleaning process is a dish wash cleaning process, including Automatic Dish Wash (ADW), hand dish wash (HDW) and industrial dish cleaning processes; optionally said use is carried out under alkaline conditions having pH 7.5 or above.

17. A process of degrading a beta-glucan comprising applying the cleaning or detergent composition of any of claims 1-15 to said beta-glucan, wherein said process is a dish washing process, preferably said beta-glucan is a beta-D-glucan, further preferably said beta-glucan is a beta-1,3-1,4 glucan, most preferably said beta-glucan is a mix-linkage beta-glucan, further most preferably said beta-glucan is a barley beta-glucan or oatmeal beta-glucan; optionally, said process is carried out under alkaline conditions having pH 7.5 or above.

18. A method for reducing or preventing soil redeposition using a cleaning or detergent composition of any of claims 1-15 or

a polypeptide having beta-glucanase activity, selected from the group consisting of:

(a) a polypeptide having at least 70% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8, or (ii) the full-

length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 89% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;perferably wherein said polypeptide having at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, more preferably wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9.,

most preferably, wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity,

wherein said method is a dish wash method.

19. Use of a cleaning or detergent composition of any of claims 1-15 or a polypeptide having beta-glucanase activity,

selected from the group consisting of:

(a) a polypeptide having at least 70% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 70% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

perferably wherein said polypeptide having at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9,

more preferably wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9.,

most preferably, wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity,

for reducing or preventing soil redeposition, wherein said use is use in dish wash or during a dish washing process.

20. Use of a cleaning or detergent composition of any of claims 1-15 or a polypeptide having beta-glucanase activity,

selected from the group consisting of:

(a) a polypeptide having at least 70% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 70% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

perferably wherein said polypeptide having at 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9,

more preferably wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9., most preferably, wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity;

for removal of cereal containing soil, especially dried-on cereal containing soil, preferably oat flakes containing soil, especially dried-on oat flakes containing soil and/or cooked oats containing soil, and/or cooked and burned-in oats containing soil, and/or uncooked oats containing soil, in dish wash or during a dish washing process.

21. Use of a cleaning or detergent composition of any of claims 1-15 or a polypeptide having beta-glucanase activity,

selected from the group consisting of:

(a) a polypeptide having at least 70% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 70% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

perferably wherein said polypeptide having at 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9,

more preferably wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9., most preferably, wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity,

for facilitating removal of starch-containing soil in the presence of one or more amylases and/or

for enhancing amylase related cleaning performance, wherein said use is use in dish wash or during a dish washing process.

22. Use of a cleaning or detergent composition of any of claims 1-15 or a polypeptide having beta-glucanase activity,

selected from the group consisting of:

(a) a polypeptide having at least 70% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8, or (ii) the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 70% sequence identity to the mature polypeptide coding sequence of the sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 8;

(d) a variant of the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, wherein said variant comprising a substitution, deletion, and/or insertion at one or more positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has beta-glucanase activity;

perferably wherein said polypeptide having at 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of the sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9,

more preferably wherein the mature polypeptide is selected from the group consisting of: amino acids 1 to 222 of SEQ ID NO: 7, amino acids 1 to 351 of SEQ ID NO: 2, amino acids 1 to 351 of SEQ ID NO: 3, amino acids 1 to 245 of SEQ ID NO: 5, amino acids 1 to 214 of SEQ ID NO: 9.,

most preferably, wherein said beta-glucanase activity is licheninase EC 3.2.1.73 activity,

for facilitating removal of protein-containing soil in the presence of one or more proteases and/or for enhancing protease related cleaning performance, wherein said use is use in dish wash or during a dish washing process.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/080150

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/42 C11D3/386 C12N5/10 C12N15/52
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE UniProt [Online] 1 October 2000 (2000-10-01), "SubName: Full=Hybrid-endo-beta-1,3-1,4 glucanase {ECO:0000313|EMBL:BAB06950.1};", XP002757188, retrieved from EBI accession no. UNIPROT:Q9K7X6 Database accession no. Q9K7X6 cited in the application sequence ----- -/--	1-22

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2017	22/03/2017

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/080150

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE UniProt [Online] 8 March 2011 (2011-03-08), "SubName: Full=Glycoside hydrolase family 16 {ECO:0000313¦EMBL:ADU30622.1};", XP002757189, retrieved from EBI accession no. UNIPROT:E6TRB0 Database accession no. E6TRB0 cited in the application sequence -----	1-22
X	DATABASE UniProt [Online] 19 March 2014 (2014-03-19), "SubName: Full=Endo-beta-1,3-1,4 glucanase {ECO:0000313¦EMBL:GAE36131.1};", XP002757190, retrieved from EBI accession no. UNIPROT:W4QVK7 Database accession no. W4QVK7 cited in the application sequence -----	1-22
X	US 6 541 233 B1 (HILLEN WOLFGANG [DE] ET AL) 1 April 2003 (2003-04-01) figure 1; sequences 1, 2 figure 2 column 1 -----	1-22
X	YANG SHAOQING ET AL: "Purification and characterization of a novel alkaline [beta]-1,3-1,4-glucanase (lichenase) from thermophilic fungusMalbranchea cinnam", JOURNAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY, BASINGSTOKE, GB, vol. 41, no. 10, 12 August 2014 (2014-08-12), pages 1487-1495, XP035387188, ISSN: 1367-5435, DOI: 10.1007/S10295-014-1494-4 [retrieved on 2014-08-12] the whole document -----	1-22
X	SAMEH MAKTOUF ET AL: "A laundry detergent compatible lichenase: Statistical optimization for production under solid state fermentation on crude millet", INDUSTRIAL CROPS AND PRODUCTS, vol. 43, 1 May 2013 (2013-05-01), pages 349-354, XP055101093, ISSN: 0926-6690, DOI: 10.1016/j.indcrop.2012.06.055 the whole document -----	1-22

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/080150

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/083096 A2 (NOVOZYMES AS [DK]) 5 June 2014 (2014-06-05) the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/080150

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

1-22(partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-22(partially)

   Cleaning composition comprising beta-glucanase according to SEQ ID NO: 7. Further compositions, uses, formulations, polynucleotides, host cells relating thereto.

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2. claims: 1-22(partially)

   As invention 1, but relating to SEQ ID NOs 2 and 3.

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3. claims: 1-22(partially)

   As invention 1, but relating to SEQ ID NO: 5.

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4. claims: 1-22(partially)

   As invention 1, but relating to SEQ ID NO:9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/080150

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6541233 B1	01-04-2003	AT 304597 T	15-09-2005
		CN 1265703 A	06-09-2000
		DE 19732751 A1	04-02-1999
		DE 59813060 D1	20-10-2005
		DK 0988384 T3	30-01-2006
		EP 0988384 A1	29-03-2000
		ES 2249840 T3	01-04-2006
		HU 0004264 A2	28-03-2001
		JP 4222723 B2	12-02-2009
		JP 2001512023 A	21-08-2001
		PL 338296 A1	23-10-2000
		US 6541233 B1	01-04-2003
		WO 9906573 A1	11-02-1999
WO 2014083096 A2	05-06-2014	EP 2925849 A2	07-10-2015
		US 2015353871 A1	10-12-2015
		WO 2014083096 A2	05-06-2014