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(54) Title: DETERGENT COMPOSITIONS WITH GALACTANASE

(57) Abstract: The present invention relates to polypeptides having endo-beta-1-6-galactanase enzyme activity, detergent composition comprising such polypeptides and uses of the composition and/or polypeptide in methods of washing, such as laundering of textiles.



DETERGENT COMPOSITIONS WITH GALACTANASE

The present invention relates to endo-beta-1-6-galactanase enzymes and cleaning compositions comprising an endo-beta-1-6-galactanase enzyme. Also described is cleaning methods and the use of the endo-beta-1-6-galactanase. In particular, the endo-beta-1-6-galactanase is useful in reducing and preventing the staining of items being washed.

When clothing is worn, they are exposed to bacteria from the body of the user and from the rest of the environment in which they are used. Some of these bacteria are capable of adhering to the clothing item and form a biofilm on the item. The presence of biofilm means that the clothing items may become sticky and therefore soil adheres to the sticky areas. This soil has shown difficult to remove by commercially available detergent compositions. Further, when very dirty laundry items are washed together with less dirty laundry items the dirt present in the wash liquor tend to stick to the biofilm. As a result hereof the laundry item is more "soiled" after wash than before wash. An example of this is the increase in location-specific staining of clothing items e.g. in the underarm region, following washing.

Enzymes have been used within the detergent industry as part of washing formulations for many years. Proteases are from a commercial perspective the most relevant enzyme in such formulations, but other enzymes including lipases, amylases, cellulases, hemicellulases or mixtures of enzymes are also often used. To improve the cost and/or the performance of enzymes there is an ongoing search for enzymes with improved or altered properties, such as new soil to be targeted, increased activity at low temperatures, increased stability, increased specific activity at a given pH, altered Ca^{2+} dependency, increased stability in the presence of other detergent ingredients (e.g. bleach, surfactants etc.) etc. Examples of common-place enzymes used in detergents include, but are not limited to subtilases and alpha-amylases.

Detergent compositions have been described and used widely in the art, but there is a continued need for improved detergent compositions to meet industrial and domestic needs. Thus, it is an objective of the present invention to provide such detergent compositions and their uses.

The inventors have now identified that polypeptides having endo-beta-1-6-galactanase activity can be used in detergent compositions for cleaning items. In particular, the polypeptides have found to be useful in relation to preventing and reducing soling which may stick to organic components, such as EPS or biofilm components on items such as textiles and/or fabric.

In a first aspect of the invention there is provided a polypeptide having endo-beta-1-6-galactanase enzyme activity. Such polypeptide may be an endo-beta-1-6-galactanase enzyme. The enzyme may be derived from the glycoside hydrolase 30 (GH30) family of enzymes; and more specifically the glycoside hydrolase 30_5 (GH30_5) family.

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A polypeptide having endo-beta-1-6-galactanase activity or an endo-beta-1-6-galactanase enzyme is any polypeptide that catalyzes endohydrolysis of (1->6)-beta-D-galactosidic linkages in arabinogalactan proteins and (1->3):(1->6)-beta-galactans to yield galactose and (1->6)-beta-galactobiose. 1,6-beta-D-galactooligosaccharides are hydrolysed with a degree of polymerization (DP) higher than 3. The two terms “polypeptide having endo-beta-1-6-galactanase activity” and “endo-beta-1-6-galactanase” may be used interchangeably herein. Endo-beta-1-6-galactanase is also known as “galactan endo-1,6-beta-galactosidase” or “endo-1,6-beta-galactanase”.

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15 The polypeptide may be derived from a bacterial source, such as a *Bacillus* or *Paenibacillus*.

The polypeptide may be a natural polypeptide or may be produced recombinantly. A natural polypeptide would be one isolated from the organism in which it is normally produced. Recombinant production would typically be the expression of the polypeptide from an exogenous or recombinant nucleic acid present in a host cell or organism in which that polypeptide is not normally produced, but would also include the increase of production of the polypeptide from a cell or organism in which it is normally produced by the introduction of exogenous or recombinant nucleic acid.

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25 Preferably, the polypeptide is an isolated polypeptide.

The polypeptide may, comprise or consist of the full-length polypeptide sequence of amino acid sequence of SEQ ID NO: 1 or an allelic variant thereof; or is a fragment thereof having endo-beta-1-6-galactanase activity. The polypeptide may alternatively comprise or consist of the mature polypeptide of SEQ ID NO: 2 or an allelic variant thereof; or is a fragment thereof having endo-beta-1-6-galactanase activity.

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Full length polypeptide – endo-beta-1-6-galactanase [SEQ ID NO: 1]

35

MQKKNLRLKCLFCLVLLVTIGGMLPATAQSIFAEGATKAVKVDETIKLDPSYQHKPFDGW
GTALVWFANVTGGWPDEIKNELADALFGEEGLNFNIGRYNIGGGDSPETTPYMRKGGAVS
GYWNRPAEFGPPEEKAEGWKEQENWWDPENPDHWNWKADKNQQWWLEAAAARGADTFEAF
SNSPPYFMTRSGYTSGNWNWDNINIKADQFENFAAYLTNVVDYLQEELEIEFRTLSPVNE

PYTGYWRALGRQEGSNWSRASQAKI INEVKKQLNEKELNTVVSAMDETHPQRFRENWEQY
 NQQTRANIGQLNVHTYWPQEHIAVRDIAKGAEKKLWMSEVDLSPGGIGQDHEDIRTGLGL
 SERITLDIQRLEPEAWVLWQAIEDEVNMNPEHENGWGLIQVDFSPDDFDKLEIYKNKKY
 YAMGNYSKFIRPGYQFINSNNSQTLAAMNKEDDSVVIVYTNYLKEAKTVEFDLSGFKIVG
 5 ENAQAVPHITSATDNLVQGTINNIADKKFSTTIKPQSITTFVVSNSVSGVDSSQKFINSKD
 AYKLINKHSDKAADIGADNEKIVQKTVVREKDSQSWNIQQITNGNTTQELYKIFNSKTGK
 VLGVANGGAVLQESDEGSLSQQWMLSTYGNGEYTLINRKDGNLLEVGGQSRAEDAGMGVW
 RPNSGNNQVWKVIKAGIVEIEPVEVVVTKKKIAPILPNQITAIYGDGEKVEKEVIWDSID
 PEKYQKENTFTVEGSIDGTDKKAIIIVSKVDRADSIKVKTVTGVALELPSEITANLLS
 10 GSNGKVEVEWEEIDPALYENVGKFVVGKVVNSPEKAFAYVQVVEPILDNLALRKSNSEF
 PKASASFTGRWDNVNHINDGTITSSRWTNWDPNAREEDWVAIEFEEVEKISKVTFHFYD
 DHGGTRPAKSLKLQYWDGEAWKDIDGTQLDVNERKELSIEFEEVSTSAIRSVMTAIPGTC
 IAISELMVWGESANPKPSMGKDPTLENILIDGKPLKDFDSNTTFYKVELHRGNSKNKINE
 IEVITNDIFATVKITLPEMLSGEATIVVTSEDGKSEKGYSVVFEENK

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Mature polypeptide - endo-beta-1-6-galactanase [SEQ ID NO: 2]

QSI FAEGATKAVKVDETIKLDPSYQHKKPFDGWTALVWFANVTGGWPDEIKNELADALFGEEGLNFNIGR
 YNIGGGDSPETTPYMRKGGAVSGYWNRAEFGPPEEKAEGWKEQENWWDPENPDHWNWKADKNQQWWLEA
 AAARGADTFEAFSNSPPYFMTRSGYTSGNWNSWDDNIKADQFENFAAYLTNVVDYLQEELEIEFRTLSPV
 20 NEPYTGYWRALGRQEGSNWSRASQAKI INEVKKQLNEKELNTVVSAMDETHPQRFRENWEQYNQQTRANI
 GQLNVHTYWPQEHIAVRDIAKGAEKKLWMSEVDLSPGGIGQDHEDIRTGLGLSERITLDIQRLEPEAWVL
 WQAIEDEVNMNPEHENGWGLIQVDFSPDDFDKLEIYKNKKKYAMGNYSKFIRPGYQFINSNNSQTLAAM
 NKEDDSVVIVYTNYLKEAKTVEFDLSGFKIVGENAQAVPHITSATDNLVQGTINNIADKKFSTTIKPQSI
 TTFVVSNSVSGVDSSQKFINSKDAYKLINKHSDKAADIGADNEKIVQKTVVREKDSQSWNIQQITNGNTTQ
 25 ELYKIFNSKTGKVLGVANGGAVLQESDEGSLSQQWMLSTYGNGEYTLINRKDGNLLEVGGQSRAEDAGMG
 VWRPNSGNNQVWKVIKAGIVEIEPVEVVVTKKKIAPILPNQITAIYGDGEKVEKEVIWDSIDPEKYQKEN
 TFTVEGSIDGTDKKAIIIVSKVDRADSIKVKTVTGVALELPSEITANLLSGSNGKVEVEWEEIDPALY
 ENVGKFVVGKVVNSPEKAFAYVQVVEPILDNLALRKSNSEFPKASASFTGRWDNVNHINDGTITSSRWT
 NWDPNAREEDWVAIEFEEVEKISKVTFHFYDDHGGTRPAKSLKLQYWDGEAWKDIDGTQLDVNERKELS
 30 IEFEEVSTSAIRSVMTAIPGTCIAISELMVWGESANPKPSMGKDPTLENILIDGKPLKDFDSNTTFYKVE
 LHRGNSKNKINEIEVITNDIFATVKITLPEMLSGEATIVVTSEDGKSEKGYSVVFEENK

Wildtype Signal peptide - endo-beta-1-6-galactanase [SEQ ID NO: 3]

MQKKNLRKLCLFCLVLLVTIGGMLPATA

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Bacillus clausii secretion signal [SEQ ID NO: 4]

MKKPLGKIVASTALLISVAFSSSIASA

Affinity tag sequence [SEQ ID NO: 5]

HHHHHHPR

- 5 The polypeptide having endo-beta-1-6-galactanase activity preferably:
- (i) has at least 60% sequence identity to the mature polypeptide shown in SEQ ID NO: 2;
 - (ii) is encoded by a polynucleotide that hybridizes under low stringency conditions with
 - (a) the mature polypeptide coding sequence shown in SEQ ID NO: 2,
 - (b) the cDNA sequence thereof, or
 - (c) the full-length complement of (a) or (b);
 - (iii) is encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence shown in SEQ ID NO: 8 or the cDNA sequence thereof;
 - (iv) is a variant of the mature polypeptide shown in SEQ ID NO: 2 comprising a substitution, deletion, and/or insertion at one or more positions; and
 - (v) is a fragment of the polypeptide of (i), (ii), (iii), or (iv) that has endo-beta-1-6-galactanase activity.

Full nucleotide encoding sequence [SEQ ID NO: 6]

**ATGCAAAAAAGAACTTAAGAAAATTATGTTTATTTTGTGTTAGTTTTACTTGTTACTATA
GGTGGAAATGTTACCAGCTACGGCA**CAATCCATCTTTGCTGAAGGAGCAACAAAGGCAGTG
AAGGTAGATGAAACGATTAACTTGATCCGAGTTATCAACATAAACCGTTTGATGGTTGG
GGTACAGCATTAGTTTGGTTTGGCAATGTAACAGGAGTTGGCCTGATGAAATAAAAAAT
GAACTGGCTGATGCCTTATTTGGAGAAGAGGGATTAAATTTTAATATCGGGCGTTACAAC
ATCGGAGGAGGAGATTCTCCGAAACGACGCCCTTATATGCGAAAAGGTGGAGCGGTTTCG
GGCTATTGGAATAGACCGGCCGAATTTGGCCCCCTGAAGAAAAGGCAGAAGGCTGGAAG
GAGCAAGAGAATTGGTGGGACCCGAAAATCCGGACCATTGGAAGCTGGAAAGCTGATAAA
AACCAACAATGGTGGCTAGAAGCAGCAGCAGCAAGAGGTGCAGATACATTTGAAGCATTT
TCTAATTCACCACCATACTTTATGACCAGAAGTGGCTACACATCTGGGAATTGGAATTCA
TGGGATGATAATATTAAAGCCGATCAATTTGAGAATTTTGCGGCATACTTAACAAATGTT
GTTGATTATTTACAAGAAGAGTTAGAAATTGAGTTTCGTACCCTTTCTCCAGTGAATGAA
CCATATACTGGCTACTGGAGAGCATTAGGAAGACAGGAAGGTTCTAACTGGTCACGGGCA
TCACAAGCTAAAATAATTAATGAAGTGAAAAAGCAACTTAACGAAAAGGAACTTAATACG
GTTGTCTCTGCCATGGATGAACTCATCCTCAAAGATTTAGAGAGAATTGGGAGCAATAT
AATCAACAACTCGAGCAAATATCGGTCAATTAAATGTCCATACGTATTGGCCACAAGAA

CATATTGCGGTTAGAGATATTGCAAAAGGTGCAGAGAAAAAAGTTTGGATGTCTGAAGTT
GATTTAAGCCCAGGTGGTATTGGTCAAGATCATGAAGATATTAGAACAGGATTAGGGTTG
TCAGAAAGAATTACACTGGATATTCAGAGATTAGAGCCTGAAGCATGGGTTCTATGGCAA
GCGATTGAAGATGAGGTAAACATGAACCCAGAACATGAAAATGGCAACTGGGGACTCATT
5 CAAGTAGATTTCTCACCGGATGATTTTGACAAGTTAGAAATCTACAAAAATAAAAAGTAT
TATGCAATGGGGAATTACAGTAAATTTATTAGACCCGGGTATCAGTTTATTAACCTCAAAT
AATAGTCAAACATTAGCTGCAATGAACAAAGAAGATGATTCAGTAGTCATCGTTTATACT
AACTATTTAAAAGAAGCGAAGACGGTTGAATTTGATTTATCTGGCTTTAAAATCGTTGGA
GAAAATGCTCAAGCAGTTCCGCATATCACTTCAGCTACGGATAATCTAGTACAAGGAACA
10 ACGATTAACATTGCTGACAAAAAGTTTTCAACAACGATTAAACCTCAGTCCATTACTACA
TTTGTAGTTTCAAACGTCTCAGGTGTTGACTCGAGTCAGAAAATTTATCAACTCAAAGAT
GCATATAAACTAATAAATAAACATAGTGACAAGGCAGCAGACATTGGTGCAGACAATGAG
AAAATTGTCCAAAAACAGTTGTACGCGAAAAAGATAGTCAAAGCTGGAATATTCAACAG
ATTACCAATGGAAATACGACACAGGAGCTATACAAAATTTTTAACTCCAAAAACAGGGAAA
15 GTGCTAGGTGTAGCAAATGGCGGTGCTGTTTTACAAGAATCTGATGAAGGGTCTTTAAGC
CAACAGTGGATGCTGTCCACCTACGGGAATGGCGAATATACACTCATTAAATAGGAAAGAT
GGCAACTTACTAGAAGTGGGCGGACAATCAAGAGCTGAAGACGCTGGTATGGGTGTTTGG
AGGCCCAATTCAGGTAATAACCAAGTCTGGAAAGTCATCAAGGCAGGAATTGTTGAAATT
GAGCCTGTTGAGGTGTTGTAACGAAGAAAAAATAGCTCCTATTCTTCCCAACCAGATA
20 ACAGCTATTTATGGTGACGGTGAAAAGGTGAAAAAGAAGTAATCTGGGATTCTATTGAT
CCAGAAAAATATCAAAAAGAAAAATACATTTACGGTAGAAGGTCTATTGATGGAACGGAT
AAAAAAGCAATAGCGACAATAATTGTTAGCAAGGTTGATCGGGCAGACTCTATTAAGGTG
AAAACGGTAACTGGTGTTGCACTAGAGCTACCAAGTGAAATTACGGCGAATCTACTTTCC
GGTTCTAATGGGAAAGTAGAAGTAGAATGGGAGGAAATCGATCCTGCTCTTTATGAAAAT
25 GTAGGTAAGTTTGTGGTAAAAGGAAAGGTGGTTAATAGCCCAGAAAAGGCATTTGCGTAT
GTTTCAAGTAGTAGAACCTATACTCGATAATCTTGCCCTAAGGAAATCAAATAGTGAATTT
CCAAAAGCTAGCGCATCGTTTACAGGAAGATGGGATAATGTAAATCATATTAATGATGGT
ACGATTACAAGTTCACGATGGACGAACTGGGATCCAAATGCTTGGAGAGAAGAAGATTGG
GTGGCAATAGAATTTGAGGAAGTGGAGAAAAATTTCCAAAGTTACTTTCCATTTTTACGAT
30 GATCATGGTGGTACGAGACCGGCTAAATCTTTAAAGCTTCAATATTGGGATGGAGAAGCT
TGGAAGGACATAGATGGTACACAATTAGACGTTAACGAAAGAAAGGAATTATCTATCGAG
TTTGAAGAAGTATCGACATCCGCCATACGATCTGTCATGACAGCAATACCCGGTACATGT
ATTGCTATATCTGAATTAATGGTTTGGGGTGAATCTGCAAATCCAAAACCAAGTATGGGT
AAAGATCCAACACTTGAAAATATTTTAATAGACGGGAAGCCACTGAAAGATTTTGATTCA
35 AATACTACTTTTTATAAGGTTGAATTGCACAGAGGAAATAGCAAAAATAAAATCAATGAA
ATCGAAGTCATAACAAATGATATTTTTGCAACAGTTAAAATTACATTGCCGGAAATGCTT
TCGGGAGAGGCGACTATTGTTGTAACCTCAGAGGATGGAAAGTCTGAAAAAGGTTATAGT
GTTGTGTTTGAAGAAAATAAGTAA

Nucleotide sequence encoding signal peptide [SEQ ID NO: 7]

ATGCAAAAAAGAACTTAAGAAAATTATGTTTATTTTGTGTTAGTTTTACTTGTTACTATA
GGTGAATGTTACCAGCTACGGCA

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Nucleotide sequence encoding mature peptide [SEQ ID NO: 8]

CAATCCATCTTTGCTGAAGGAGCAACAAAGGCAGTGAAGGTAGATGAAACGATTAAACTT
GATCCGAGTTATCAACATAAACCGTTTGATGGTTGGGGTACAGCATTAGTTTGGTTTGCG
AATGTAACAGGAGGTTGGCCTGATGAAATAAAAAATGAACTGGCTGATGCCTTATTTGGA
10 GAAGAGGGATTAAATTTTAAATATCGGGCGTTACAACATCGGAGGAGGAGATTCTCCGGAA
ACGACGCCTTATATGCGAAAAGGTGGAGCGGTTTCGGGCTATTGGAATAGACCGGCCGAA
TTTGGCCCCCTGAAGAAAAGGCAGAAGGCTGGAAGGAGCAAGAGAATTGGTGGGACCCG
GAAAATCCGGACCATTTGAACTGGAAAGCTGATAAAAACCAACAATGGTGGCTAGAAGCA
GCAGCAGCAAGAGGTGCAGATACATTTGAAGCATTTTCTAATTCACCACCATACTTTATG
15 ACCAGAAGTGGCTACACATCTGGGAATTGGAATTCATGGGATGATAATATTAAAGCCGAT
CAATTTGAGAATTTTGCGGCATACTTAACAAATGTTGTTGATTATTTACAAGAAGAGTTA
GAAATTGAGTTTCGTACCCTTTCTCCAGTGAATGAACCATATACTGGCTACTGGAGAGCA
TTAGGAAGACAGGAAGGTTCTAACTGGTCACGGGCATCACAAGCTAAAATAATTAATGAA
GTGAAAAAGCAACTTAACGAAAAGGAACTTAATACGGTTGTCTCTGCCATGGATGAACT
20 CATCCTCAAAGATTTAGAGAGAATTGGGAGCAATATAATCAACAAACTCGAGCAAATATC
GGTCAATTAAATGTCCATACGTATTGGCCACAAGACATATTGCGGTTAGAGATATTGCA
AAAGGTGCAGAGAAAAAACTTTGGATGTCTGAAGTTGATTTAAGCCCAGGTGGTATTGGT
CAAGATCATGAAGATATTAGAACAGGATTAGGGTTGTCAGAAAGAATTACACTGGATATT
CAGAGATTAGAGCCTGAAGCATGGGTTCTATGGCAAGCGATTGAAGATGAGGTAAACATG
25 AACCCAGAACATGAAAATGGCAACTGGGGACTCATTCAAGTAGATTTCTCACC GGATGAT
TTTGACAAGTTAGAAATCTACAAAAATAAAAAAGTATTATGCAATGGGGAATTACAGTAAA
TTTATTAGACCCGGGTATCAGTTTATTA ACTCAAATAATAGTCAAACATTAGCTGCAATG
AACAAAGAAGATGATTCAGTAGTCATCGTTTATACTAACTATTTAAAAGAAGCGAAGACG
GTTGAATTTGATTTATCTGGCTTTAAAATCGTTGGAGAAAATGCTCAAGCAGTTCCGCAT
30 ATCACTTCAGCTACGGATAATCTAGTACAAGGAACAACGATTAACATTGCTGACAAAAAG
TTTTCAACAACGATTAAACCTCAGTCCATTACTACATTTGTAGTTTCAAACGTCTCAGGT
GTTGACTCGAGTCAGAAATTTATCAACTCAAAGATGCATATAAACTAATAAATAAACAT
AGTGACAAGGCAGCAGACATTGGTGCAGACAATGAGAAAATTGTCCAAAAACAGTTGTA
CGCGAAAAAGATAGTCAAAGCTGGAATATTCAACAGATTACCAATGGAAATACGACACAG
35 GAGCTATACAAAATTTTAACTCCAAAACAGGGAAAGTGCTAGGTGTAGCAAATGGCGGT
GCTGTTTTACAAGAATCTGATGAAGGGTCTTTAAGCCAACAGTGGATGCTGTCCACCTAC
GGGAATGGCGAATATACACTCATTAATAGGAAAGATGGCAACTTACTAGAAGTGGGCGGA
CAATCAAGAGCTGAAGACGCTGGTATGGGTGTTTGGAGGCCCAATTCAGGTAATAACCAA

GTCTGGAAAGTCATCAAGGCAGGAATTGTTGAAATTGAGCCTGTTGAGGTTGTTGTAACG
 AAGAAAAAATAGCTCCTATTCTTCCCAACCAGATAACAGCTATTTATGGTGACGGTGAA
 AAGGTGGAAAAAGAAGTAATCTGGGATTCTATTGATCCAGAAAAATATCAAAAAGAAAAAT
 ACATTTACGGTAGAAGGTTCTATTGATGGAACGGATAAAAAAGCAATAGCGACAATAATT
 5 GTTAGCAAGGTTGATCGGGCAGACTCTATTAAGGTGAAAACGGTAACTGGTGTGCACTA
 GAGCTACCAAGTGAAATTACGGCGAATCTACTTTCCGGTTCTAATGGGAAAGTAGAAGTA
 GAATGGGAGGAAATCGATCCTGCTCTTTATGAAAATGTAGGTAAGTTTGTGGTAAAGGA
 AAGGTGGTTAATAGCCCAGAAAAGGCATTTGCGTATGTTTCAGGTAGTAGAACCTATACTC
 GATAATCTTGCCCTAAGGAAATCAAATAGTGAATTTCCAAAAGCTAGCGCATCGTTTACA
 10 GGAAGATGGGATAATGTAAATCATATTAATGATGGTACGATTACAAGTTCACGATGGACG
 AACTGGGATCCAAATGCTTGGAGAGAAGAAGATTGGGTGGCAATAGAATTTGAGGAAGTG
 GAGAAAAATTTCCAAAGTTACTTTCCATTTTTACGATGATCATGGTGGTACGAGACCGGCT
 AAATCTTTAAAGCTTCAATATTGGGATGGAGAAGCTTGGAAGGACATAGATGGTACACAA
 TTAGACGTTAACGAAAGAAAGGAATTATCTATCGAGTTTGAAGAAGTATCGACATCCGCC
 15 ATACGATCTGTCATGACAGCAATACCCGGTACATGTATTGCTATATCTGAATTAATGGTT
 TGGGGTGAATCTGCAAATCCAAAACCAAGTATGGGTAAAGATCCAACACTTGAAAATATT
 TTAATAGACGGGAAGCCACTGAAAGATTTTGATTCAAATACTACTTTTTATAAGGTTGAA
 TTGCACAGAGGAAATAGCAAAAATAAATCAATGAAATCGAAGTCATAACAAATGATATT
 TTTGCAACAGTTAAAATTACATTGCCGGAAATGCTTTCGGGAGAGGCGACTATTGTTGTA
 20 ACTTCAGAGGATGGAAAGTCTGAAAAAGGTTATAGTGTGTTGTTGAAGAAAATAAG

Hence, the polypeptide may have a sequence identity to the mature polypeptide 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 85%, at
 least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at
 25 least 97%, at least 98%, at least 99%, or 100%, and which has endo-beta-1-6-galactanase
 activity. The polypeptides may differ by up to 10 amino acids, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10,
 from the mature polypeptide shown in SEQ ID NO: 2. The polypeptide as defined in (i) may
 have the sequence of the mature polypeptide shown in SEQ ID NO: 2.

30 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 (EMBOSS:
 The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-
 277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap
 35 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:

$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$

The polypeptide having endo-beta-1-6-galactanase activity may be encoded by a polynucleotide having a sequence identity to the mature polypeptide coding sequence shown in SEQ ID NO: 8 or the cDNA sequence thereof of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In a further embodiment, the polypeptide has been isolated.

The polypeptide may also be a variant of the mature polypeptide shown in SEQ ID NO: 2 comprising a substitution, deletion, and/or insertion at one or more (e.g., several) positions. The number of amino acid substitutions, deletions and/or insertions introduced into the mature polypeptide shown in SEQ ID NO: 2 is up to 10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. 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.

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 (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for

endo-beta-1-6-galactanase activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. 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. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. 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 by Reidhaar-Olson and Sauer, 1988, *Science* 241: 53-57; Bowie and Sauer, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2152-2156; WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (e.g., Lowman *et al.*, 1991, *Biochemistry* 30: 10832-10837; U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis (Derbyshire *et al.*, 1986, *Gene* 46: 145; Ner *et al.*, 1988, *DNA* 7: 127).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides expressed by host cells (Ness *et al.*, 1999, *Nature Biotechnology* 17: 893-896). 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 (Cooper *et al.*, 1993, *EMBO J.* 12: 2575-2583; Dawson *et al.*, 1994, *Science* 266: 776-779).

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. Examples of cleavage sites include, but are not limited to, the sites disclosed in Martin *et al.*, 2003, *J. Ind. Microbiol. Biotechnol.* 3: 568-576; Svetina *et al.*, 2000, *J. Biotechnol.* 76: 245-251; Rasmussen-Wilson *et al.*, 1997, *Appl. Environ. Microbiol.* 63: 3488-3493; Ward *et al.*, 1995, *Biotechnology* 13: 498-503; and Contreras *et al.*, 1991, *Biotechnology* 9: 378-381; Eaton *et al.*, 1986, *Biochemistry* 25: 505-512; Collins-Racie *et al.*, 1995, *Biotechnology* 13: 982-987; Carter *et al.*, 1989, *Proteins: Structure, Function, and Genetics* 6: 240-248; and Stevens, 2003, *Drug Discovery World* 4: 35-48.

The polypeptide having endo-beta-1-6-galactanase activity may be encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence shown in SEQ ID NO: 8, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii) (Sambrook *et al.*, 1989, *Molecular Cloning, A Laboratory Manual*, 2d edition, Cold Spring Harbor, New York).

Alternatively, the polynucleotide hybridizes under any of low-medium; medium-high, high or very high stringency conditions with (i) the mature polypeptide coding sequence shown in SEQ ID NO: 8, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii).

The polynucleotide shown in SEQ ID NO: 8 or a subsequence thereof, as well as the polypeptide shown in SEQ ID NO: 2 or a fragment thereof, may also be used to design nucleic acid probes to identify and clone DNA encoding polypeptides having endo-beta-1-6-galactanase activity from strains of different genera or species according to methods well known in the art. 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 , ^3H , ^{35}S , biotin, or avidin).

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 endo-beta-1-6-galactanase 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 SEQ ID NO: 8 or a subsequence thereof, the carrier material is used in a Southern blot. Such hybridization includes that the polynucleotide hybridizes to a labeled nucleic acid probe corresponding to (i) SEQ ID NO: 8; (ii) the mature polypeptide coding sequence of SEQ ID NO: 8; (iii) the cDNA
5 sequence thereof; (iv) the full-length complement thereof; or (v) a subsequence thereof; under very low, low stringency conditions, low-medium stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions 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
10 in the art.

The polynucleotide encoding the polypeptide having endo-beta-1-6-galactanase activity may be an isolated polynucleotide. The polynucleotide preferably comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 8.

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
20 fragments with shared structural features. See, e.g., Innis *et al.*, 1990, *PCR: A Guide to Methods and Application*, Academic Press, New York. 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
25 polypeptide encoding region of the polynucleotide.

Modification of a polynucleotide encoding a polypeptide 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
30 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 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
35 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. For a general description of nucleotide substitution, see, e.g., Ford *et al.*, 1991, *Protein Expression and Purification* 2: 95-107.

Thus, in one embodiment, the polynucleotide may comprise or consist of the polynucleotide sequence set forth in SEQ ID NO: 8, wherein the codons have been modified by nucleotide substitutions to correspond to the codon usage of the host organism intended for production of the polypeptide of the present invention.

The polynucleotide encoding the polypeptides with endo-beta-1-6-galactanase activity may be contained in a nucleic acid construct wherein the polynucleotide is operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

The polynucleotide may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the polynucleotide prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides utilizing recombinant DNA methods are well known in the art.

The control sequence may be a promoter, a polynucleotide that is recognized by a host cell for expression of a polynucleotide encoding a polypeptide of the present invention. The promoter contains transcriptional control sequences that mediate the expression of the polypeptide. The promoter may be any polynucleotide that shows transcriptional activity in the host cell including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a bacterial host cell are the promoters obtained from the *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus licheniformis* penicillinase gene (*penP*), *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus subtilis* *xylA* and *xylB* genes, *Bacillus thuringiensis* *cryIIIA* gene (Agaisse and Lereclus, 1994, *Molecular Microbiology* 13: 97-107), *E. coli lac* operon, *E. coli trc* promoter (Egon *et al.*, 1988, *Gene* 69: 301-315), *Streptomyces coelicolor* agarase gene (*dagA*), and prokaryotic beta-lactamase gene (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* 75: 3727-3731), as well as the *tac* promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in Gilbert *et al.*, 1980, *Scientific American* 242: 74-94; and in Sambrook *et al.*, 1989, *supra*. Examples of tandem promoters are disclosed in WO 99/43835.

Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*),
 5 *Aspergillus oryzae* TAKA amylase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Fusarium oxysporum* trypsin-like protease (WO 96/00787), *Fusarium venenatum* amyloglucosidase (WO 00/56900), *Fusarium venenatum* Daria (WO 00/56900), *Fusarium venenatum* Quinn (WO 00/56900), *Rhizomucor miehei* lipase, *Rhizomucor miehei* aspartic proteinase, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei*
 10 cellobiohydrolase I, *Trichoderma reesei* cellobiohydrolase II, *Trichoderma reesei* endoglucanase I, *Trichoderma reesei* endoglucanase II, *Trichoderma reesei* endoglucanase III, *Trichoderma reesei* endoglucanase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* xylanase III, *Trichoderma reesei* beta-xylosidase, and *Trichoderma reesei* translation elongation factor, as well as the NA2-tpi promoter (a modified promoter from an
 15 *Aspergillus* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus* triose phosphate isomerase gene; non-limiting examples include modified promoters from an *Aspergillus niger* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus nidulans* or *Aspergillus oryzae* triose phosphate isomerase gene); and mutant, truncated, and
 20 hybrid promoters thereof. Other promoters are described in U.S. Patent No. 6,011,147.

In a yeast host, useful promoters are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* galactokinase (GAL1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH1, ADH2/GAP),
 25 *Saccharomyces cerevisiae* triose phosphate isomerase (TPI), *Saccharomyces cerevisiae* metallothionein (CUP1), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are described by Romanos *et al.*, 1992, *Yeast* 8: 423-488.

The control sequence may also be a transcription terminator, which is recognized by a host cell
 30 to terminate transcription. The terminator is operably linked to the 3'-terminus of the polynucleotide encoding the polypeptide. Any terminator that is functional in the host cell may be used in the present invention.

Preferred terminators for bacterial host cells are obtained from the genes for *Bacillus clausii*
 35 alkaline protease (*aprH*), *Bacillus licheniformis* alpha-amylase (*amyL*), and *Escherichia coli* ribosomal RNA (*rrnB*).

Preferred terminators for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase, *Aspergillus oryzae* TAKA amylase, *Fusarium oxysporum* trypsin-like protease, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei* cellobiohydrolase I, *Trichoderma reesei* cellobiohydrolase II, *Trichoderma reesei* endoglucanase I, *Trichoderma reesei* endoglucanase II, *Trichoderma reesei* endoglucanase III, *Trichoderma reesei* endoglucanase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* xylanase III, *Trichoderma reesei* beta-xylosidase, and *Trichoderma reesei* translation elongation factor.

Preferred terminators for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be an mRNA stabilizer region downstream of a promoter and upstream of the coding sequence of a gene which increases expression of the gene.

Examples of suitable mRNA stabilizer regions are obtained from a *Bacillus thuringiensis cryIIIA* gene (WO 94/25612) and a *Bacillus subtilis* SP82 gene (Hue *et al.*, 1995, *Journal of Bacteriology* 177: 3465-3471).

The control sequence may also be a leader, a nontranslated region of an mRNA that is important for translation by the host cell. The leader is operably linked to the 5'-terminus of the polynucleotide encoding the polypeptide. Any leader that is functional in the host cell may be used.

Preferred leaders for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase and *Aspergillus nidulans* triose phosphate isomerase.

Suitable leaders for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP).

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3'-terminus of the polynucleotide and, when transcribed, is recognized by the host cell as a

signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence that is functional in the host cell may be used.

Preferred polyadenylation sequences for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase *Aspergillus oryzae* TAKA amylase, and *Fusarium oxysporum* trypsin-like protease.

Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, *Mol. Cellular Biol.* 15: 5983-5990.

The control sequence may also be a signal peptide coding region that encodes a signal peptide linked to the N-terminus of a polypeptide and directs the polypeptide into the cell's secretory pathway. The 5'-end of the coding sequence of the polynucleotide may inherently contain a signal peptide coding sequence naturally linked in translation reading frame with the segment of the coding sequence that encodes the polypeptide. Alternatively, the 5'-end of the coding sequence may contain a signal peptide coding sequence that is foreign to the coding sequence. A foreign signal peptide coding sequence may be required where the coding sequence does not naturally contain a signal peptide coding sequence. Alternatively, a foreign signal peptide coding sequence may simply replace the natural signal peptide coding sequence in order to enhance secretion of the polypeptide. However, any signal peptide coding sequence that directs the expressed polypeptide into the secretory pathway of a host cell may be used.

Effective signal peptide coding sequences for bacterial host cells are the signal peptide coding sequences obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* beta-lactamase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus stearothermophilus* neutral proteases (*nprT*, *nprS*, *nprM*), and *Bacillus subtilis* *prsA*. Further signal peptides are described by Simonen and Palva, 1993, *Microbiological Reviews* 57: 109-137.

Effective signal peptide coding sequences for filamentous fungal host cells are the signal peptide coding sequences obtained from the genes for *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Aspergillus oryzae* TAKA amylase, *Humicola insolens* cellulase, *Humicola insolens* endoglucanase V, *Humicola lanuginosa* lipase, and *Rhizomucor miehei* aspartic proteinase.

Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase. Other useful signal peptide coding sequences are described by Romanos *et al.*, 1992, *supra*.

- 5 The control sequence may also be a propeptide coding sequence that encodes a propeptide positioned at the N-terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to an active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding sequence may be obtained from
- 10 the genes for *Bacillus subtilis* alkaline protease (*aprE*), *Bacillus subtilis* neutral protease (*nprT*), *Myceliophthora thermophila* laccase (WO 95/33836), *Rhizomucor miehei* aspartic proteinase, and *Saccharomyces cerevisiae* alpha-factor.

- Where both signal peptide and propeptide sequences are present, the propeptide sequence is
- 15 positioned next to the N-terminus of a polypeptide and the signal peptide sequence is positioned next to the N-terminus of the propeptide sequence.

- It may also be desirable to add regulatory sequences that regulate expression of the polypeptide relative to the growth of the host cell. Examples of regulatory sequences are those that cause
- 20 expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory sequences in prokaryotic systems include the *lac*, *tac*, and *trp* operator systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the *Aspergillus niger* glucoamylase promoter, *Aspergillus oryzae* TAKA alpha-amylase promoter, and *Aspergillus oryzae* glucoamylase promoter, *Trichoderma*
- 25 *reesei* cellobiohydrolase I promoter, and *Trichoderma reesei* cellobiohydrolase II promoter may be used. Other examples of regulatory sequences are those that allow for gene amplification. In eukaryotic systems, these regulatory sequences include the dihydrofolate reductase gene that is amplified in the presence of methotrexate, and the metallothionein genes that are amplified with heavy metals. In these cases, the polynucleotide encoding the polypeptide would be
- 30 operably linked to the regulatory sequence.

- The polynucleotide may be present in a recombinant expression vector which comprises a polynucleotide of the present invention, a promoter, and transcriptional and translational stop signals. The various nucleotide and control sequences may be joined together to produce a
- 35 recombinant expression vector that may include one or more convenient restriction sites to allow for insertion or substitution of the polynucleotide encoding the polypeptide at such sites. Alternatively, the polynucleotide may be expressed by inserting the polynucleotide or a nucleic acid construct comprising the polynucleotide into an appropriate vector for expression. In

creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression. In one embodiment, the expression vector comprises the polynucleotide sequence set forth in SEQ ID NO: 8 operably linked to the appropriate control sequences for expression. In a further
5 embodiment, polynucleotide sequence codons have been modified by nucleotide substitutions to correspond to the codon usage of the host organism intended for production of the polypeptide of the present invention.

The recombinant expression vector may be any vector (*e.g.*, a plasmid or virus) that can be
10 conveniently subjected to recombinant DNA procedures and can bring about expression of the polynucleotide. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may be a linear or closed circular plasmid.

The vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an
15 extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one that, when introduced into the host cell, is integrated into the genome and replicated together
20 with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids that together contain the total DNA to be introduced into the genome of the host cell, or a transposon, may be used.

The vector preferably contains one or more selectable markers that permit easy selection of
25 transformed, transfected, transduced, or the like cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are *Bacillus licheniformis* or *Bacillus subtilis* *dal* genes,
30 or markers that confer antibiotic resistance such as ampicillin, chloramphenicol, kanamycin, neomycin, spectinomycin, or tetracycline resistance. Suitable markers for yeast host cells include, but are not limited to, ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *adeA* (phosphoribosylaminoimidazole-succinocarboxamide synthase), *adeB* (phosphoribosyl-
35 aminoimidazole synthase), *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenylyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus*

cell are *Aspergillus nidulans* or *Aspergillus oryzae* *amdS* and *pyrG* genes and a *Streptomyces hygroscopicus* *bar* gene. Preferred for use in a *Trichoderma* cell are *adeA*, *adeB*, *amdS*, *hph*, and *pyrG* genes.

- 5 The selectable marker may be a dual selectable marker system as described in WO 2010/039889. In one aspect, the dual selectable marker is an *hph-tk* dual selectable marker system.

10 The vector preferably contains an element(s) that permits integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the polynucleotide's sequence encoding the polypeptide or any other element of the vector for integration into the genome by homologous or non-homologous recombination. Alternatively, the vector may contain additional polynucleotides for directing integration by homologous recombination into the genome of the host cell at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should contain a sufficient number of nucleic acids, such as 100 to 10,000 base pairs, 400 to 10,000 base pairs, and 800 to 10,000 base pairs, which have a high degree of sequence identity to the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding polynucleotides. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

25

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. The origin of replication may be any plasmid replicator mediating autonomous replication that functions in a cell. The term "origin of replication" or "plasmid replicator" means a polynucleotide that enables a plasmid or vector to replicate *in vivo*.

30

Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in *E. coli*, and pUB110, pE194, pTA1060, and pAM β 1 permitting replication in *Bacillus*.

35

Examples of origins of replication for use in a yeast host cell are the 2 micron origin of replication, ARS1, ARS4, the combination of ARS1 and CEN3, and the combination of ARS4 and CEN6.

Examples of origins of replication useful in a filamentous fungal cell are AMA1 and ANS1 (Gems *et al.*, 1991, *Gene* 98: 61-67; Cullen *et al.*, 1987, *Nucleic Acids Res.* 15: 9163-9175; WO 00/24883). Isolation of the AMA1 gene and construction of plasmids or vectors comprising the gene can be accomplished according to the methods disclosed in WO 00/24883.

5

More than one copy of a polynucleotide of the present invention may be inserted into a host cell to increase production of a polypeptide. An increase in the copy number of the polynucleotide can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the polynucleotide where
10 cells containing amplified copies of the selectable marker gene, and thereby additional copies of the polynucleotide, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

15

The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, *e.g.*, Sambrook *et al.*, 1989, *supra*).

20

The polynucleotide may be present in, and therefore express the polypeptide having endo-beta-1-6-galactanase activity in a recombinant host cell, wherein the cell comprises a polynucleotide described above operably linked to one or more control sequences that direct the production of a polypeptide of the present invention (such as the constructs or vectors discussed above). A construct or vector comprising a polynucleotide is introduced into a host cell so that the construct or vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector as described earlier. The term "host cell" encompasses any progeny of a parent cell that
25 is not identical to the parent cell due to mutations that occur during replication.

The host cell may be any cell useful in the recombinant production of a polypeptide of the present invention, *e.g.*, a prokaryote or a eukaryote.

30

The prokaryotic host cell may be any Gram-positive or Gram-negative bacterium. Gram-positive bacteria include, but are not limited to, *Bacillus*, *Clostridium*, *Enterococcus*, *Geobacillus*, *Lactobacillus*, *Lactococcus*, *Oceanobacillus*, *Staphylococcus*, *Streptococcus*, and *Streptomyces*. Gram-negative bacteria include, but are not limited to, *Campylobacter*, *E. coli*, *Flavobacterium*, *Fusobacterium*, *Helicobacter*, *Ilyobacter*, *Neisseria*, *Pseudomonas*,
35 *Salmonella*, and *Ureaplasma*.

The bacterial host cell may be any *Bacillus* cell including, but not limited to, *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*, and *Bacillus thuringiensis* cells. Preferably the *Bacillus* cell is *Bacillus subtilis*.

- 5 The bacterial host cell may also be any *Streptococcus* cell including, but not limited to, *Streptococcus equisimilis*, *Streptococcus pyogenes*, *Streptococcus uberis*, and *Streptococcus equi* subsp. *Zooepidemicus* cells.

The bacterial host cell may also be any *Streptomyces* cell including, but not limited to,
10 *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, and *Streptomyces lividans* cells.

The introduction of DNA into a *Bacillus* cell may be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, *Mol. Gen. Genet.* 168: 111-115), competent cell transformation
15 (see, e.g., Young and Spizizen, 1961, *J. Bacteriol.* 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, *J. Mol. Biol.* 56: 209-221), electroporation (see, e.g., Shigekawa and Dower, 1988, *Biotechniques* 6: 742-751), or conjugation (see, e.g., Koehler and Thorne, 1987, *J. Bacteriol.* 169: 5271-5278). The introduction of DNA into an *E. coli* cell may be effected by protoplast transformation (see, e.g., Hanahan, 1983, *J. Mol. Biol.* 166: 557-580) or
20 electroporation (see, e.g., Dower *et al.*, 1988, *Nucleic Acids Res.* 16: 6127-6145). The introduction of DNA into a *Streptomyces* cell may be effected by protoplast transformation, electroporation (see, e.g., Gong *et al.*, 2004, *Folia Microbiol. (Praha)* 49: 399-405), conjugation (see, e.g., Mazodier *et al.*, 1989, *J. Bacteriol.* 171: 3583-3585), or transduction (see, e.g., Burke *et al.*, 2001, *Proc. Natl. Acad. Sci. USA* 98: 6289-6294). The introduction of DNA into a
25 *Pseudomonas* cell may be effected by electroporation (see, e.g., Choi *et al.*, 2006, *J. Microbiol. Methods* 64: 391-397) or conjugation (see, e.g., Pinedo and Smets, 2005, *Appl. Environ. Microbiol.* 71: 51-57). The introduction of DNA into a *Streptococcus* cell may be effected by natural competence (see, e.g., Perry and Kuramitsu, 1981, *Infect. Immun.* 32: 1295-1297), protoplast transformation (see, e.g., Catt and Jollick, 1991, *Microbios* 68: 189-207),
30 electroporation (see, e.g., Buckley *et al.*, 1999, *Appl. Environ. Microbiol.* 65: 3800-3804), or conjugation (see, e.g., Clewell, 1981, *Microbiol. Rev.* 45: 409-436). However, any method known in the art for introducing DNA into a host cell can be used.

The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell.
35

The host cell may be a fungal cell. "Fungi" as used herein includes the phyla *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, and *Zygomycota* as well as the *Oomycota* and all mitosporic

fungi (as defined by Hawksworth *et al.*, In, *Ainsworth and Bisby's Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK).

The fungal host cell may be a yeast cell. "Yeast" as used herein includes *ascosporogenous* yeast (Endomycetales), *basidiosporogenous* yeast, and yeast belonging to the Fungi *Imperfecti* (Blastomycetes). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in *Biology and Activities of Yeast* (Skinner, Passmore, and Davenport, editors, *Soc. App. Bacteriol. Symposium Series No. 9*, 1980).

The yeast host cell may be a *Candida*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, or *Yarrowia* cell, such as a *Kluyveromyces lactis*, *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis*, *Saccharomyces oviformis*, or *Yarrowia lipolytica* cell.

The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision *Eumycota* and *Oomycota* (as defined by Hawksworth *et al.*, 1995, *supra*). The filamentous fungi are generally characterized by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative.

The filamentous fungal host cell may be an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Bjerkandera*, *Ceriporiopsis*, *Chrysosporium*, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trametes*, or *Trichoderma* cell.

For example, the filamentous fungal host cell may be an *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Bjerkandera adusta*, *Ceriporiopsis aneirina*, *Ceriporiopsis caregiea*, *Ceriporiopsis gilvescens*, *Ceriporiopsis pannocinta*, *Ceriporiopsis rivulosa*, *Ceriporiopsis subrufa*, *Ceriporiopsis subvermispora*, *Chrysosporium inops*, *Chrysosporium keratinophilum*, *Chrysosporium lucknowense*, *Chrysosporium merdarium*, *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium tropicum*, *Chrysosporium zonatum*, *Coprinus cinereus*, *Coriolus hirsutus*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*,

Fusarium negundi, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum*, *Humicola insolens*, *Humicola lanuginosa*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*,
 5 *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Phlebia radiata*, *Pleurotus eryngii*, *Thielavia terrestris*, *Trametes villosa*, *Trametes versicolor*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride* cell.

10 Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known *per se*. Suitable procedures for transformation of *Aspergillus* and *Trichoderma* host cells are described in EP 238023, Yelton *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474, and Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Suitable methods for transforming *Fusarium* species are
 15 described by Malardier *et al.*, 1989, *Gene* 78: 147-156, and WO 96/00787. Yeast may be transformed using the procedures described by Becker and Guarente, *In* Abelson, J.N. and Simon, M.I., editors, *Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology*, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito *et al.*, 1983, *J. Bacteriol.* 153: 163; and Hinnen *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* 75: 1920.

20 The polypeptides of the invention and otherwise as described herein may be produced by methods comprising (a) cultivating a cell, which in its wild-type form produces the polypeptide, under conditions conducive for production of the polypeptide; and optionally, (b) recovering the polypeptide. In one aspect, the cell is a *Bacillus* cell. In another aspect, the cell is a *Trichoderma*
 25 *subtilis* cell. Alternatively, polypeptide may be produced by a method comprising (a) cultivating a recombinant host cell of the present invention under conditions conducive for production of the polypeptide; and optionally, (b) recovering the polypeptide.

The host cells are cultivated in a nutrient medium suitable for production of the polypeptide using
 30 methods known in the art. For example, the cells may be cultivated by shake flask cultivation, or small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures
 35 known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (e.g., in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be

recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

The polypeptide may be detected using methods known in the art that are specific for the polypeptides. These detection methods include, but are not limited to, use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the polypeptide.

The polypeptide may be recovered using methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures including, but not limited to, collection, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation. In one aspect, a fermentation broth comprising the polypeptide is recovered.

The polypeptide may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing), differential solubility (e.g., ammonium sulfate precipitation), SDS-PAGE, or extraction (see, e.g., *Protein Purification*, Janson and Ryden, editors, VCH Publishers, New York, 1989) to obtain substantially pure polypeptides.

The polypeptide may also be produced by cultivating the recombinant host cell further comprising a polynucleotide encoding a second polypeptide of interest; preferably an enzyme of interest; more preferably a secreted enzyme of interest; even more preferably a hydrolase, isomerase, ligase, lyase, oxidoreductase, or a transferase; and most preferably the secreted enzyme is an alpha-galactosidase, alpha-glucosidase, aminopeptidase, amylase, asparaginase, beta-galactosidase, beta-glucosidase, beta-xylosidase, carbohydrase, carboxypeptidase, catalase, cellobiohydrolase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, endoglucanase, esterase, green fluorescent protein, glucano-transferase, glucoamylase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or a xylanase.

The second polypeptide of interest is heterologous or homologous to the host cell.

The recombinant host cell further producing a second polypeptide of interest may be a fungal host cell; preferably a filamentous fungal host cell; more preferably an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Bjerkandera*, *Ceriporiopsis*, *Chrysosporium*, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*,

- Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes, or Trichoderma cell; most preferably an Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae,
- 5 Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus,
- 10 Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochrom, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens,
- 15 Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, or Trichoderma viride cell.
- 20 Alternatively, the recombinant host cell may be a bacterial host cell; preferably a prokaryotic host cell; more preferably a Gram-positive host cell; even more preferably a Bacillus, Clostridium, Enterococcus, Geobacillus, Lactobacillus, Lactococcus, Oceanobacillus, Staphylococcus, Streptococcus, or Streptomyces host cell; and most preferably a Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans,
- 25 Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, and Bacillus thuringiensis host cell.

30 The method of producing the second polypeptide of interest may comprise cultivating the host cell under conditions conducive for production of the second polypeptide of interest. The method may also include the additional step of recovering the second polypeptide of interest.

35 The polypeptide having endo-beta-1-6-galactanase activity and or a host cell producing it may be comprised in a fermentation broth formulation or a cell composition. The fermentation broth product further comprises additional ingredients used in the fermentation process, such as, for example, cells (including, the host cells containing the gene encoding the polypeptide of the present invention which are used to produce the polypeptide of interest), cell debris, biomass, fermentation media and/or fermentation products. In some embodiments, the composition is a

cell-killed whole broth containing organic acid(s), killed cells and/or cell debris, and culture medium.

Such fermentation broth formulation and cell compositions may comprise a first organic acid component comprising at least one 1-5 carbon organic acid and/or a salt thereof and a second organic acid component comprising at least one 6 or more carbon organic acid and/or a salt thereof. In a specific embodiment, the first organic acid component is acetic acid, formic acid, propionic acid, a salt thereof, or a mixture of two or more of the foregoing and the second organic acid component is benzoic acid, cyclohexanecarboxylic acid, 4-methylvaleric acid, phenylacetic acid, a salt thereof, or a mixture of two or more of the foregoing.

The composition may contain an organic acid(s), and optionally further contains killed cells and/or cell debris. The killed cells and/or cell debris may be removed from a cell-killed whole broth to provide a composition that is free of these components.

Fermentation broth formulations or cell compositions may further comprise a preservative and/or anti-microbial (e.g., bacteriostatic) agent, including, but not limited to, sorbitol, sodium chloride, potassium sorbate, and others known in the art.

The cell-killed whole broth or composition may contain the unfractionated contents of the fermentation materials derived at the end of the fermentation. Typically, the cell-killed whole broth or composition contains the spent culture medium and cell debris present after the microbial cells (e.g., filamentous fungal cells) are grown to saturation, incubated under carbon-limiting conditions to allow protein synthesis. The cell-killed whole broth or composition may contain the spent cell culture medium, extracellular enzymes, and killed filamentous fungal cells. In some embodiments, the microbial cells present in the cell-killed whole broth or composition can be permeabilized and/or lysed using methods known in the art.

A whole broth or cell composition as described herein is typically a liquid, but may contain insoluble components, such as killed cells, cell debris, culture media components, and/or insoluble enzyme(s). In some embodiments, insoluble components may be removed to provide a clarified liquid composition. Whole broth formulations and cell compositions may be produced by a method described in WO 90/15861 or WO 2010/096673.

In a second aspect of the invention there is provided a detergent composition comprising a polypeptide as defined in the first aspect of the invention.

The detergent composition of the first aspect may comprise the polypeptide having endo-beta-1-6-galactanase activity in an amount corresponding to at least 1 mg of endo-beta-1-6-galactanase protein, such as at least 5 mg of protein, preferably at least 10 mg of protein, more preferably at least 15 mg of protein, even more preferably at least 20 mg of protein, most preferably at least 30 mg of protein, and even most preferably at least 40 mg of protein per liter of wash liquor. Thus, the detergent composition may comprise at least 0.1% endo-beta-1-6-galactanase protein, preferably at least 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.8%, 1.0%, 1.2%, 1.5%, or 2.0% of endo-beta-1-6-galactanase protein.

The concentration of the polypeptide having endo-beta-1-6-galactanase activity in the detergent composition is typically in the range of 0.001-10 ppm, 0.002-10 ppm, 0.001-10 ppm, 0.002-10 ppm, 0.01-10 ppm, 0.02-10 ppm, 0.1-10 ppm, 0.2-10 ppm or such as 1-40 ppm, 2-20 ppm, preferably 0.01-20 ppm, more preferably 0.01-10 ppm active enzyme protein per liter of wash liquor.

The concentration of the polypeptide having endo-beta-1-6-galactanase activity in the detergent composition is typically in the range of 0.01-10 ppm enzyme protein, 0.1-10 ppm enzyme protein, 1-40 ppm enzyme protein, preferably 1-20 ppm enzyme protein, more preferably 1-10 ppm enzyme protein.

When the detergent composition is used in a wash, the concentration of the polypeptide having endo-beta-1-6-galactanase activity in the wash liquor is typically in the range of 0.01-100 ppm enzyme protein, preferably 0.05-50 ppm enzyme protein, more preferably 0.1-50 ppm enzyme protein, more preferably 0.1-30 ppm enzyme protein, more preferably 0.5-20 ppm enzyme protein, even more preferably 1-10 ppm enzyme protein, and most preferably 0.01-1 ppm enzyme protein.

The polypeptide having endo-beta-1-6-galactanase activity may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g. an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in, for example, WO 92/19709 and WO 92/19708. The polypeptide may also be incorporated in the detergent formulations disclosed in WO 97/07202.

The detergent composition may further comprise at least one detergent adjunct ingredient.

Preferably, the detergent adjunct ingredient is selected from the group consisting of surfactants, builders, flocculating aid, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of

hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, builders and co-builders, fabric huing agents, anti-foaming agents, dispersants, processing aids, and/or pigments.

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The choice of detergent components may include, for textile care, the consideration of the type of textile to be cleaned, the type and/or degree of soiling, the temperature at which cleaning is to take place, and the formulation of the detergent product. A component as those listed below may comprise additional functionalities as will be appreciated by the skilled artisan. 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.

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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.

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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.

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When included therein, the detergent will usually contain from about 1% to about 40% by weight 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, alkoxyated quaternary ammonium (AQA) compounds, ester quats, and combinations thereof.

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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

10 alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxyated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxyated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides

15 (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.

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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.

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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.

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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.

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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. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in detergents may be utilized. Non-limiting examples of

builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), 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),
 5 triethanolamine (TEA, also known as 2,2',2''-nitrilotriethan-1-ol), and (carboxymethyl)inulin (CMI), and combinations thereof.

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 a co-builder alone, or in combination
 10 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). Further non-limiting examples include citrate, chelators such as aminocarboxylates, aminopolycarboxylates and phosphonates, and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2''-nitrilotriacetic acid (NTA),
 15 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), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis(methylenephosphonic acid) (DTMPA or DTPMPA), *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
 25 (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), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof. Further
 30 exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053

The detergent may contain 0-30% by weight, such as about 1% to about 20%, of a bleaching system. Any bleaching system comprising components known in the art for use in cleaning detergents may be utilized. Suitable bleaching system components include sources of hydrogen
 35 peroxide; sources of peracids; and bleach catalysts or boosters.

Sources of hydrogen peroxide

Suitable sources of hydrogen peroxide are inorganic persalts, including alkali metal salts such

as sodium percarbonate and sodium perborates (usually mono- or tetrahydrate), and hydrogen peroxide—urea (1/1).

Sources of peracids

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Peracids may be (a) incorporated directly as preformed peracids or (b) formed in situ in the wash liquor from hydrogen peroxide and a bleach activator (perhydrolysis) or (c) formed in situ in the wash liquor from hydrogen peroxide and a perhydrolase and a suitable substrate for the latter, e.g., an ester.

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a) Suitable preformed peracids include, but are not limited to, peroxycarboxylic acids such as peroxybenzoic acid and its ring-substituted derivatives, peroxy- α -naphthoic acid, peroxyphthalic acid, peroxyauric acid, peroxysearic acid, ϵ -phthalimidoperoxycaproic acid [phthalimidoperoxyhexanoic acid (PAP)], and o-carboxybenzamidoperoxycaproic acid; aliphatic and aromatic diperoxydicarboxylic acids such as diperoxydodecanedioic acid, diperoxyazelaic acid, diperoxysebacic acid, diperoxybrassylic acid, 2-decyldiperoxybutanedioic acid, and diperoxyphthalic, -isophthalic and -terephthalic acids; perimidic acids; peroxymonosulfuric acid; peroxydisulfuric acid; peroxyphosphoric acid; peroxyasilic acid; and mixtures of said compounds. It is understood that the peracids mentioned may in some cases be best added as suitable salts, such as alkali metal salts (e.g., Oxone®) or alkaline earth-metal salts.

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b) Suitable bleach activators include those belonging to the class of esters, amides, imides, nitriles or anhydrides and, where applicable, salts thereof. Suitable examples are tetraacetylenediamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene-1-sulfonate (ISONOBS), sodium 4-(dodecanoyloxy)benzene-1-sulfonate (LOBS), sodium 4-(decanoyloxy)benzene-1-sulfonate, 4-(decanoyloxy)benzoic acid (DOBA), sodium 4-(nonanoyloxy)benzene-1-sulfonate (NOBS), and/or those disclosed in WO98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particularly preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that they are 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.

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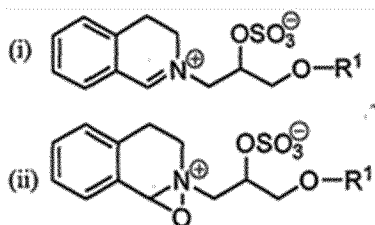
Bleach catalysts and boosters

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The bleaching system may also include a bleach catalyst or booster.

Some non-limiting examples of bleach catalysts that may be used in the compositions of the present invention include manganese oxalate, manganese acetate, manganese-collagen, cobalt-amine catalysts and manganese triazacyclononane (MnTACN) catalysts; particularly preferred are complexes of manganese with 1,4,7-trimethyl-1,4,7-triazacyclononane (Me3-TACN) or 1,2,4,7-tetramethyl-1,4,7-triazacyclononane (Me4-TACN), in particular Me3-TACN, such as the dinuclear manganese complex [(Me3-TACN)Mn(O)3Mn(Me3-TACN)](PF6)2, and [2,2',2''-nitrilotris(ethane-1,2-diylazanylylidene-κN-methanylylidene)triphenolato-κ3O]manganese(III). The bleach catalysts may also be other metal compounds, such as iron or cobalt complexes.

In some embodiments, where a source of a peracid is included, an organic bleach catalyst or bleach booster may be used having one of the following formulae:



(iii) and mixtures thereof; wherein each R1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R1 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 R1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylononyl, 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. 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 textile or hard surface treatment step.

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 diquatonium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated.

The detergent compositions of the present invention may also include fabric hueing agents such as dyes or pigments, which when formulated in detergent compositions can deposit onto a fabric when said fabric is contacted with a wash liquor comprising said detergent compositions and thus altering the tint of said fabric through absorption/reflection of visible light. Fluorescent whitening agents emit at least some visible light. In contrast, fabric hueing agents alter the tint of a surface as they absorb at least a portion of the visible light spectrum. Suitable fabric hueing agents include dyes and dye-clay conjugates, and may also include pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct Blue, Direct Red, Direct Violet, Acid Blue, Acid Red, Acid Violet, Basic Blue, Basic Violet and Basic Red, or mixtures thereof, for example as described in WO2005/03274, WO2005/03275, WO2005/03276 and EP1876226 (hereby incorporated by reference). The detergent composition preferably comprises from about 0.00003 wt% to about 0.2 wt%, from about 0.00008 wt% to about 0.05 wt%, or even from about 0.0001 wt% to about 0.04 wt% fabric hueing agent. The composition may comprise from 0.0001 wt% to 0.2 wt% fabric hueing agent, this may be especially preferred when the composition is in the form of a unit dose pouch. Suitable hueing agents are also disclosed in, e.g. WO 2007/087257 and WO2007/087243.

The detergent composition may comprise one or more additional enzymes such as a protease, lipase, cutinase, an amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, 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. In a preferred embodiment, the detergent additive or the detergent composition comprises a protease.

Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants 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 colour 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 WO99/001544.

Other cellulases are endo-beta-1,4-glucanase enzyme having a sequence of at least 97% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2 of WO 2002/099091 or a family 44 xyloglucanase, which a xyloglucanase enzyme having a sequence of at least 60% identity to positions 40-559 of SEQ ID NO: 2 of WO 2001/062903.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S) Carezyme Premium™ (Novozymes A/S), Celluclean™ (Novozymes A/S), Celluclean Classic™ (Novozymes A/S), Cellusoft™ (Novozymes A/S), Whitezyme™ (Novozymes A/S), Clazinas™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

Suitable proteases include those of bacterial, fungal, plant, viral or animal origin e.g. vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants 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 according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. 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
 5 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
 10 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.

15 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,
 20 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, 24, 27, 42, 55, 59, 60, 66, 74, 85, 96, 97, 98, 99, 100, 101, 102, 104, 116, 118, 121, 126, 127, 128, 154, 156, 157, 158, 161, 164, 176, 179, 182, 185, 188, 189, 193, 198, 199, 200, 203, 206, 211, 212, 216, 218, 226, 229,
 25 230, 239, 246, 255, 256, 268 and 269 wherein the positions correspond to the positions of the *Bacillus Lentus* protease shown in SEQ ID NO 1 of WO 2016/001449. More preferred the subtilase variants may comprise one or more of the mutations: S3T, V4I, S9R, S9E, A15T, S24G, S24R, K27R, N42R, S55P, G59E, G59D, N60D, N60E, V66A, N74D, N85S, N85R, , G96S, G96A, S97G, S97D, S97A, S97SD, S99E, S99D, S99G, S99M, S99N, S99R, S99H, S101A,
 30 V102I, V102Y, V102N, S104A, G116V, G116R, H118D, H118N, N120S, S126L, P127Q, S128A, S154D, A156E, G157D, G157P, S158E, Y161A, R164S, Q176E, N179E, S182E, Q185N, A188P, G189E, V193M, N198D, V199I, Y203W, S206G, L211Q, L211D, N212D, N212S, M216S, A226V, K229L, Q230H, Q239R, N246K, N255W, N255D, N255E, L256E, L256D T268A or R269H. The protease variants are preferably variants of the *Bacillus Lentus* protease
 35 (Savinase®) shown in SEQ ID NO 1 of WO 2016/001449, the *Bacillus amylolichenifaciens* protease (BPN') shown in SEQ ID NO 2 of WO2016/001449. The protease variants preferably have at least 80 % sequence identity to SEQ ID NO 1 or SEQ ID NO 2 of WO 2016/001449.

A protease variant comprising a substitution at one or more positions corresponding to positions 171, 173, 175, 179, or 180 of SEQ ID NO: 1 of WO2004/067737, wherein said protease variant has a sequence identity of at least 75% but less than 100% to SEQ ID NO: 1 of WO2004/067737.

5 Suitable commercially available protease enzymes include those sold under the trade names Alcalase®, Duralase™, Durazym™, Relase®, Relase® Ultra, Savinase®, Savinase® Ultra, Primase®, Polarzyme®, Kannase®, Liquanase®, Liquanase® Ultra, Ovozime®, Coronase®, Coronase® Ultra, Blaze®, Blaze Evity® 100T, Blaze Evity® 125T, Blaze Evity® 150T, Neutrase®, Everlase® and Esperase® (Novozymes A/S), those sold under the tradename
 10 Maxatase®, Maxacal®, Maxapem®, Purafect Ox®, Purafect OxP®, Puramax®, FN2®, FN3®, FN4®, Excellase®, Excellenz P1000™, Excellenz P1250™, Eraser®, Preferenz P100™, Purafect Prime®, Preferenz P110™, Effectenz P1000™, Purafect®™, Effectenz P1050™, Purafect Ox®™, Effectenz P2000™, Purafast®, Properase®, Opticlean® and Optimase® (Danisco/DuPont), Axapem™ (Gist-Brocades N.V.), BLAP (sequence shown in Figure 29 of
 15 US5352604) and variants hereof (Henkel AG) and KAP (*Bacillus alkalophilus* subtilisin) from Kao.

Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutant enzymes are included. Examples include lipase from *Thermomyces*,
 20 e.g. from *T. lanuginosus* (previously named *Humicola lanuginosa*) as described in EP258068 and EP305216, cutinase from *Humicola*, e.g. *H. insolens* (WO96/13580), lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g. *P. alcaligenes* or *P. pseudoalcaligenes* (EP218272), *P. cepacia* (EP331376), *P. sp.* strain SD705 (WO95/06720 & WO96/27002), *P. wisconsinensis* (WO96/12012), GDSL-type *Streptomyces* lipases
 25 (WO10/065455), cutinase from *Magnaporthe grisea* (WO10/107560), cutinase from *Pseudomonas mendocina* (US5,389,536), lipase from *Thermobifida fusca* (WO11/084412), *Geobacillus stearothermophilus* lipase (WO11/084417), lipase from *Bacillus subtilis* (WO11/084599), and lipase from *Streptomyces griseus* (WO11/150157) and *S. pristinaespiralis* (WO12/137147).

30 Other examples are lipase variants such as those described in EP407225, WO92/05249, WO94/01541, WO94/25578, WO95/14783, WO95/30744, WO95/35381, WO95/22615, WO96/00292, WO97/04079, WO97/07202, WO00/34450, WO00/60063, WO01/92502, WO07/87508 and WO09/109500.

35 Preferred commercial lipase products include Lipolase™, Lipex™, Lipolex™ and Lipoclean™ (Novozymes A/S), Lumafast (originally from Genencor) and Lipomax (originally from Gist-Brocades).

Still other examples are lipases sometimes referred to as acyltransferases or perhydrolases, e.g. acyltransferases with homology to *Candida antarctica* lipase A (WO10/111143), acyltransferase from *Mycobacterium smegmatis* (WO05/56782), perhydrolases from the CE 7 family (WO09/67279), and variants of the *M. smegmatis* perhydrolase in particular the S54V variant
 5 used in the commercial product Gentle Power Bleach from Huntsman Textile Effects Pte Ltd (WO10/100028).

Suitable amylases which can be used together with the enzyme of the invention may be an alpha-amylase or a glucoamylase and may be of bacterial or fungal origin. Chemically modified
 10 or protein engineered mutants 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: 2 in WO 95/10603 or variants having
 15 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. in WO 02/010355 Preferred variants of
 20 SEQ ID NO: 6 in WO 02/010355 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 obtained 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
 25 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 obtained from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues
 30 36-483 of SEQ ID NO: 4 of WO 2006/066594 are those having the substitutions:

35 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 in WO 99/019467. Preferred variants of SEQ ID NO: 6 in WO 99/019467 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 of WO 96/023873. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7 of WO 96/023873 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, using SEQ ID 2 of WO 96/023873 for numbering. More preferred variants are those having a deletion in two positions selected from 181, 182, 183 and 184, such as 181 and 182, 182 and 183, or positions 183 and 184. Most preferred amylase variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 WO 96/023873 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 or 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 of WO 09/061380 thereof. Preferred variants of SEQ ID NO: 2 of WO 09/061380 are those having a truncation of the C-terminus and/or a substitution, a deletion or an insertion in one or 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 of WO 09/061380 are those having the substitution in one or 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 of WO 09/061380 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.

5

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 in WO01/66712. 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.

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Other examples are amylase variants such as those described in WO2011/098531, WO2013/001078 and WO2013/001087.

Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™, Stainzyme™, Stainzyme Plus™, Natalase™, Liquozyme X and BAN™ (from Novozymes A/S), and Rapidase™, Purastar™/Effectenz™, Powerase and Preferenz S100 (from Genencor International Inc./DuPont).

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A peroxidase according to the invention is a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, as set out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), or any fragment obtained therefrom, exhibiting peroxidase activity.

25

Suitable peroxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinopsis*, e.g., from *C. cinerea* (EP 179,486), and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

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A peroxidase according to the invention also includes a haloperoxidase enzyme, such as chloroperoxidase, bromoperoxidase and compounds exhibiting chloroperoxidase or bromoperoxidase activity. Haloperoxidases are classified according to their specificity for halide ions. Chloroperoxidases (E.C. 1.11.1.10) catalyze formation of hypochlorite from chloride ions.

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In an embodiment, the haloperoxidase of the invention is a chloroperoxidase. Preferably, the haloperoxidase is a vanadium haloperoxidase, *i.e.*, a vanadate-containing haloperoxidase. In a preferred method of the present invention the vanadate-containing haloperoxidase is combined with a source of chloride ion.

5

Haloperoxidases have been isolated from many different fungi, in particular from the fungus group dematiaceous hyphomycetes, such as *Caldariomyces*, *e.g.*, *C. fumago*, *Alternaria*, *Curvularia*, *e.g.*, *C. verruculosa* and *C. inaequalis*, *Drechslera*, *Ulocladium* and *Botrytis*.

10 Haloperoxidases have also been isolated from bacteria such as *Pseudomonas*, *e.g.*, *P. pyrocinia* and *Streptomyces*, *e.g.*, *S. aureofaciens*.

In a preferred embodiment, the haloperoxidase is derivable from *Curvularia* sp., in particular *Curvularia verruculosa* or *Curvularia inaequalis*, such as *C. inaequalis* CBS 102.42 as described
15 in WO 95/27046; or *C. verruculosa* CBS 147.63 or *C. verruculosa* CBS 444.70 as described in WO 97/04102; or from *Drechslera hartlebii* as described in WO 01/79459, *Dendryphiella salina* as described in WO 01/79458, *Phaeotrichoconis crotalarie* as described in WO 01/79461, or *Geniculosporium* sp. as described in WO 01/79460.

20 An oxidase according to the invention include, in particular, any laccase enzyme comprised by the enzyme classification EC 1.10.3.2, or any fragment obtained therefrom exhibiting laccase activity, or a compound exhibiting a similar activity, such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5).

25 Preferred laccase enzymes are enzymes of microbial origin. The enzymes may be obtained from plants, bacteria or fungi (including filamentous fungi and yeasts).

Suitable examples from fungi include a laccase derivable from a strain of *Aspergillus*, *Neurospora*, *e.g.*, *N. crassa*, *Podospira*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*,
30 *Trametes*, *e.g.*, *T. villosa* and *T. versicolor*, *Rhizoctonia*, *e.g.*, *R. solani*, *Coprinopsis*, *e.g.*, *C. cinerea*, *C. comatus*, *C. friesii*, and *C. plicatilis*, *Psathyrella*, *e.g.*, *P. condelleana*, *Panaeolus*, *e.g.*, *P. papilionaceus*, *Myceliophthora*, *e.g.*, *M. thermophila*, *Schytalidium*, *e.g.*, *S. thermophilum*, *Polyporus*, *e.g.*, *P. pinsitus*, *Phlebia*, *e.g.*, *P. radiata* (WO 92/01046), or *Coriolus*, *e.g.*, *C. hirsutus* (JP 2238885).

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Suitable examples from bacteria include a laccase derivable from a strain of *Bacillus*.

A laccase obtained from *Coprinopsis* or *Myceliophthora* is preferred; in particular a laccase obtained from *Coprinopsis cinerea*, as disclosed in WO 97/08325; or from *Myceliophthora thermophila*, as disclosed in WO 95/33836.

- 5 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, liquids, in particular stabilized
10 liquids, or slurries.

Non-dusting granulates may be produced, *e.g.* as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to
15 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. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol
20 such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

Any detergent components known in the art for use in detergents may also be utilized. Other optional detergent components include anti-corrosion agents, anti-shrink agents, anti-soil
25 redeposition agents, anti-wrinkling agents, bactericides, binders, corrosion inhibitors, disintegrants/disintegration agents, dyes, enzyme stabilizers (including boric acid, borates, CMC, and/or polyols such as propylene glycol), fabric conditioners including clays, fillers/processing aids, fluorescent whitening agents/optical brighteners, foam boosters, foam (suds) regulators, perfumes, soil-suspending agents, softeners, suds suppressors, tarnish
30 inhibitors, and wicking agents, either alone or in combination. Any ingredient known in the art for use in detergents may be utilized. The choice of such ingredients is well within the skill of the artisan.

The detergent compositions can also contain dispersants. In particular powdered detergents
35 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. Suitable dispersants are for example described in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc.

The detergent compositions of the present invention may also include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine *N*-oxide polymers, copolymers of *N*-vinylpyrrolidone and *N*-vinylimidazole, polyvinylloxazolidones and polyvinylimidazoles or mixtures thereof. When present in a subject composition, the dye transfer inhibiting agents may be present at levels from about 0.0001 % to about 10%, from about 0.01% to about 5% or even from about 0.1% to about 3% by weight of the composition.

The detergent compositions of the present invention will preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agent or optical brighteners. Where present the brightener is preferably at a level of about 0.01% to about 0.5%. Any fluorescent whitening agent suitable for use in a laundry detergent composition may be used in the composition of the present invention. The most commonly used fluorescent whitening agents are those belonging to the classes of diaminostilbene-sulfonic acid derivatives, diarylpyrazoline derivatives and bisphenyl-distyryl derivatives. Examples of the diaminostilbene-sulfonic acid derivative type of fluorescent whitening agents include the sodium salts of: 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2-anilino-4-(*N*-methyl-*N*-2-hydroxyethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(4-phenyl-1,2,3-triazol-2-yl)stilbene-2,2'-disulfonate and sodium 5-(2*H*-naphtho[1,2-*d*][1,2,3]triazol-2-yl)-2-[(*E*)-2-phenylvinyl]benzenesulfonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt of 4,4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenyl-styryl)-disulfonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins.

Suitable fluorescent brightener levels include lower levels of from about 0.01, from 0.05, from about 0.1 or even from about 0.2 wt % to upper levels of 0.5 or even 0.75 wt%.

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 terephthalate based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides see for example Chapter 7 in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc. Another type of soil release polymers are amphiphilic alkoxylated grease cleaning

polymers comprising a core structure and a plurality of alkoxyate 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 derivatives 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.

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.

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, anti-shrink agents, anti-wrinkling agents, bactericides, binders, carriers, dyes, enzyme stabilizers, fabric softeners, fillers, foam regulators, hydrotropes, perfumes, pigments, sod suppressors, solvents, and structurants for liquid detergents and/or structure elasticizing agents.

The detergent composition of the second aspect of the invention may be for use in washing an item. Preferably, the detergent composition is for use in preventing, reducing or removing one or more of:

- (a) stickiness from an item,
- (b) adherence of soil to an item,
- (c) deposition or re-deposition of soil on an item during a wash cycle; and/or
- (d) mal-odour of an item.

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The bacteria, forming a biofilm and/or EPS, may be selected from the group consisting of *Acinetobacter* sp., *Aeromicrobium* sp., *Brevundimonas* sp., *Microbacterium* sp., *Micrococcus luteus*, *Pseudomonas* sp., *Staphylococcus epidermidis*, and *Stenotrophomonas* sp.

- 10 The present invention therefore also concerns removal or reduction of mal-odour on textile or fabric. One example of such unpleasant smelling compounds is E-2-nonenal. The mal-odour can be present on newly washed textile or fabric which is still wet. Or the mal-odour can be present on newly washed textile or fabric, which has subsequently been dried. The mal-odour may also be present on textile or fabric, which has been stored for some time after wash. The
- 15 present invention concerns the reduction or removal of mal-odour such as E-2-nonenal from wet or dry textile or fabric.

- In one embodiment, the soil is a pigment soil. The detergent composition may be used for use in pre-treating stains on an item. The detergent composition may also be used in maintaining or
- 20 improving whiteness of an item. When the soil, e.g. a pigment soil, does not adhere to the item, the item appears cleaner and as such the polypeptide having endo-beta-1-6-galactanase activity may maintain or improve the whiteness of the item, in particular textile items.

- A detergent composition according to any previous claim wherein the item is a textile. The textile
- 25 can be made of cotton, Polyester, Polyamide, Polyacryl and/or silk, and combinations thereof, such as Cotton/Polyester.

- A detergent composition according to any one of the preceding claims, wherein said detergent composition is a liquid detergent composition or a powder detergent composition.

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The detergent composition of the invention may be in any convenient form, e.g., 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.

- 35 The detergent composition may take the form of a unit dose product. A unit dose product is the packaging of a single dose in a non-reusable container. It is increasingly used in detergents for laundry. A detergent unit dose product is the packaging (e.g., in a pouch made from a water soluble film) of the amount of detergent used for a single wash.

Pouches can be configured as single or multicompartments. It can be of any form, shape and material which is suitable for hold the composition, e.g. without allowing the release of the composition to release of the composition from the pouch prior to water contact. The pouch is made from water soluble film which encloses an inner volume. Said inner volume can be divided into compartments of the pouch. Preferred films are polymeric materials preferably polymers which are formed into a film or sheet. Preferred polymers, copolymers or derivatives thereof are selected polyacrylates, and water soluble acrylate copolymers, methyl cellulose, carboxy methyl cellulose, sodium dextrin, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, malto dextrin, poly methacrylates, most preferably polyvinyl alcohol copolymers and, hydroxypropyl methyl cellulose (HPMC). Preferably the level of polymer in the film for example PVA is at least about 60%. Preferred average molecular weight will typically be about 20,000 to about 150,000. Films can also be of blended compositions comprising hydrolytically degradable and water soluble polymer blends such as polylactide and polyvinyl alcohol (known under the Trade reference M8630 as sold by MonoSol LLC, Indiana, USA) plus plasticisers like glycerol, ethylene glycerol, propylene glycol, sorbitol and mixtures thereof. The pouches can comprise a solid laundry cleaning composition or part components and/or a liquid cleaning composition or part components separated by the water soluble film. The compartment for liquid components can be different in composition than compartments containing solids: US2009/0011970 A1.

Detergent ingredients can be separated physically from each other by compartments in water dissolvable pouches or in different layers of tablets. Thereby negative storage interaction between components can be avoided. Different dissolution profiles of each of the compartments can also give rise to delayed dissolution of selected components in the wash solution.

A liquid detergent composition has a physical form, which is not solid (or gas). It may be a pourable liquid, a paste, a pourable gel or a non-pourable gel. It may be either isotropic or structured, preferably isotropic. It may be a formulation useful for washing in automatic washing machines or for hand washing.

A liquid or gel detergent, which is not unit dosed, may be aqueous, typically containing at least 20% by weight and up to 95% water, such as up to about 70% water, up to about 65% water, up to about 55% water, up to about 45% water, up to about 35% water. Other types of liquids, including without limitation, alkanols, amines, diols, ethers and polyols may be included in an aqueous liquid or gel.

An aqueous liquid or gel detergent may contain from 0-30% organic solvent.

A liquid or gel detergent may also be non-aqueous, wherein the water content is below 10%, preferably below 5%.

A liquid detergent composition may comprise a surfactant and a detergent builder in a total concentration of at least 3% by weight, and a detergent enzyme contained within a microcapsule, wherein the membrane of the microcapsule is produced by cross-linking of a polybranched polyamine having a molecular weight of more than 1 kDa. Encapsulating enzymes in a microcapsule with a semipermeable membrane of the invention, and having a water activity inside these capsules (prior to addition to the liquid detergent) higher than in the liquid detergent, the capsules will undergo a (partly) collapse when added to the detergent (water is oozing out), thus leaving a more concentrated and more viscous enzyme containing interior in the capsules.

The collapse of the membrane may also result in a reduced permeability. This can be further utilized by addition of stabilizers/polymers, especially ones that are not permeable through the membrane. The collapse and resulting increase in viscosity will reduce/hinder the diffusion of hostile components (e.g., surfactants or sequestrants) into the capsules, and thus increase the storage stability of the enzyme in the liquid detergent. Components in the liquid detergent that are sensitive to the enzyme (e.g., components that act as substrate for the enzyme) are also protected against degradation by the enzyme. During wash the liquid detergent is diluted by water, thus increasing the water activity. Water will now diffuse into the capsules (osmosis). The capsules will swell and the membrane will either become permeable to the enzyme so they can leave the capsules, or simply burst and in this way releasing the enzyme. The concept is very efficient in stabilizing the enzymes against hostile components in liquid detergent, and vice versa also protects enzyme sensitive components in the liquid detergent from enzymes.

Examples of detergent components which are sensitive to, and can be degraded by, enzymes include (relevant enzyme in parenthesis): xanthan gum (xanthanase), polymers with ester bonds (lipase), hydrogenated castor oil (lipase), perfume (lipase), methyl ester sulfonate surfactants (lipase), cellulose and cellulose derivatives (e.g. CMC) (cellulase), and dextrin and cyclodextrin (amylase).

Such sensitive detergent ingredients can be encapsulated, and thus stabilized, in microcapsules.

Sensitive detergent ingredients are prone to degradation during storage. Such detergent ingredients include bleaching compounds, bleach activators, perfumes, polymers, builder, surfactants, etc. Generally, the microcapsules can be used to separate incompatible components/compounds in detergents.

Addition of the microcapsules to detergents can be used to influence the visual appearance of the detergent product, such as an opacifying effect (small microcapsules) or an effect of distinctly visible particles (large microcapsules). The microcapsules may also be colored.

The microcapsules can be used to reduce the enzyme dust levels during handling and processing of enzyme products.

Microcapsules are typically produced by forming water droplets into a continuum that is non-miscible with water - i.e., typically by preparing a water-in-oil emulsion - and subsequently formation of the membrane by interfacial polymerization via addition of a cross-linking agent. After eventual curing the capsules can be harvested and further rinsed and formulated by methods known in the art. The capsule formulation is subsequently added to the detergent.

The payload, the major membrane constituents and eventual additional component that are to be encapsulated are found in the water phase. In the continuum is found components that stabilize the water droplets towards coalescence (emulsifiers, emulsion stabilizers, surfactants etc.) and the cross-linking agent is also added via the continuum.

The emulsion can be prepared by any methods known in the art, e.g., by mechanical agitation, dripping processes, membrane emulsification, microfluidics, sonication etc. In some cases, simple mixing of the phases automatically will result in an emulsion, often referred to as self-emulsification. Using methods resulting in a narrow size distribution is an advantage.

The cross-linking agent(s) is typically subsequently added to the emulsion, either directly or more typically by preparing a solution of the crosslinking agent in a solvent which is soluble in the continuous phase. The emulsion and cross-linking agent or solution hereof can be mixed by conventional methods used in the art, e.g., by simple mixing or by carefully controlling the flows of the emulsion and the cross-linking agent solution through an in-line mixer.

In some cases, curing of the capsules is needed to complete the membrane formation. Curing is often simple stirring of the capsules for some time to allow the interfacial polymerization reaction to end. In other cases, the membrane formation can be stopped by addition of reaction quencher.

The capsules may be post modified, e.g., by reacting components onto the membrane to hinder or reduce flocculation of the particles in the detergent as described in WO 99/01534.

The produced capsules can be isolated or concentrated by methods known in the art, e.g., by filtration, centrifugation, distillation or decantation of the capsule dispersion.

The resulting capsules can be further formulated, e.g., by addition of surfactants to give the product the desired properties for storage, transport and later handling and addition to the

detergent. Other microcapsule formulation agents include rheology modifiers, biocides (e.g., Proxel), acid/base for adjustment of pH (which will also adjust inside the microcapsules), and water for adjustment of water activity.

5 The capsule forming process may include the following steps:

- Preparation of the initial water and oil phase(s),
- Forming a water-in-oil emulsion,
- Membrane formation by interfacial polymerization,
- 10 - Optional post modification,
- Optional isolation and/or formulation,
- Addition to detergent.

The process can be either a batch process or a continuous or semi-continuous process.

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Microencapsulation of enzymes, as used in the present invention, may be carried out by interfacial polymerization, wherein the two reactants in a polymerization reaction meet at an interface and react rapidly. The basis of this method is a reaction of a polyamine with an acid derivative, usually an acid halide, acting as a crosslinking agent. The polyamine is preferably substantially water-soluble (when in free base form). Under the right conditions, thin flexible membranes form rapidly at the interface. One way of carrying out the polymerization is to use an aqueous solution of the enzyme and the polyamine, which are emulsified with a non-aqueous solvent (and an emulsifier), and a solution containing the acid derivative is added. An alkaline agent may be present in the enzyme solution to neutralize the acid formed during the reaction.

20 Polymer (polyamide) membranes form instantly at the interface of the emulsion droplets. The polymer membrane of the microcapsule is typically of a cationic nature, and thus bind/complex with compounds of an anionic nature.

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The diameter of the microcapsules is determined by the size of the emulsion droplets, which is controlled, for example by the stirring rate.

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Emulsions can be classified as either simple emulsions, wherein the dispersed liquid phase is a simple homogeneous liquid, or a more complex emulsion, wherein the dispersed liquid phase is a heterogeneous combination of liquid or solid phases, such as a double emulsion or a multiple-emulsion. For example, a water-in-oil double emulsion or multiple emulsion may be formed wherein the water phase itself further contains an emulsified oil phase; this type of emulsion may be specified as an oil-in-water-in oil (o/w/o) emulsion. Alternatively, a water-in-oil emulsion may be formed wherein the water phase contains a dispersed solid phase often referred to as a

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suspension- emulsion. Other more complex emulsions can be described. Because of the inherent difficulty in describing such systems, the term emulsion is used to describe both simple and more complex emulsions without necessarily limiting the form of the emulsion or the type and number of phases present

5 The rigidity/flexibility and permeability of the membrane is mainly influenced by the choice of polyamine. The polyamine according to the invention is a polybranched polyamine. Each branch, preferably ending with a primary amino group serves as a tethering point in the membrane network, thereby giving the favorable properties of the invention. A polybranched polyamine according to the present invention is a polyamine having more than two branching points and
10 more than two reactive amino groups (capable of reacting with the crosslinking agent, i.e., primary and secondary amino groups). The polybranched polyamine is used as starting material when the emulsion is prepared – it is not formed in situ from other starting materials. To obtain the attractive properties of the invention, the polybranched structure of the polyamine must be present as starting material.

15 There is a close relation between number of branching points and number of primary amines, since primary amines will always be positioned at the end of a branch: A linear amine can only contain two primary amines. For each branching point hypothetically introduced in such a linear di-amine will allow one or more primary amine(s) to be introduced at the end of the introduced
20 branch(es). In this context, we understand the primary amino group as part of the branch, i.e., the endpoint of the branch. For example, both tris(2-aminoethyl) amine and 1,2,3-propanetriamine is considered as molecules having one branching point. For the invention, the polyamine has at least four primary amines. Branching points can be introduced from an aliphatic hydrocarbon chain as in the previously stated examples or from unsaturated carbon bonds, such
25 as in, e.g., 3,3'-diaminobenzidine, or from tertiary amino groups, such as in N,N,N',N'-tetrakis-(2-aminoethyl)ethylenediamine.

In addition to the number of branching points, we have found that the compactness of the reactive amino groups is of high importance. A substance such as, e.g., N,N,N',N'-tetrakis-(12-
30 aminododecyl)ethylenediamine would not be suitable. Neither would a peptide or protein, such as an enzyme, be suitable for membrane formation. Thus, the polybranched polyamine is not a peptide or protein.

In an embodiment, the reactive amino groups constitute at least 15% of the molecular weight of
35 the polybranched polyamine, such as more than 20%, or more than 25%. Preferably, the molecular weight of the polybranched polyamine is at least 1 kDa; more preferably, the molecular weight of the polybranched polyamine is at least 1.3 kDa.

In a preferred embodiment, the polybranched polyamine is a polyethyleneimine (PEI), and modifications thereof, having more than two branching points and more than two reactive amino groups; wherein the reactive amino groups constitute at least 15% of the molecular weight of the PEI, such as more than 20%, or more than 25%. Preferably, the molecular weight of the PEI is at least 1 kDa. Combinations of different polybranched polyamines may be used for preparing such microcapsules.

The advantageous properties (e.g., enzyme storage stability, reduced enzyme leakage, reduced in-flux of detergent ingredients) of the microcapsule of the invention may be improved by adding one or more small amines with a molecular weight of less than 1 kDa. The small amine is preferably substantially water-soluble (when in free base form) and can be a material such as ethylene diamine, hexamethylene diamine, hexane diamine, diethylene tetramine, ethylene tetramine, diamino benzene, piperazine, tetramethylene pentamine or, preferably, diethylene triamine (DETA). The small amines may be added in an amount of up to 50%, preferably up to 40%, up to 30%, up to 20%, up to 10%, or up to 5%, by weight of the total content of small amine and polybranched polyamine, when preparing the microcapsule of the invention.

A crosslinking agent as described herein is a molecule with at least two groups/sites capable of reacting with amines to form covalent bonds. The crosslinking agent is preferably oil soluble and can be in the form of an acid anhydride or acid halide, preferably an acid chloride. For example, it can be adipoyl chloride, sebacoyl chloride, dodecanedioic acid chloride, phthaloyl chloride, terephthaloyl chloride, isophthaloyl chloride, or trimesoyl chloride; but preferably, the crosslinking agent is terephthaloyl chloride or trimesoyl chloride.

The microcapsules may be added to the liquid detergent composition in an amount corresponding to from 0.0001% to 5% (w/w) active enzyme protein (AEP); preferably from 0.001% to 5%, more preferably from 0.005% to 5%, more preferably from 0.005% to 4%, more preferably from 0.005% to 3%, more preferably from 0.005% to 2%, even more preferably from 0.01% to 2%, and most preferably from 0.01% to 1% (w/w) active enzyme protein.

The detergent composition may be in the form of a laundry soap bar and used for hand washing laundry, fabrics and/or textiles. The term laundry soap bar includes laundry bars, soap bars, combo bars, syndet bars and detergent bars. The types of bar usually differ in the type of surfactant they contain, and the term laundry soap bar includes those containing soaps from fatty acids and/or synthetic soaps. The laundry soap bar has a physical form which is solid and not a liquid, gel or a powder at room temperature. The term solid is defined as a physical form which does not significantly change over time, i.e. if a solid object (e.g. laundry soap bar) is placed inside a container, the solid object does not change to fill the container it is placed in. The bar is a solid typically in bar form but can be in other solid shapes such as round or oval.

The laundry soap bar may contain one or more additional enzymes, protease inhibitors such as peptide aldehydes (or hydrosulfite adduct or hemiacetal adduct), boric acid, borate, borax and/or phenylboronic acid derivatives such as 4-formylphenylboronic acid, one or more soaps or synthetic surfactants, polyols such as glycerine, pH controlling compounds such as fatty acids, citric acid, acetic acid and/or formic acid, and/or a salt of a monovalent cation and an organic anion wherein the monovalent cation may be for example Na^+ , K^+ or NH_4^+ and the organic anion may be for example formate, acetate, citrate or lactate such that the salt of a monovalent cation and an organic anion may be, for example, sodium formate.

The laundry soap bar may also contain complexing agents like EDTA and HEDP, perfumes and/or different type of fillers, surfactants e.g. anionic synthetic surfactants, builders, polymeric soil release agents, detergent chelators, stabilizing agents, fillers, dyes, colorants, dye transfer inhibitors, alkoxylated polycarbonates, suds suppressers, structurants, binders, leaching agents, bleaching activators, clay soil removal agents, anti-redeposition agents, polymeric dispersing agents, brighteners, fabric softeners, perfumes and/or other compounds known in the art.

The laundry soap bar may be processed in conventional laundry soap bar making equipment such as but not limited to: mixers, plodders, e.g. a two stage vacuum plodder, extruders, cutters, logo-stampers, cooling tunnels and wrappers. The invention is not limited to preparing the laundry soap bars by any single method. The premix of the invention may be added to the soap at different stages of the process. For example, the premix containing a soap, endo-beta-1-6-galactanase, optionally one or more additional enzymes, a protease inhibitor, and a salt of a monovalent cation and an organic anion may be prepared and the mixture is then plodded. The endo-beta-1-6-galactanase and optional additional enzymes may be added at the same time as the protease inhibitor for example in liquid form. Besides the mixing step and the plodding step, the process may further comprise the steps of milling, extruding, cutting, stamping, cooling and/or wrapping.

The endo-beta-1-6-galactanase may be formulated as a granule for example as a co-granule that combines one or more enzymes. Each enzyme will then be present in more granules securing a more uniform distribution of enzymes in the detergent. This also reduces the physical segregation of different enzymes due to different particle sizes. Methods for producing multi-enzyme co-granulates for the detergent industry are disclosed in the [IP.com](#) disclosure IPCOM000200739D.

Another example of formulation of enzymes by the use of co-granulates are disclosed in WO 2013/188331, which relates to a detergent composition comprising (a) a multi-enzyme co-granule; (b) less than 10 wt zeolite (anhydrous basis); and (c) less than 10 wt phosphate salt (anhydrous basis), wherein said enzyme co-granule comprises from 10 to 98 wt% moisture sink components and the composition additionally comprises from 20 to 80 wt% detergent moisture sink components.

WO 2013/188331 also relates to a method of treating and/or cleaning a surface, preferably a fabric surface comprising the steps of (i) contacting said surface with the detergent composition as claimed and described herein in an aqueous wash liquor, (ii) rinsing and/or drying the surface.

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The multi-enzyme co-granule may comprise a endo-beta-1-6-galactanase and (a) one or more enzymes selected from the group consisting of first- wash lipases, cleaning cellulases, xyloglucanases, perhydrolases, peroxidases, lipoxygenases, laccases and mixtures thereof; and (b) one or more enzymes selected from the group consisting of hemicellulases, proteases, care
10 cellulases, cellobiose dehydrogenases, xylanases, phospho lipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, tannases, pentosanases, lichenases glucanases, arabinosidases, hyaluronidase, chondroitinase, amylases, other galactanases and mixtures thereof.

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In a third aspect of the invention there is provided a use of either (i) a polypeptide having endo-beta-1-6-galactanase activity or (ii) a detergent composition comprising a polypeptide having endo-beta-1-6-galactanase activity, in the washing of an item. Both the polypeptide and the detergent composition may be as deinfed in the first aspect of the invention. In other words,
20 each feature of either the polypeptide and/or the detergent composition described in the first aspect equally applies to the polypeptide and detergent compositions being used in the third aspect of the invention.

In a fourth aspect of the invention there is provided a method of washing an item comprising the
25 steps of:

- a. Exposing an item to a polypeptide according to the first aspect of the invention or a detergent composition according to the second aspect of the invention;
- b. Completing at least one wash cycle; and
- c. Optionally rinsing the item.

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Preferably, the item is a textile.

The pH of the wash liquor (i.e. the solution in which washing takes place) is in the range of 7 to 10, such as in the range of 7 to 9, in the range of 7 to 8 or in the range of 7 to 7.5.

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The wash liquor may also have a temperature in the range of 5°C to 95°C, or in the range of 10°C to 80°C, in the range of 10°C to 70°C, in the range of 10°C to 60°C, in the range of 10°C to

50°C, in the range of 15°C to 40°C or in the range of 20°C to 30°C. In one embodiment, the temperature of the wash liquor is 30°C.

It is preferred if the wash cycle is performed at (and/or the wash liquor has) a temperature of 40°C or less, or more preferably at a temperature of 30°C or less, or even more preferably at a temperature of 20°C or less.

The method for washing an item may further comprise the step of draining of the wash liquor or part of the wash liquor after completion of a wash cycle. The wash liquor can then be re-used in a subsequent wash cycle or in a subsequent rinse cycle. The item may be exposed to the wash liquor during a first and optionally a second or a third wash cycle.

In one embodiment, the item is rinsed after being exposed to the wash liquor. The item can be rinsed with water or with water comprising a conditioner, such as a fabric conditioner.

Exemplary assays that may be used to test wash performance and methods are the Launder-O-Meter (LOM) Model Wash System and the Mini Launder-O-Meter (MiniLOM) Model Wash System.

The Launder-O-Meter (LOM) is a medium scale model wash system that can be applied to test up to 20 different wash conditions simultaneously. A LOM is basically a large temperature controlled water bath with 20 closed metal beakers rotating inside it. Each beaker constitutes one small washing machine and during an experiment, each will contain a solution of a specific detergent/enzyme system to be tested along with the soiled and unsoiled fabrics it is tested on. Mechanical stress is achieved by the beakers being rotated in the water bath and by including metal balls in the beaker.

The LOM model wash system is mainly used in medium scale testing of detergents and enzymes at European wash conditions. In a LOM experiment, factors such as the ballast to soil ratio and the fabric to wash liquor ratio can be varied. Therefore, the LOM provides the link between small scale experiments, such as AMSA and mini-wash, and the more time consuming full scale experiments in front loader washing machines.

MiniLOM is a modified mini wash system of the Launder-O-Meter (LOM), which is a medium scale model wash system that can be applied to test up to 20 different wash conditions simultaneously. A LOM is basically a large temperature controlled water bath with 20 closed metal beakers rotating inside it. Each beaker constitutes one small washing machine and during an experiment, each will contain a solution of a specific detergent/enzyme system to be tested

along with the soiled and unsoiled fabrics it is tested on. Mechanical stress is achieved by the beakers being rotated in the water bath and by including metal balls in the beaker.

The LOM model wash system is mainly used in medium scale testing of detergents and enzymes at European wash conditions. In a LOM experiment, factors such as the ballast to soil ratio and the fabric to wash liquor ratio can be varied. Therefore, the LOM provides the link between small scale experiments, such as AMSA and mini-wash, and the more time consuming full scale experiments in front loader washing machines.

10 In miniLOM, washes are performed in 50 ml test tubes placed in Stuart rotator.

In a fifth aspect of the invention there is provided an item washed according to the method of the fourth aspect of the invention.

15 **Commercial Detergent compositions**

The below mentioned detergent compositions can be used in combination with the endo-beta-1-6-galactanase of the invention.

Biotex black (liquid)

20 5-15% Anionic surfactants, <5% Nonionic surfactants, perfume, enzymes, DMDM and hydantoin.

Composition of Ariel Sensitive White & Color, liquid detergent composition

25 Aqua, Alcohol Ethoxy Sulfate, Alcohol Ethoxylate, Amino Oxide, Citrid Acid, C12-18 topped palm kernel fatty acid, Protease, Glycosidase, Amylase, Ethanol, 1,2 Propanediol, Sodium Formate, Calcium Chloride, Sodium hydroxide, Silicone Emulsion, Trans-sulphated EHDQ (the ingredients are listed in descending order).

Composition of WFK IEC-A model detergent (powder)

30 Ingredients: Linear sodium alkyl benzene sulfonate 8,8 %, Ethoxylated fatty alcohol C12-18 (7 EO) 4,7 %, Sodium soap 3,2 %, Anti foam DC2-4248S 3,9 %, Sodium aluminium silicate zeolite 4A 28,3 %, Sodium carbonate 11,6 %, Sodium salt of a copolymer from acrylic and maleic acid (Sokalan CP5) 2,4 %, Sodium silicate 3,0 %, Carboxymethylcellulose 1,2 %, Dequest 2066 2,8 %, Optical whitener 0,2 %, Sodium sulfate 6,5 %, Protease 0,4 %.

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Composition of model detergent A (liquid)

Ingredients: 12% LAS, 11% AEO Biosoft N25-7 (NI), 7% AEOS (SLES), 6% MPG (monopropylene glycol), 3% ethanol, 3% TEA, 2.75% cocoa soap, 2.75% soya soap, 2%

glycerol, 2% sodium hydroxide, 2% sodium citrate, 1% sodium formiate, 0.2% DTMPA and 0.2% PCA (all percentages are w/w)

Composition of Ariel Actilift (liquid)

- 5 Ingredients: 5-15% Anionic surfactants; <5% Non-ionic surfactants, Phosphonates, Soap; Enzymes, Optical brighteners, Benzisothiazolinone, Methylisothiazolinone, Perfumes, Alpha-isomethyl ionone, Citronellol, Geraniol, Linalool.

Composition of Ariel Actilift Colour&Style (liquid)

- 10 Ingredients: 5-15% Anionic surfactants; <5% Non-ionic surfactants, Phosphonates, Soap; Enzymes, Perfumes, Benzisothiazolinone, Methylisothiazolinone, Alpha-isomethyl ionone, Butylphenyl methylpropional, Citronellol, Geraniol, Linalool.

Composition of Persil Small & Mighty (liquid)

- 15 Ingredients: 15-30% Anionic surfactants, Non-ionic surfacts, 5-15% Soap, < 5% Polycarboxylates, Perfume, Phosphates, Optical Brighteners

Persil 2 in1 with Comfort Passion Flower Powder

- Sodium sulfate, Sodium carbonate, Sodium Dodecylbenzenesulfonate, Bentonite, Sodium
20 Carbonate Peroxide, Sodium Silicate, Zeolite, Aqua, Citric acid, TAED, C12-15 Pareth-7, Stearic Acid, Parfum, Sodium Acrylic Acid/MA Copolymer, Cellulose Gum, Corn Starch Modified, Sodium chloride, Tetrasodium Etidronate, Calcium Sodium EDTMP, Disodium Anilinomorpholinotriazinyl-aminostilbenesulfonate, Sodium bicarbonate, Phenylpropyl Ethyl Methicone, Butylphenyl Methylpropional, Glyceryl Stearates, Calcium carbonate, Sodium
25 Polyacrylate, Alpha-Isomethyl Ionone, Disodium Distyrylbiphenyl Disulfonate, Cellulose, Protease, Limonene, PEG-75, Titanium dioxide, Dextrin, Sucrose, Sodium Polyaryl Sulphonate, CI 12490, CI 45100, CI 42090, Sodium Thiosulfate, CI 61585.

Persil Biological Powder

- 30 Sucrose, Sorbitol, Aluminum Silicate, Polyoxymethylene Melamine, Sodium Polyaryl Sulphonate, CI 61585, CI 45100, Lipase, Amylase, Xanthan gum, Hydroxypropyl methyl cellulose, CI 12490, Disodium Distyrylbiphenyl Disulfonate, Sodium Thiosulfate, CI 42090, Mannanase, CI 11680, Etidronic Acid, Tetrasodium EDTA.

35 Persil Biological Tablets

Sodium carbonate, Sodium Carbonate Peroxide, Sodium bicarbonate, Zeolite, Aqua, Sodium Silicate, Sodium Lauryl Sulfate, Cellulose, TAED, Sodium Dodecylbenzenesulfonate, Hemicellulose, Lignin, Lauryl Glucoside, Sodium Acrylic Acid/MA Copolymer, Bentonite, Sodium

chloride, Parfum, Tetrasodium Etidronate, Sodium sulfate, Sodium Polyacrylate, Dimethicone, Disodium Anilinomorpholinotriazinylaminostilbenesulfonate, Dodecylbenzene Sulfonic Acid, Trimethylsiloxysilicate, Calcium carbonate, Cellulose, PEG-75, Titanium dioxide, Dextrin, Protease, Corn Starch Modified, Sucrose, CI 12490, Sodium Polyaryl Sulphonate, Sodium

5 Thiosulfate, Amylase, Kaolin,

Persil Colour Care Biological Powder

Subtilisin, Imidazolidinone, Hexyl Cinnamal, Sucrose, Sorbitol, Aluminum Silicate, Polyoxymethylene Melamine, CI 61585, CI 45100, Lipase, Amylase, Xanthan gum,

10 Hydroxypropyl methyl cellulose, CI 12490, Disodium Distyrylbiphenyl Disulfonate, Sodium Thiosulfate, CI 42090, Mannanase, CI 11680, Etidronic Acid, Tetrasodium EDTA.

Persil Colour Care Biological Tablets

Sodium bicarbonate, Sodium carbonate, Zeolite, Aqua, Sodium Silicate, Sodium Lauryl Sulfate,

15 Cellulose Gum, Sodium Dodecylbenzenesulfonate, Lauryl Glucoside, Sodium chloride, Sodium Acrylic Acid/MA Copolymer, Parfum, Sodium Thioglycolate, PVP, Sodium sulfate, Tetrasodium Etidronate, Sodium Polyacrylate, Dimethicone, Bentonite, Dodecylbenzene Sulfonic Acid, Trimethylsiloxysilicate, Calcium carbonate, Cellulose, PEG-75, Titanium dioxide, Dextrin, Protease, Corn Starch Modified, Sucrose, Sodium Thiosulfate, Amylase, CI 74160, Kaolin.

Persil Dual Action Capsules Bio

MEA-Dodecylbenzenesulfonate, MEA-Hydrogenated Cocoate, C12-15 Pareth-7, Dipropylene Glycol, Aqua, Tetrasodium Etidronate, Polyvinyl Alcohol, Glycerin, Aziridine, homopolymer ethoxylated, Propylene glycol, Parfum, Sodium Diethylenetriamine Pentamethylene

25 Phosphonate, Sorbitol, MEA-Sulfate, Ethanolamine, Subtilisin, Glycol, Butylphenyl Methylpropional, Boronic acid, (4-formylphenyl), Hexyl Cinnamal, Limonene, Linalool, Disodium Distyrylbiphenyl Disulfonate, Alpha-Isomethyl Ionone, Geraniol, Amylase, Polymeric Blue Colourant, Polymeric Yellow Colourant, Talc, Sodium chloride, Benzisothiazolinone, Mannanase, Denatonium Benzoate.

Persil 2 in1 with Comfort Sunshiny Days Powder

Sodium sulfate, Sodium carbonate, Sodium Dodecylbenzenesulfonate, Bentonite, Sodium Carbonate Peroxide, Sodium Silicate, Zeolite, Aqua, Citric acid, TAED, C12-15 Pareth-7, Parfum, Stearic Acid, Sodium Acrylic Acid/MA Copolymer, Cellulose Gum, Corn Starch Modified,

35 Sodium chloride, Tetrasodium Etidronate, Calcium Sodium EDTMP, Disodium Anilinomorpholinotriazinyl-aminostilbenesulfonate, Sodium bicarbonate, Phenylpropyl Ethyl Methicone, Butylphenyl Methylpropional, Glyceryl Stearates, Calcium carbonate, Sodium Polyacrylate, Geraniol, Disodium Distyrylbiphenyl Disulfonate, Cellulose, Protease, PEG-75,

Titanium dioxide, Dextrin, Sucrose, Sodium Polyaryl Sulphonate, CI 12490, CI 45100, CI 42090, Sodium Thiosulfate, CI 61585.

Persil Small & Mighty 2in1 with Comfort Sunshiny Days

5 Aqua, C12-15 Pareth-7, Sodium Dodecylbenzenesulfonate, Propylene glycol, Sodium Hydrogenated Cocoate, Triethanolamine, Glycerin, TEA-Hydrogenated Cocoate, Parfum, Sodium chloride, Polyquaternium-10, PVP, Polymeric Pink Colourant, Sodium sulfate, Disodium Distyrylbiphenyl Disulfonate, Butylphenyl Methylpropional, Styrene/Acrylates Copolymer, Hexyl Cinnamal, Citronellol, Eugenol, Polyvinyl Alcohol, Sodium acetate, Isopropyl alcohol, Polymeric
10 Yellow Colourant, Sodium Lauryl Sulfate.

Persil Small & Mighty Bio

Aqua, MEA-Dodecylbenzenesulfonate, Propylene glycol, Sodium Laureth Sulfate, C12-15 Pareth-7, TEA-Hydrogenated Cocoate, MEA-Citrate, Aziridine homopolymer ethoxylated, MEA-
15 Etidronate, Triethanolamine, Parfum, Acrylates Copolymer, Sorbitol, MEA-Sulfate, Sodium Sulfite, Disodium Distyrylbiphenyl Disulfonate, Butylphenyl Methylpropional, Styrene/Acrylates Copolymer, Citronellol, Sodium sulfate, Peptides, salts, sugars from fermentation (process), Subtilisin, Glycerin, Boronic acid, (4-formylphenyl), Geraniol, Pectate Lyase, Amylase, Sodium Lauryl Sulfate, Mannanase, CI 42051.

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Persil Small & Mighty Capsules Biological

MEA-Dodecylbenzenesulfonate, MEA-Hydrogenated Cocoate, C12-15 Pareth-7, Dipropylene Glycol, Aqua, Glycerin, Polyvinyl Alcohol, Parfum, Aziridine homopolymer ethoxylated, Sodium Diethylenetriamine Pentamethylene Phosphonate, Propylene glycol, Sorbitol, MEA-Sulfate,
25 Ethanolamine, Subtilisin, Glycol, Butylphenyl Methylpropional, Hexyl Cinnamal, Starch, Boronic acid, (4-formylphenyl), Limonene, Linalool, Disodium Distyrylbiphenyl Disulfonate, Alpha-Isomethyl Ionone, Geraniol, Amylase, Talc, Polymeric Blue Colourant, Sodium chloride, Benzisothiazolinone, Denatonium Benzoate, Polymeric Yellow Colourant, Mannanase.

Persil Small & Mighty Capsules Colour Care

MEA-Dodecylbenzenesulfonate, MEA-Hydrogenated Cocoate, C12-15 Pareth-7, Dipropylene Glycol, Aqua, Glycerin, Polyvinyl Alcohol, Parfum, Aziridine homopolymer ethoxylated, Sodium Diethylenetriamine Pentamethylene Phosphonate, Propylene glycol, MEA-Sulfate, Ethanolamine, PVP, Sorbitol, Butylphenyl Methylpropional, Subtilisin, Hexyl Cinnamal, Starch,
35 Limonene, Linalool, Boronic acid, (4-formylphenyl), Alpha-Isomethyl Ionone, Geraniol, Talc, Polymeric Blue Colourant, Denatonium Benzoate, Polymeric Yellow Colourant.

Persil Small & Mighty Colour Care

Aqua, MEA-Dodecylbenzenesulfonate, Propylene glycol, Sodium Laureth Sulfate, C12-15 Pareth-7, TEA-Hydrogenated Cocoate, MEA-Citrate, Aziridine homopolymer ethoxylated, MEA-Etidronate, Triethanolamine, Parfum, Acrylates Copolymer, Sorbitol, MEA-Sulfate, Sodium Sulfite, Glycerin, Butylphenyl Methylpropional, Citronellol, Sodium sulfate, Peptides, salts, sugars from fermentation (process), Styrene/Acrylates Copolymer, Subtilisin, Boronic acid, (4-formylphenyl), Geraniol, Pectate Lyase, Amylase, Sodium Lauryl Sulfate, Mannanase, CI 61585, CI 45100.

Composition of Fairy Non Bio (liquid)

Ingredients: 15-30% Anionic Surfactants, 5-15% Non-Ionic Surfactants, Soap, Benzisothiazolinone, Methylisothiazolinone, Perfumes

Composition of Model detergent T (powder)

Ingredients: 11% LAS, 2% AS/AEOS, 2% soap, 3% AEO, 15.15% sodium carbonate, 3% sodium silicate, 18.75% zeolite, 0.15% chelant, 2% sodium citrate, 1.65% AA/MA copolymer, 2.5% CMC and 0.5% SRP (all percentages are w/w).

Composition of Model detergent X (powder)

Ingredients: 16.5% LAS, 15% zeolite, 12% sodium disilicate, 20% sodium carbonate, 1% socalan, 35.5% sodium sulfate (all percentages are w/w).

Composition of Ariel Actilift Colour&Style (powder)

Ingredients: 15-30% Anionic surfactants, <5% Non-ionic surfactants, Phosphonates, Polycarboxylates, Zeolites; Enzymes, Perfumes, Hexyl cinnamal.

Composition of Ariel Actilift (powder)

Ingredients: 5-15% Anionic surfactants, Oxygen-based bleaching agents, <5% Non-ionic surfactants, Phosphonates, Polycarboxylates, Zeolites, Optical brighteners, Enzymes, Perfumes, Butylphenyl Methylpropional, Coumarin, Hexyl Cinnamal.

Composition of Persil Megaperls (powder)

Ingredients: 15 - 30 % of the following: anionic surfactants, oxygen-based bleaching agent and zeolites, less than 5 % of the following: non-ionic surfactants, phosphonates, polycarboxylates, soap, Further ingredients: Perfumes, Hexyl cinnamal, Benzyl salicylate, Linalool, optical brighteners, Enzymes and Citronellol.

Tide Liquid, Original: Linear alkylbenzene sulfonate, propylene glycol, citric acid, sodium hydroxide, borax, ethanolamine, ethanol, alcohol sulfate, polyethyleneimine ethoxylate, sodium fatty acids, diquaternium ethoxysulfate, protease, diethylene glycol, laureth-9,

alkyldimethylamine oxide, fragrance, amylase, disodium diaminostilbene disulfonate, DTPA, sodium formate, calcium formate, polyethylene glycol 4000, mannanase, Liquitint™ Blue, dimethicone.

- 5 **Liquid Tide, Free and Gentle:** Water, sodium alcoholethoxy sulfate, propylene glycol, borax, ethanol, linear alkylbenzene sulfonate sodium, salt, polyethyleneimine ethoxylate, diethylene glycol, trans sulfated & ethoxylated hexamethylene diamine, alcohol ethoxylate, linear alkylbenzene sulfonate, MEA salt, sodium formate, sodium alkyl sulfate, DTPA, amine oxide, calcium formate, disodium diaminostilbene, disulfonate, amylase, protease, dimethicone,
10 benzisothiazolinone

- Tide Coldwater Liquid, Fresh Scent:** Water, alcoholethoxy sulfate, linear alkylbenzene sulfonate, diethylene glycol, propylene glycol, ethanolamine, citric acid, Borax, alcohol sulfate, sodium hydroxide, polyethyleneimine, ethoxylate, sodium fatty acids, ethanol, protease,
15 Laureth-9, diquaternium ethoxysulfate, lauramine oxide, sodium cumene, sulfonate, fragrance, DTPA, amylase, disodium, diaminostilbene, disulfonate, sodium formate, disodium distyrylbiphenyl disulfonate, calcium formate, polyethylene glycol 4000, mannanase, pectinase, Liquitint™ Blue, dimethicone

- 20 **Tide TOTALCARE™ Liquid, Cool Cotton:** Water, alcoholethoxy sulfate, propylene glycol, sodium fatty acids, laurtrimonium chloride, ethanol, sodium hydroxide, sodium cumene sulfonate, citric acid, ethanolamine, diethylene glycol, silicone polyether, borax, fragrance, polyethyleneimine ethoxylate, protease, Laureth-9, DTPA, polyacrylamide quaternium chloride, disodium diaminostilbene disulfonate, sodium formate, Liquitint™ Orange, dipropylethyl
25 tetraamine, dimethicone, cellulose.

- Liquid Tide Plus Bleach Alternative™, Vivid White and Bright, Original and Clean Breeze:** Water, sodium alcoholethoxy sulfate, sodium alkyl sulfate, MEA citrate, linear alkylbenzene sulfonate, MEA salt, propylene glycol, diethylene glycol, polyethyleneimine ethoxylate, ethanol,
30 sodium fatty acids, ethanolamine, lauramine oxide, borax, Laureth-9, DTPA, sodium cumene sulfonate, sodium formate, calcium formate, linear alkylbenzene sulfonate, sodium salt, alcohol sulfate, sodium hydroxide, diquaternium ethoxysulfate, fragrance, amylase, protease, mannanase, pectinase, disodium diaminostilbene disulfonate, benzisothiazolinone, Liquitint™ Blue, dimethicone, dipropylethyl tetraamine.

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Liquid Tide HE, Original Scent: Water, Sodium alcoholethoxy sulfate, MEA citrate, Sodium Alkyl Sulfate, alcohol ethoxylate, linear alkylbenzene sulfonate, MEA salt, sodium fatty acids, polyethyleneimine ethoxylate, diethylene glycol, propylene glycol, diquaternium ethoxysulfate,

borax, polyethyleneimine, ethoxylate propoxylate, ethanol, sodium cumene sulfonate, fragrance, DTPA, disodium diaminostilbene disulfonate, Mannanase, cellulase, amylase, sodium formate, calcium formate, Lauramine oxide, Liquitint™ Blue, Dimethicone / polydimethyl silicone.

- 5 **Tide TOTALCARE HE Liquid, renewing Rain:** Water, alcoholethoxy sulfate, linear alkylbenzene sulfonate, alcohol ethoxylate, citric acid, Ethanolamine, sodium fatty acids, diethylene glycol, propylene glycol, sodium hydroxide, borax, polyethyleneimine ethoxylate, silicone polyether, ethanol, protease, sodium cumene sulfonate, diquaternium ethoxysulfate, Laureth-9, fragrance, amylase, DTPA, disodium diaminostilbene disulfonate, disodium
10 distyrylbiphenyl disulfonate, sodium formate, calcium formate, mannanase, Liquitint™ Orange, dimethicone, polyacrylamide quaternium chloride, cellulase, dipropylethyl tetraamine.

- Tide liquid HE Free:** Water, alcoholethoxy sulfate, diethylene glycol, monoethanolamine citrate, sodium formate, propylene glycol, linear alkylbenzene sulfonates, ethanolamine, ethanol,
15 polyethyleneimine ethoxylate, amylase, benzisothiazolin, borax, calcium formate, citric acid, diethylenetriamine pentaacetate sodium, dimethicone, diquaternium ethoxysulfate, disodium diaminostilbene disulfonate, Laureth-9, mannanase, protease, sodium cumene sulfonate, sodium fatty acids.

- 20 **Tide Coldwater HE Liquid, Fresh Scent:** Water, alcoholethoxy sulfate, MEA Citrate, alcohol sulfate, Alcohol ethoxylate, Linear alkylbenzene sulfonate MEA, sodium fatty acids, polyethyleneimine ethoxylate, diethylene glycol, propylene glycol, diquaternium ethoxysulfate, borax, polyethyleneimine ethoxylate propoxylate, ethanol, sodium cumene sulfonate, fragrance, DTPA, disodium diaminostilbene disulfonate, protease, mannanase, cellulase, amylase, sodium
25 formate, calcium formate, lauramine oxide, Liquitint™ Blue, dimethicone.

- Tide for Coldwater HE Free Liquid:** Water, sodium alcoholethoxy sulfate, MEA Citrate, Linear alkylbenzene sulfonate: sodium salt, Alcohol ethoxylate, Linear alkylbenzene sulfonate: MEA salt, sodium fatty acids, polyethyleneimine ethoxylate, diethylene glycol, propylene glycol,
30 diquaternium ethoxysulfate, Borax, protease, polyethyleneimine ethoxylate propoxylate, ethanol, sodium cumene sulfonate, Amylase, citric acid, DTPA, disodium diaminostilbene disulfonate, sodium formate, calcium formate, dimethicone.

- Tide Simply Clean & Fresh:** Water, alcohol ethoxylate sulfate, linear alkylbenzene sulfonate
35 Sodium/Mea salts, propylene glycol, diethylene glycol, sodium formate, ethanol, borax, sodium fatty acids, fragrance, lauramine oxide, DTPA, Polyethylene amine ethoxylate, calcium formate, disodium diaminostilbene disulfonate, dimethicone, tetramine, Liquitint™ Blue.

Tide Pods, Ocean Mist, Mystic Forest, Spring Meadow: linear alkylbenzene sulfonates, C12-16 Parath-9, propylene glycol, alcoholethoxy sulfate, water, polyethyleneimine ethoxylate, glycerine, fatty acid salts, PEG-136 polyvinyl acetate, ethylene Diamine disuccinic salt, monoethanolamine citrate, sodium bisulfite, diethylenetriamine pentaacetate sodium, disodium distyrylbiphenyl disulfonate, calcium formate, mannanase, exyloglucanase, sodium formate, hydrogenated castor oil, natalase, dyes, termamyl, subtilisin, benzisothiazolin, perfume.

Tide to Go: Deionized water, Dipropylene Glycol Butyl Ether, Sodium Alkyl Sulfate, Hydrogen Peroxide, Ethanol, Magnesium Sulfate, Alkyl Dimethyl Amine Oxide, Citric Acid, Sodium Hydroxide, Trimethoxy Benzoic Acid, Fragrance.

Tide Stain Release Liquid: Water, Alkyl Ethoxylate, Linear Alkylbenzenesulfonate, Hydrogen Peroxide, Diquaternium Ethoxysulfate, Ethanolamine, Disodium Distyrylbiphenyl Disulfonate, tetrabutyl Ethylidenebisphenol, F&DC Yellow 3, Fragrance.

Tide Stain Release Powder: Sodium percarbonate, sodium sulfate, sodium carbonate, sodium aluminosilicate, nonanoyloxy benzene sulfonate, sodium polyacrylate, water, sodium alkylbenzenesulfonate, DTPA, polyethylene glycol, sodium palmitate, amylase, protease, modified starch, FD&C Blue 1, fragrance.

Tide Stain Release, Pre Treater Spray: Water, Alkyl Ethoxylate, MEA Borate, Linear Alkylbenzenesulfonate, Propylene Glycol, Diquaternium Ethoxysulfate, Calcium Chlorideenzyme, Protease, Ethanolamine, Benzoisothiazolinone, Amylase, Sodium Citrate, Sodium Hydroxide, Fragrance.

Tide to Go Stain Eraser: Water, Alkyl Amine Oxide, Dipropylene Glycol Phenyl Ether, Hydrogen Peroxide, Citric Acid, Ethylene Diamine Disuccinic Acid Sodium salt, Sodium Alkyl Sulfate, Fragrance.

Tide boost with Oxi: Sodium bicarbonate, sodium carbonate, sodium percarbonate, alcohol ethoxylate, sodium chloride, maleic/acrylic copolymer, nonanoyloxy benzene sulfonate, sodium sulfate, colorant, diethylenetriamine pentaacetate sodium salt, hydrated aluminosilicate (zeolite), polyethylene glycol, sodium alkylbenzene sulfonate, sodium palmitate, starch, water, fragrance.

Tide Stain Release boost Duo Pac: Polyvinyl Alcoholpouch film, wherein there is packed a liquid part and a powder part. **Liquid Ingredients:** Dipropylene Glycol, diquaternium Ethoxysulfate, Water, Glycerin, Liquitint™ Orange, **Powder Ingredients:** sodium percarbonate, nonanoyloxy benzene sulfonate, sodium carbonate, sodium sulfate, sodium

aluminosilicate, sodium polyacrylate, sodium alkylbenzenesulfonate, maleic/acrylic copolymer, water, amylase, polyethylene glycol, sodium palmitate, modified starch, protease, glycerine, DTPA, fragrance.

- 5 **Tide Ultra Stain Release:** Water, sodium alcoholethoxy sulfate, linear alkyl benzene sulfonate, sodium/MEA salts, MEA citrate, propylene glycol, polyethyleneimine ethoxylate, ethanol, diethylene glycol, polyethyleneimine propoxyethoxylate, sodium fatty acids, protease, borax, sodium cumene sulfonate, DTPA, fragrance, amylase, disodium diaminostilbene disulfonate, calcium formate, sodium formate, gluconase, dimethicone, Liquitint™ Blue, mannanase.

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Ultra Tide with a Touch of Downy® Powdered Detergent, April Fresh/Clean Breeze/April Essence: Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Bentonite, Water, Sodium Percarbonate, Sodium Polyacrylate, Silicate, Alkyl Sulfate, Nonanoyloxybenzenesulfonate, DTPA, Polyethylene Glycol 4000, Silicone, Ethoxylate, fragrance, Polyethylene Oxide, Palmitic Acid, Disodium Diaminostilbene Disulfonate, Protease, Liquitint™ Red, FD&C Blue 1, Cellulase.

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Ultra Tide with a Touch of Downy Clean Breeze: Water, sodium alcoholethoxy sulfate, MEA citrate, linear alkyl benzene sulfonate: sodium/MEA salts, propylene glycol, polyethyleneimine ethoxylate, ethanol, diethylene glycol, polyethyleneimine, propoxyethoxylate, diquaternium ethoxysulfate, alcohol sulfate, dimethicone, fragrance, borax, sodium fatty acids, DTPA, protease, sodium bisulfite, disodium diaminostilbene disulfonate, amylase, gluconase, castor oil, calcium formate, MEA, styrene acrylate copolymer, sodium formate, Liquitint™ Blue.

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Ultra Tide with Downy Sun Blossom: Water, sodium alcoholethoxy sulfate, MEA citrate, linear alkyl benzene sulfonate: sodium/MEA salts, propylene glycol, ethanol, diethylene glycol, polyethyleneimine propoxyethoxylate, polyethyleneimine ethoxylate, alcohol sulfate, dimethicone, fragrance, borax, sodium fatty acids, DTPA, protease, sodium bisulfite, disodium diaminostilbene disulfonate, amylase, castor oil, calcium formate, MEA, styrene acrylate copolymer, propanaminium propanamide, gluconase, sodium formate, Liquitint™ Blue.

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Ultra Tide with Downy April Fresh/ Sweet Dreams: Water, sodium alcoholethoxy sulfate, MEA citrate, linear alkyl benzene sulfonate: sodium/MEA salts, propylene glycol, polyethyleneimine ethoxylate, ethanol, diethylene glycol, polyethyleneimine propoxyethoxylate, diquaternium ethoxysulfate, alcohol sulfate, dimethicone, fragrance, borax, sodium fatty acids, DTPA, protease, sodium bisulfite, disodium diaminostilbene disulfonate, amylase, gluconase, castor oil, calcium formate, MEA, styrene acrylate copolymer, propanaminium propanamide, sodium formate, Liquitint™ Blue.

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Ultra Tide Free Powdered Detergent: Sodium Carbonate, Sodium Aluminosilicate, Alkyl Sulfate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Water, Sodium polyacrylate, Silicate, Ethoxylate, Sodium percarbonate, Polyethylene Glycol 4000, Protease, Disodium

5 Diaminostilbene Disulfonate, Silicone, Cellulase.

Ultra Tide Powdered Detergent, Clean Breeze/Spring Lavender/mountain Spring: Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Alkyl Sulfate, Sodium Percarbonate, Water, Sodium Polyacrylate, Silicate,

10 Nonanoyloxybenzenesulfonate, Ethoxylate, Polyethylene Glycol 4000, Fragrance, DTPA, Disodium Diaminostilbene Disulfonate, Palmitic Acid, Protease, Silicone, Cellulase.

Ultra Tide HE (high Efficiency) Powdered Detergent, Clean Breeze: Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Water,

15 Nonanoyloxybenzenesulfonate, Alkyl Sulfate, Sodium Polyacrylate, Silicate, Sodium Percarbonate, Ethoxylate, Polyethylene Glycol 4000, Fragrance, DTPA, Palmitic Acid, Disodium Diaminostilbene Disulfonate, Protease, Silicone, Cellulase.

Ultra Tide Coldwater Powdered Detergent, Fresh Scent: Sodium Carbonate, Sodium

20 Aluminosilicate, Sodium Sulfate, Sodium Percarbonate, Alkyl Sulfate, Linear Alkylbenzene Sulfonate, Water, Nonanoyloxybenzenesulfonate, Sodium Polyacrylate, Silicate, Ethoxylate, Polyethylene Glycol 4000, DTPA, Fragrance, Natalase, Palmitic Acid, Protease, Disodium, Diaminostilbene Disulfonate, FD&C Blue 1, Silicone, Cellulase, Alkyl Ether Sulfate.

Ultra Tide with bleach Powdered Detergent, Clean Breeze: Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Sodium Percarbonate, Nonanoyloxybenzenesulfonate, Alkyl Sulfate, Water, Silicate, Sodium Polyacrylate, Ethoxylate, Polyethylene Glycol 4000, Fragrance, DTPA, Palmitic Acid, Protease, Disodium Diaminostilbene Disulfonate, Silicone, FD&C Blue 1, Cellulase, Alkyl Ether Sulfate.

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Ultra Tide with Febreze Freshness™ Powdered Detergent, Spring Renewal: Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Sodium Percarbonate, Alkyl Sulfate, Water, Sodium Polyacrylate, Silicate, Nonanoyloxybenzenesulfonate, Ethoxylate, Polyethylene Glycol 4000, DTPA, Fragrance,

30 Cellulase, Protease, Disodium Diaminostilbene Disulfonate, Silicone, FD&C Blue 1.

Liquid Tide Plus with Febreze Freshness – Sport HE Active Fresh:

Water, Sodium alcoholethoxy sulfate, MEA citrate, linear alkylbenzene sulfonate, sodium salt, linear alkylbenzene sulfonate: MEA salt, alcohol ethoxylate, sodium fatty acids, propylene glycol, diethylene glycol, polyethyleneimine ethoxylate propoxylate, diquaternium ethoxysulfate, Ethanol, sodium cumene sulfonate, borax, fragrance, DTPA, Sodium bisulfate, disodium

5 diaminostilbene disulfonate, Mannanase, cellulase, amylase, sodium formate, calcium formate, Lauramine oxide, Liquitint™ Blue, Dimethicone / polydimethyl silicone.

Tide Plus Febreeze Freshness Spring & Renewal:

Water, sodium alcoholethoxy sulfate, linear alkyl benzene sulfonate: sodium/MEA salts, MEA

10 citrate, propylene glycol, polyethyleneimine ethoxylate, fragrance, ethanol, diethylene glycol, polyethyleneimine propoxyethoxylate, protease, alcohol sulfate, borax, sodium fatty acids, DTPA, disodium diaminostilbene disulfonate, MEA, mannanase, gluconase, sodium formate, dimethicone, Liquitint™ Blue, tetramine.

15 **Liquid Tide Plus with Febreeze Freshness, Sport HE Victory Fresh:** Water, Sodium alcoholethoxy sulfate, MEA citrate, linear alkylbenzene sulfonate, sodium salt, linear alkylbenzene sulfonate: MEA salt, alcohol ethoxylate, sodium fatty acids, propylene glycol, diethylene glycol, polyethyleneimine ethoxylate propoxylate, diquaternium ethoxysulfate, ethanol, sodium cumene sulfonate, borax, fragrance, DTPA, Sodium bisulfate, disodium

20 diaminostilbene disulfonate, Mannanase, cellulase, amylase, sodium formate, calcium formate, Lauramine oxide, Liquitint™ Blue, Dimethicone / polydimethyl silicone.

Tide Vivid White + Bright Powder, Original:

Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate,

25 Sodium Percarbonate, Nonanoyloxybenzenesulfonate, Alkyl Sulfate, Water, Silicate, Sodium Polyacrylate Ethoxylate, Polyethylene Glycol 4000, Fragrance, DTPA, Palmitic Acid, Protease, Disodium Diaminostilbene Disulfonate, Silicone, FD&C Blue 1, Cellulase, Alkyl Ether Sulfate.

Ingredient	Amount (in wt %)
Anionic deterative surfactant (such as alkyl benzene sulphonate, alkyl ethoxylated sulphate and mixtures)	from 8 wt % to 15 wt % thereof)
Non-ionic deterative surfactant (such as alkyl ethoxylated alcohol)	from 0.5 wt % to 4 wt %
Cationic deterative surfactant (such as quaternary ammonium compounds)	from 0 to 4 wt %
Other deterative surfactant (such as zwitterionic deterative surfactants, amphoteric surfactants and mixtures thereof)	from 0 wt % to 4 wt %

Carboxylate polymer (such as co-polymers of maleic acid and acrylic acid)	from 1 wt % to 4 wt %
Polyethylene glycol polymer (such as a polyethylene glycol polymer comprising poly vinyl acetate side chains)	from 0.5 wt % to 4 wt %
Polyester soil release polymer (such as Repel-o-tex from and/or Texcare polymers)	0.1 to 2 wt %
Cellulosic polymer (such as carboxymethyl cellulose, methyl cellulose and combinations thereof)	from 0.5 wt % to 2 wt %
Other polymer (such as amine polymers, dye transfer inhibitor polymers, hexamethylenediamine derivative polymers, and mixtures thereof)	from 0 wt % to 4 wt %
Zeolite builder and phosphate builder (such as zeolite 4A and/or sodium tripolyphosphate)	from 0 wt % to 4 wt %
Other builder (such as sodium citrate and/or citric acid)	from 0 wt % to 3 wt %
Carbonate salt (such as sodium carbonate and/or sodium bicarbonate)	from 15 wt % to 30 wt %
Silicate salt (such as sodium silicate)	from 0 wt % to 10 wt %
Filler (such as sodium sulphate and/or bio-fillers)	from 10 wt % to 40 wt %
Source of available oxygen (such as sodium percarbonate)	from 10 wt % to 20 wt %
Bleach activator (such as tetraacetyethylene diamine (TAED) and/or nonanoyloxybenzenesulphonate (NOBS))	from 2 wt % to 8 wt %
Bleach catalyst (such as oxaziridinium-based bleach catalyst and/or transition metal bleach catalyst)	from 0 wt % to 0.1 wt %
Other bleach (such as reducing bleach and/or pre- formed peracid)	from 0 wt % to 10 wt %
Chelant (such as ethylenediamine-N'N'-disuccinic acid (EDDS) and/or hydroxyethane diphosphonic acid(HEDP))	from 0.2 wt % to 1 wt %
Photobleach (such as zinc and/or aluminium sulphonated phthalocyanine)	from 0 wt % to 0.1 wt %
Hueing agent (such as direct violet 99, acid red 52, acid blue 80, direct violet 9, solvent violet 13 and any combination thereof)	from 0 wt % to 1 wt %
Brightener (such as brightener 15 and/or brightener 49)	from 0.1 wt % to 0.4 wt %

Protease (such as Savinase, Savinase Ultra, Purafect, FN3, FN4 and any combination thereof)	from 0.1 wt % to 0.4 wt %
Amylase (such as Termamyl, Termamyl ultra, Natalase, Optisize, Stainzyme, Stainzyme Plus, and any combination thereof)	from 0.05 wt % to 0.2 wt %
Cellulase (such as Carezyme and/or Celluclean)	from 0.05 wt % to 0.2 wt %
Lipase (such as Lipex, Lipolex, Lipoclean and any combination thereof)	from 0.2 to 1 wt %
Other enzyme (such as xyloglucanase, cutinase, pectate lyase, mannanase, bleaching enzyme)	from 0 wt % to 2 wt %
Fabric softener (such as montmorillonite clay and/or polydimethylsiloxane (PDMS))	from 0 wt % to 4 wt %
Flocculant (such as polyethylene oxide)	from 0 wt % to 1 wt %
Suds suppressor (such as silicone and/or fatty acid)	from 0 wt % to 0.1 wt %
Perfume (such as perfume microcapsule, spray-on perfume, starch encapsulated perfume accords, perfume loaded zeolite, and any combination thereof)	from 0.1 wt % to 1 wt %
Aesthetics (such as coloured soap rings and/or coloured speckles/noodles)	from 0 wt % to 1 wt %
Miscellaneous	balance

Ingredient	Amount
Carboxyl group-containing polymer (comprising from about 60% to about 70% by mass of an acrylic acid-based monomer (A); and from about 30% to about 40% by mass of a sulfonic acid group-containing monomer (B); and wherein the average molecular weight is from about 23,000 to about 50,000 preferably in the range of from about 25,000 to about 38,000 as described in WO2014032269.	from about 0.5 wt% to about 1.5 wt%
Amylase (Stainzyme Plus(R), having an enzyme activity of 14 mg active enzyme/ g)	from about 0.1wt% to about 0.5 wt%
Anionic deterative surfactant (such as alkyl benzene sulphonate, alkyl ethoxylated sulphate and mixtures thereof)	from about 8 wt% to about 15 wt%

Non-ionic deterative surfactant (such as alkyl ethoxylated alcohol)	from about 0.5 wt% to 4 wt%
Cationic deterative surfactant (such as quaternary ammonium compounds)	from about 0 wt% to about 4 wt%
Other deterative surfactant (such as zwitterionic deterative surfactants, amphoteric surfactants and mixtures thereof)	from about 0 wt% to 4 wt%
Carboxylate polymer (such as co-polymers of maleic acid and acrylic acid)	from about 1 wt% to about 4 wt%
Polyethylene glycol polymer (such as a polyethylene glycol polymer comprising poly vinyl acetate side chains)	from about 0 wt% to about 4 wt%
Polyester soil release polymer (such as Repel-O- Tex(R) and/or Texcare(R) polymers)	from about 0.1 wt% to about 2 wt%
Cellulosic polymer (such as carboxymethyl cellulose, methyl cellulose and combinations thereof)	from about 0.5wt% to about 2 wt%
Other polymer (such as amine polymers, dye transfer inhibitor polymers, hexamethylenediamine derivative polymers, and mixtures thereof)	from about 0 wt% to about 4 wt%
Zeolite builder and phosphate builder (such as zeolite 4A and/or sodium tripolyphosphate)	from about 0 wt% to about 4 wt%
Other builder (such as sodium citrate and/or citric acid)	from about 0 wt% to about 3 wt%
Carbonate salt (such as sodium carbonate and/or sodium bicarbonate)	from about 15 wt% to about 30 wt%
Silicate salt (such as sodium silicate)	from about 0 wt% to about 10 wt%
Filler (such as sodium sulphate and/or bio-fillers)	from about 10 wt% to about 40 wt%

Source of available oxygen (such as sodium percarbonate)	from about 10wt% to about 20 wt%
Bleach activator (such as tetraacetyethylene diamine (TAED) and/or nonanoyloxybenzenesulphonate (NOBS)	from about 2 wt% to about 8 wt%
Bleach catalyst (such as oxaziridinium-based bleach catalyst and/or transition metal bleach catalyst)	from about 0 wt% to about 0.1 wt%
Other bleach (such as reducing bleach and/or pre formed peracid)	from about 0 wt% to about 10 wt%
Chelant (such as ethylenediamine-N'N'-disuccinic acid (EDDS) and/or hydroxyethane diphosphonic acid (HEDP)	from about 0.2wt% to about 1wt%
Photobleach (such as zinc and/or aluminium sulphonated phthalocyanine)	from about 0 wt% to about 0.1 wt%
Hueing agent (such as direct violet 99, acid red 52, acid blue 80, direct violet 9, solvent violet 13 and any combination thereof)	from about 0 wt% to about 0.5 wt%
Brightener (such as brightener 15 and/or brightener 49)	from about 0.1wt% to about 0.4 wt%
Protease (such as Savinase, Polarzyme, Purafect, FN3, FN4 and any combination thereof, typically having an enzyme activity of from about 20 mg to about 100mg active enzyme/g)	from about 0.1wt% to about 1.5 wt%
Amylase (such as Termamyl(R), Termamyl Ultra(R), Natalase(R), Optisize HT Plus(R), Powerase(R), Stainzyme(R) and any combination thereof, typically having an enzyme activity of from about 10 mg to about 50 mg active enzyme/ g)	from about 0.05 wt% to about 0.2 wt%
Cellulase (such as Carezyme(R), Celluzyme(R) and/or Celluclean(R), typically having an enzyme activity of about from 10 to 50mg active enzyme/ g)	from about 0.05 wt% to 0.5 wt%
Lipase (such as Lipex(R), Lipolex(R), Lipoclean(R) and any combination thereof, typically having an enzyme activity of from about 10 mg to about 50 mg active enzyme/ g)	from about 0.2 wt% to about 1 wt%

Other enzyme (such as xyloglucanase (e.g., Whitezyme(R)), cutinase, pectate lyase, mannanase, bleaching enzyme, typically having an enzyme activity of from about 10 mg to about 50 mg active enzyme/g)	from 0 wt% to 2 wt%
Fabric softener (such as montmorillonite clay and/or polydimethylsiloxane (PDMS))	from 0 wt% to 15 wt%
Flocculant (such as polyethylene oxide)	from 0 wt% to 1 wt%
Suds suppressor (such as silicone and/or fatty acid)	from 0 wt% to 0.1wt%
Perfume (such as perfume microcapsule, spray-on perfume, starch encapsulated perfume accords, perfume loaded zeolite, and any combination thereof)	from 0.1 wt% to 1 wt%
Aesthetics (such as colored soap rings and/or colored speckles/noodles)	from 0 wt% to 1wt%
Miscellaneous	Balance

All enzyme levels expressed as rug active enzyme protein per 100 g detergent composition. Surfactant ingredients can be obtained from BASF, Ludwigshafen, Germany (Lutensol(R)); Shell Chemicals, London, UK; Stepan, Northfield, Ill, USA; Huntsman, Huntsman, Salt Lake City, Utah, USA; Clariant, Sulzbach, Germany (Praepagen(R)).

Sodium tripolyphosphate can be obtained from Rhodia, Paris, France. Zeolite can be obtained from Industrial Zeolite (UK) Ltd, Grays, Essex, UK. Citric acid and sodium citrate can be obtained from Jungbunzlauer, Basel, Switzerland. NOBS is sodium nonanoyloxybenzenesulfonate, supplied by Eastman, Batesville, Ark., USA.

TAED is tetraacetythylenediamine, supplied under the Peractive(R) brand name by Clariant GmbH, Sulzbach, Germany.

Sodium carbonate and sodium bicarbonate can be obtained from Solvay, Brussels, Belgium. Polyacrylate, polyacrylate/maleate copolymers can be obtained from BASF, Ludwigshafen, Germany.

Repel-O-Tex(R) can be obtained from Rhodia, Paris, France. Texcare(R) can be obtained from Clariant, Sulzbach, Germany. Sodium percarbonate and sodium carbonate can be obtained from Solvay, Houston, Tex., USA.

Sodium salt of Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer (EDDS) was supplied by Octel, Ellesmere Port, UK.

Hydroxy ethane di phosphonate (HEDP) was supplied by Dow Chemical, Midland, Mich., USA.

Enzymes Savinase(R), Savinase(R) Ultra, Stainzyme(R) Plus, Lipex(R), Lipolex(R),

Lipoclean(R), Celluclean(R), Carezyme(R), Natalase(R), Stainzyme(R), Stainzyme(R) Plus, Termamyl(R), Termamyl(R) ultra, and Mannaway(R) can be obtained from Novozymes, Bagsvaerd, Denmark.

Enzymes Purafect(R), FN3, FN4 and Optisize can be obtained from Genencor International Inc.,

5 Palo Alto, California, US.

Direct violet 9 and 99 can be obtained from BASF DE, Ludwigshafen, Germany.

Solvent violet 13 can be obtained from Ningbo Lixing Chemical Co., Ltd. Ningbo, Zhejiang, China.

10 Brighteners can be obtained from Ciba Specialty Chemicals, Basel, Switzerland.

Definitions

15 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.

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Biofilm: A biofilm may be produced by any group of microorganisms in which cells stick to each other on a surface, such as a textile, dishware or hard surface. 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.

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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 benefit 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.

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35 Biofilm producing bacteria can be found among the following species: Acinetobacter sp., Aeromicrobium sp., Brevundimonas sp., Microbacterium sp., Micrococcus luteus, Pseudomonas sp., Staphylococcus epidermidis, and Stenotrophomonas sp.

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.

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.

Color difference (L value): A Lab color space is a color-opponent space with dimension L for lightness. L value, L^* represents the darkest black at $L^* = 0$, and the brightest white at $L^* = 100$. In the context of the present invention L value is also referred to as color difference.

Control sequences: The term "control sequences" means nucleic acid sequences necessary for expression of a polynucleotide encoding a mature polypeptide of the present invention. Each control sequence may be native (*i.e.*, from the same gene) or foreign (*i.e.*, from a different gene) to the polynucleotide encoding the polypeptide or native or foreign to each other. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the polynucleotide encoding a polypeptide.

Detergent adjunct ingredient: The detergent adjunct ingredient is different to the enzyme of this invention. The precise nature of these additional adjunct components, and levels of incorporation thereof, will depend on the physical form of the composition and the nature of the operation for which it is to be used. Suitable adjunct materials include, but are not limited to the components described below such as surfactants, builders, flocculating aid, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, builders and co-builders, fabric hueing agents, anti-foaming agents, dispersants, processing aids, and/or pigments.

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. The detergent composition may be used to e.g. clean textiles 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; fabric fresheners; fabric softeners; and textile and laundry pre-spotters/pretreatment). In addition to containing the enzyme of the invention, the detergent formulation may contain one or more additional enzymes (such as proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidaes, haloperoxygenases, catalases and mannanases, 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.

Enzyme Detergency benefit: The term “enzyme detergency benefit” is defined herein as the advantageous effect an enzyme may add to a detergent compared to the same detergent without the enzyme. Important detergency benefits which can be provided by enzymes are stain removal with no or very little visible soils after washing and/or cleaning, prevention or reduction of redeposition of soils released in the washing process (an effect that also is termed anti-redeposition), restoring fully or partly the whiteness of textiles which originally were white but after repeated use and wash have obtained a greyish or yellowish appearance (an effect that also is termed whitening). Textile care benefits, which are not directly related to catalytic stain removal or prevention of redeposition of soils, are also important for enzyme detergency benefits. Examples of such textile care benefits are prevention or reduction of dye transfer from one fabric to another fabric or another part of the same fabric (an effect that is also termed dye transfer inhibition or anti-backstaining), removal of protruding or broken fibers from a fabric surface to decrease pilling tendencies or remove already existing pills or fuzz (an effect that also is termed anti-pilling), improvement of the fabric-softness, colour clarification of the fabric and removal of particulate soils which are trapped in the fibers of the fabric or garment. Enzymatic bleaching is a further enzyme detergency benefit where the catalytic activity generally is used to catalyse the formation of bleaching components such as hydrogen peroxide or other peroxides.

Emulsion: An emulsion is a temporary or permanent dispersion of one liquid phase within a second liquid phase. The second liquid is generally referred to as the continuous phase.

Surfactants are commonly used to aid in the formation and stabilization of emulsions. Not all surfactants are equally able to stabilize an emulsion. The type and amount of a surfactant needs to be selected for optimum emulsion utility especially with regard to preparation and physical stability of the emulsion, and stability during dilution and further processing. Physical stability refers to maintaining an emulsion in a dispersion form. Processes such as coalescence, aggregation, adsorption to container walls, sedimentation and creaming, are forms of physical instability, and should be avoided. Examples of suitable surfactants are described in WO 97/24177, page 19-21; and in WO 99/01534.

Expression: The term "expression" includes any step involved in the production of a polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Expression vector: The term "expression vector" means a linear or circular DNA molecule that comprises a polynucleotide encoding a polypeptide and is operably linked to control sequences that provide for its expression.

Fermentation broth: The term "fermentation broth" as used herein refers to a preparation produced by cellular fermentation that undergoes no or minimal recovery and/or purification. For example, fermentation broths are produced when microbial cultures are grown to saturation, incubated under carbon-limiting conditions to allow protein synthesis (e.g., expression of enzymes by host cells) and secretion into cell culture medium. The fermentation broth can contain unfractionated or fractionated contents of the fermentation materials derived at the end of the fermentation. Typically, the fermentation broth is unfractionated and comprises the spent culture medium and cell debris present after the microbial cells (e.g., filamentous fungal cells) are removed, e.g., by centrifugation. In some embodiments, the fermentation broth contains spent cell culture medium, extracellular enzymes, and viable and/or nonviable microbial cells.

Fragment: The term "fragment" means a polypeptide 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 endo-beta-1-6-galactanase activity.

Host cell: The term "host cell" means any cell type that is susceptible to transformation, transfection, transduction, or the like with a nucleic acid construct or expression vector comprising a polynucleotide of the present invention. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication.

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). An isolated substance may be present in a fermentation broth sample; e.g. a host cell may be genetically modified to express the polypeptide of the invention. The fermentation broth from that host cell will comprise the isolated polypeptide.

Laundrying: The term "laundrying" relates to both household laundrying and industrial laundrying and means the process of treating textiles with a solution containing a cleaning or detergent composition of the present invention. The laundrying process can for example be carried out using e.g. a household or an industrial washing machine or can be carried out by hand.

By the term "mal-odour" is meant an odour which is not desired on clean items. The cleaned item should smell fresh and clean without mal-odours adhered to the item. One example of mal-odour is compounds with an unpleasant smell, which may be produced by microorganisms. Another example is unpleasant smells can be sweat or body odour adhered to an item which has been in contact with human or animal. Another example of mal-odour can be the odour from spices, which sticks to items for example curry or other exotic spices which smells strongly.

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.

Mature polypeptide coding sequence: The term "mature polypeptide coding sequence" means a polynucleotide that encodes a mature polypeptide having endo-beta-1-6-galactanase activity.

Nucleic acid construct: The term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic, which comprises one or more control sequences.

Operably linked: The term “operably linked” means a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs expression of the coding sequence.

- 5 Sequence identity: The relatedness between two amino acid sequences or between two nucleotide 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 (EMBOSS: The European
10 Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), 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:
- 15
$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

- For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The
20 European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), 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:
- 25
$$(\text{Identical Deoxyribonucleotides} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment}).$$

Soil: The term “soil” means dirt or other unwanted matter, such as micro-organisms, found on the item to be washed. Soiled clothes are dirty clothes requiring washing.

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Stringency conditions:

- 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
35 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 2X SSC, 0.2% SDS at 45°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 2X SSC, 0.2% SDS at 50°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 2X SSC, 0.2% SDS at 55°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 2X SSC, 0.2% SDS at 60°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 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 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 endo-beta-1-6-galactanase activity

Textile: The term “textile” means any textile material including yarns, yarn intermediates, fibers, non-woven materials, natural materials, synthetic materials, and any other textile material, fabrics made of these materials and products made from fabrics (e.g., garments and other articles). The textile or fabric may be in the form of knits, wovens, denims, non-wovens, felts,

yarns, and toweling. The textile may be cellulose based such as natural cellulosics, including cotton, flax/linen, jute, ramie, sisal or coir or manmade cellulosics (e.g. originating from wood pulp) including viscose/rayon, cellulose acetate fibers (tricell), lyocell or blends thereof. The textile or fabric may also be non-cellulose based such as natural polyamides including wool, camel, cashmere, mohair, rabbit and silk or synthetic polymers such as nylon, aramid, polyester, acrylic, polypropylene and spandex/elastane, or blends thereof as well as blends of cellulose based and non-cellulose based fibers. Examples of blends are blends of cotton and/or rayon/viscose with one or more companion material such as wool, synthetic fiber (e.g. polyamide fiber, acrylic fiber, polyester fiber, polyvinyl chloride fiber, polyurethane fiber, polyurea fiber, aramid fiber), and/or cellulose-containing fiber (e.g. rayon/viscose, ramie, flax/linen, jute, cellulose acetate fiber, lyocell). Fabric may be conventional washable laundry, for example stained household laundry. When the term fabric or garment is used it is intended to include the broader term textiles as well. In the context of the present invention, the term "textile" also covers fabrics.

Variant: The term "variant" means a polypeptide having endo-beta-1-6-galactanase activity comprising an alteration, *i.e.*, a substitution, insertion, and/or deletion, at one or more (e.g., several) positions. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding an amino acid adjacent to and immediately following the amino acid occupying a position.

Wash cycle: The term "wash cycle" is defined herein as a washing operation wherein textiles are immersed in the wash liquor, mechanical action of some kind is applied to the textile in order to release stains and to facilitate flow of wash liquor in and out of the textile and finally the superfluous wash liquor is removed. After one or more wash cycles, the textile is generally rinsed and dried.

Wash liquor: The term "wash liquor" is defined herein as the solution or mixture of water and detergent components optionally including the enzyme of the invention.

Whiteness: The term "Whiteness" is defined herein as greying or yellowing of a textile. Greying and yellowing can be due to soil redeposition, body soils, colouring from e.g. iron and copper ions or dye transfer. Whiteness might include one or several issues from the list below: colourant or dye effects; incomplete stain removal (e.g. body soils, sebum etc.); redeposition (greying, yellowing or other discolourations of the object) (removed soils reassociate with other parts of textile, soiled or unsoiled); chemical changes in textile during application; and clarification or brightening of colours.

All percentages and ratios are calculated by weight (% w/w) unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated.

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

Examples

Examples embodying an aspect of the invention will now be described with reference to the following figures in which:

Figure 1 – MiniLoM test of endo-beta-1-6-galactanase showing results of 0.5, 2, 5 and 10 ppm on biofilm on cotton swatches when washed in dirty wash liquor, results shown in ΔY values.

Figure 2 – MiniLoM test of endo-beta-1-6-galactanase showing results of 0.25, 0.5, 1, 2, 5 and 10 ppm on biofilm on polyester-cotton swatches when washed in dirty wash liquor, results shown in ΔY values.

Example 1 - Cloning of GH30_5 from *Bacillus* sp

A bacterial strain was isolated from a soil sample obtained from United States in 2012. Chromosomal DNA from a pure culture was isolated by QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and subjected to full genome sequencing using Illumina technology. The assembled genome sequence and subsequent analysis of the 16S ribosomal subunit gene sequences against the ARB-SILVA database (The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Quast et al. Nucl. Acids Res. 41 (2013): D590-D596.) confirmed the identity of the strain as belonging to the *Bacillus* species.

The genome sequence was analyzed for protein sequences that have glycosyl hydrolase domains (*according to the CAZY definition*). One GH30_5 gene and corresponding sequence (SEQ ID NO: 1) was identified from the *Bacillus* sp.

The bacterial GH30_5 encoding gene was cloned by conventional techniques from *Bacillus* sp (SEQ ID NO: 8) and inserted into a *Bacillus* expression vector as described in WO 12/025577. The DNA encoding the mature peptide was cloned in frame to a *Bacillus clausii* secretion signal (BcSP; with the following amino acid sequence: MKKPLGKIVASTALLISVAFSSSIASA (SEQ ID NO: 4). BcSP replaced the native secretion signal in the gene.

Downstream of the BcSP sequence an affinity tag sequence was introduced to ease the purification process (His-tag; with the following amino acid sequence: HHHHHHPR (SEQ ID NO: 5) The gene that was expressed therefore comprised the BcSP sequence followed by the His-tag sequence followed by the mature wild type GH30_5 sequence (as shown in SEQ ID NO: 1).

The final expression plasmid (BcSP-His-tag-GH30_5) was transformed into a *Bacillus subtilis* expression host. The GH30_5 BcSP-fusion gene was integrated by homologous recombination into the *Bacillus subtilis* host cell genome upon transformation.

The gene construct was expressed under the control of a triple promoter system (as described in WO 99/43835). The gene coding for chloramphenicol acetyltransferase was used as maker (as described in (Diderichsen *et al.*, 1993, *Plasmid* 30: 312-315)). Transformants were selected on LB media agar supplemented with 6 microgram of chloramphenicol per ml. One recombinant *Bacillus subtilis* clone containing the GH30_5 expression construct was selected and was cultivated on a rotary shaking table in 500 ml baffled Erlenmeyer flasks each containing 100 ml yeast extract-based media. After 3-5 days cultivation time at 30 °C to 37°C, enzyme containing supernatants were harvested by centrifugation and the enzymes were purified by His-tag purification.

His tag purification method

His-tagged GH30_5 enzyme was purified by immobilized metal chromatography (IMAC) using Ni^{2+} as the metal ion on 5 mL HisTrap Excel columns (GE Healthcare Life Sciences). The purification took place at pH 7 and the bound proteins were eluted with imidazole. The purity of the purified enzymes was checked by SDS-PAGE and the concentration of each enzyme determined by Abs 280 nm after a buffer exchange in 20mM Tris pH 7.

Example 2 – Mini-LOM

This example shows the wash results from a MiniLOM test where Polyester/cotton (65 % Polyester and 45% cotton wfk 20A) is used to grow biofilm on. The Biofilm is made by *Brevundimonas vesicularis*.

Biofilm preparation:

The *Brevundimonas vesicularis* was taken from a -80 °C freeze culture and plated out on TSA Agar plates. After 3 days 4 tubes with 10 mL TSB (from Oxoid) was inoculated with bacteria culture from the plates. The TSB tubes were thoroughly mixed by whirl mixer before they were incubated overnight at 30 °C with shaking applied at 200 rpm.

The overnight culture was then collected in a 50 ml centrifuge tube and centrifuged at 3000 rpm for 5 min. the supernatant was discarded and the pellet was dissolved in 5 ml TSB per tube (20 ml in total). 150 ml of the culture was diluted 10X with 1350 µl 100% TSB in an Eppendorf tube. A 20X dilution was made by taking 500µl of the 10X dilution to 500 100% TSB in an Eppendorf tube. 4 x 100 µL of the 10x and 20x dilutions and blank (100% TSB) was added to a 96 brønd nunc micro titter plate.

The plate was measured at 600 nm on Fluostar Omega securing that one of the dilutions was below 0.6. The OD600 of the Overnight culture was determined and diluted to an OD of 0.03 in 50% TSB.

Prewashed Wfk 20A cotton (65 % Polyester and 45% cotton) was cut in circular swatches with a 2cm diameter and placed in autoclave bags and autoclaved at the "liquid program" on the Systec autoclave with 121°C in 15 min and then cooling down to 80°C before it is opened. Sterile 30A swatches were transferred with a sterile tweezer to each well in a 12 well micro titter plate. 1.62 ml of the diluted overnight culture was added to each of the wells. The swatches were incubated at 15°C with 70 rpm shaking applied on a Innova 2100 Platform shaker. After incubation in 48 h the swatches were rinsed 2x in 3 ml 0.9% NaCL. The swatches were used immediately after the rinse for the wash trial.

Pre-washed Wfk 20A is cut in circular swatches with a diameter of 2 cm. The swatches were marked by a sizzer cut to differentiate them from the 1 day biofilm swatches after the washes.

Preparation of "Dirty Detergent":

3.33 g Model A detergent was weighed in a weighing boat and 0.7 g 09V pigment soil was added on top of the Model A detergent. This was done in a fume hood using disposable gloves. The weighing boat was added to 1L of 15°dH water. The soil was dissolved at maximum speed for at least 10 minutes, to dissolve the soil completely.

Wash method:

A heating cabinet is heated to 30°C. 1 litre of Dirty detergent according to preparation method
5 above is used. Five 50 mL plastic tubes are used with each tube represented a wash. Five
circular (2 cm diameter) biofilm swatches were added to the beakers prepared according to the
Biofilm preparation method. Five circular (2 cm diameter) clean pieces of PO (tracers) were
added to each of the tubes.

10 Enzymes prepared in correct dilutions were prepared and kept on ice (0.25, 0.5, 1, 2, 5 and 10
ppm).

10 ml of "Dirty detergent" (see preparation method) was added to the first tube and enzymes
added immediately after. The lid was then added to the tube, and the tube was put on the Mini-
15 LOM rotator (Stuart Rotator SB3).

The rotation speed of MiniLOM rotator was 20rpm. When the first tube was placed on the Mini-
LOM rotator the time measurement of the wash was started. Dirty detergent and enzymes were
then added to the other tubes, and placed on the Rotator. When all the tubes were added to the
20 Rotator, it was placed the 30°C heating cabinet for 60 minutes from the time that the lid was
added to the first tube.

The rinse procedure was done by removing the rotator from the cabinet and placing it on the
operating table with the rotation on. Tube no 1 was taken and the wash water discarded with the
25 swatches left behind in the tubes. 20 ml of 15 °dH was added to the tube and it was added back
to the rotator. This was then done for all the test tubes in the trial. The rinse was done at ambient
room temperature for 10 minutes.

A second rinse was done repeating the above described steps. After the second wash the
30 swatches were removed from each of the tubes and added to a piece of paper and dried
overnight at room temperature in darkness.

Evaluation was done by photographing the swatches in Verivide and analyzed by the software
Digieye V2.62. The delta Y-values were used to present the data. Delta Y value is obtained by
35 subtracting the measured Y value of the blank (no enzyme) treated swatches from Y-values of
the enzyme treated swatches (Figure 1 and 2).

Table 1 wash results from MiniLOM test for GH30_5 (SEQ ID NO 1) on polyester/cotton (65 %

Polyester and 45% cotton) with biofilm.

Table 1

0.1 ppm	0.25 ppm	0.5 ppm	1 ppm	2 ppm	5 ppm	10 ppm
0	1	1	2	3	5	7

5 **Example 3 - β -1,6-galactanase activity of the purified GH30_5 enzyme**

β -1,6-galactanase activity of the purified GH30_5 enzyme was verified using (+)-arabinogalactan from larch wood (Aldrich) as substrate which contains galactan side chains with β -1,6 linkage. 10 μ g/ml purified enzyme was incubated with 0.1% larch wood arabinogalactan substrate in 18 mM 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris) buffer pH 7 in a total volume of 750 μ l in a 1.5 ml microcentrifuge tube at 37°C for 3 hours with 500 rpm agitation in a Thermomixer comfort (Eppendorf). The enzymatic reaction was terminated by heating the tube to 80°C for 20 minutes, likewise in a Thermomixer comfort (Eppendorf), whereafter the sample was cooled to room temperature. Two independent purified samples of the GH30_5 enzyme were tested and a blank reaction without GH30_5 enzyme was run in parallel.

The samples were analyzed for carbohydrate compounds using a Dionex ICS-3000 ion chromatography system (Thermo Scientific) with a CarboPac PA1 column and a ED electrochemical detector. The carbohydrates were eluted from the column at a flow rate of 0.8 ml/min with a gradient of sodium hydroxide from 15 mM to 85 mM and with a gradient of sodium acetate from 0 mM to 175 mM with the total length of the run of 20 minutes.

Treatment of the larch wood arabinogalactan leads to release of a galactobiose compound (DP2) which can be detected as an increase in the peak area of the peak corresponding to the DP2 compound. The peak area for the peak corresponding to the DP2 compound is listed in the table below for the reaction without GH30_5 enzyme and as an average for the reactions containing the purified GH30_5 enzyme. Incubating the larch wood arabinogalactan with GH30_5 enzyme leads to formation of more DP2 galactobiose compound than when the larch wood arabinogalactan is incubated without GH30_5 enzyme, which demonstrates that the GH30_5 has activity towards the arabinogalactan substrate and has β -1,6-galactanase activity.

Table. β -1,6-galactanase activity of the purified GH30_5 enzyme.

Sample	Enzyme concentration	DP2 peak area	Δ DP2 peak area (DP2 peak area _{sample} - DP2 peak area _{blank})

Without enzyme	GH30_5	0 µg/ml	8.8 nC*min	-
With enzyme	GH30_5	10 µg/ml	87.1 nC*min	78.3 nC*min

CLAIMS

- 5 1. A polypeptide having endo-beta-1-6-galactanase enzyme activity wherein the polypeptide:
- (i) has at least 60% sequence identity to the mature polypeptide shown in SEQ ID NO: 2;
 - (ii) is encoded by a polynucleotide that hybridizes under low stringency conditions
10 with
 - (a) the mature polypeptide coding sequence shown in SEQ ID NO: 8
 - (b) the cDNA sequence thereof, or
 - (c) the full-length complement of (i) or (ii);
 - (iii) is encoded by a polynucleotide having at least 60% sequence identity to the
15 mature polypeptide coding sequence shown in SEQ ID NO: 8 or the cDNA sequence thereof;
 - (iv) is a variant of the mature polypeptide shown in SEQ ID NO: 2 comprising a substitution, deletion, and/or insertion at one or more positions; or
 - (v) is a fragment of the polypeptide of (i), (ii), (iii), or (iv) that has endo-beta-1-6-
20 galactanase activity.
2. A polypeptide according to claim 1 wherein the polypeptide is an endo-beta-1-6-galactanase enzyme.
- 25 3. A polypeptide according to claim 1 or 2 wherein the polypeptide is derived from a bacterial source.
4. A polypeptide according to any previous claim wherein the polypeptide is derived from *Bacillus*.
- 30 5. A polypeptide according to any previous claim wherein the polypeptide is produced recombinantly.
6. A detergent composition comprising a polypeptide having endo-beta-1-6-galactanase
35 enzyme activity.
7. A detergent composition according to claim 6 wherein the polypeptide is an endo-beta-1-6-galactanase enzyme.

8. A detergent composition according to claim 6 or 7 wherein the polypeptide is derived from a bacterial source.
- 5 9. A detergent composition according to any of claims 6 to 8 wherein the polypeptide is derived from *Bacillus*.
10. A detergent composition according to any of claims 6 to 9 wherein the polypeptide is produced recombinantly.
- 10 11. A detergent composition according to any of claims 6 to 10 wherein the polypeptide:
- (i) has at least 60% sequence identity to the mature polypeptide shown in SEQ ID NO: 2;
 - (ii) is encoded by a polynucleotide that hybridizes under low stringency conditions with
 - 15 (a) the mature polypeptide coding sequence shown in SEQ ID NO: 8
 - (b) the cDNA sequence thereof, or
 - (c) the full-length complement of (i) or (ii);
 - (iii) is encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence shown in SEQ ID NO: 8 or the cDNA sequence thereof;
 - (iv) is a variant of the mature polypeptide shown in SEQ ID NO: 2 comprising a substitution, deletion, and/or insertion at one or more positions; and
 - (v) is a fragment of the polypeptide of (i), (ii), (iii), or (iv) that has endo-beta-1-6-galactanase activity.
- 25
12. A detergent composition according to any of claims 6 to 11 wherein the polypeptide is present at a concentration of 0.1 to 10 ppm, preferably 0.01-1ppm.
- 30 13. A detergent composition according to any of claims 6 to 12 further comprising at least one detergent adjunct ingredient.
14. A detergent composition according to claim 13, wherein the detergent adjunct ingredient is selected from the group consisting of surfactants, builders, flocculating aid, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric
- 35

softeners, carriers, hydrotropes, builders and co-builders, fabric huing agents, anti-foaming agents, dispersants, processing aids, and/or pigments.

- 5 15. A detergent composition according to any of claims 6 to 14 wherein the composition is for use in washing an item.
- 10 16. A detergent composition according to any of claims 6 to 15 wherein the composition is for use in preventing, reducing or removing one or more of:
- (a) stickiness from an item,
 - (b) adherence of soil to an item,
 - (c) deposition or re-deposition of soil on an item during a wash cycle; and/or
 - (d) mal-odour of an item.
- 15 17. A detergent composition according to claim 16 wherein the soil is a pigment soil.
18. A detergent composition according to any previous claim wherein the composition is for use in pre-treating stains on an item.
- 20 19. A detergent composition according to any of claims 6 to 18 wherein the composition is for use in maintaining or improving whiteness of an item.
20. A detergent composition according to any of claims 6 to 19 wherein the item is a textile.
- 25 21. A detergent composition according to any of claims 6 to 20, wherein said detergent composition is a liquid detergent composition or a powder detergent composition.
22. Use of either (i) a polypeptide as defined in claims 1 to 5 or (ii) a detergent composition as defined in claim 6 to 21, in the washing of an item.
- 30 23. A method of washing an item comprising the steps of:
- a. Exposing an item to either (i) a polypeptide as defined in claims 1 to 5 or (ii) a detergent composition as defined in claim 6 to 21;
 - b. Completing at least one wash cycle; and
 - 35 c. Optionally rinsing the item.
24. A method according to claim 23 wherein the item is a textile.

25. A method according to claim 23 or 24 wherein the wash cycle is performed at a temperature of 40°C or less, or more preferably at a temperature of 30°C or less, or even more preferably at a temperature of 20°C or less.
- 5 26. An item washed according to the method of any of claims 23 to 25.
27. A polypeptide having endo-beta-1-6-galactanase activity substantially as described herein with reference to the examples and figures.
- 10 28. A detergent composition substantially as described herein with reference to the examples and figures.
29. A use of (i) a polypeptide having endo-beta-1-6-galactanase activity or (ii) a detergent composition comprising a polypeptide having endo-beta-1-6-galactanase activity
15 substantially as described herein with reference to the examples and figures.
30. A method of washing an item substantially as described herein with reference to the examples and figures.

20

1/2

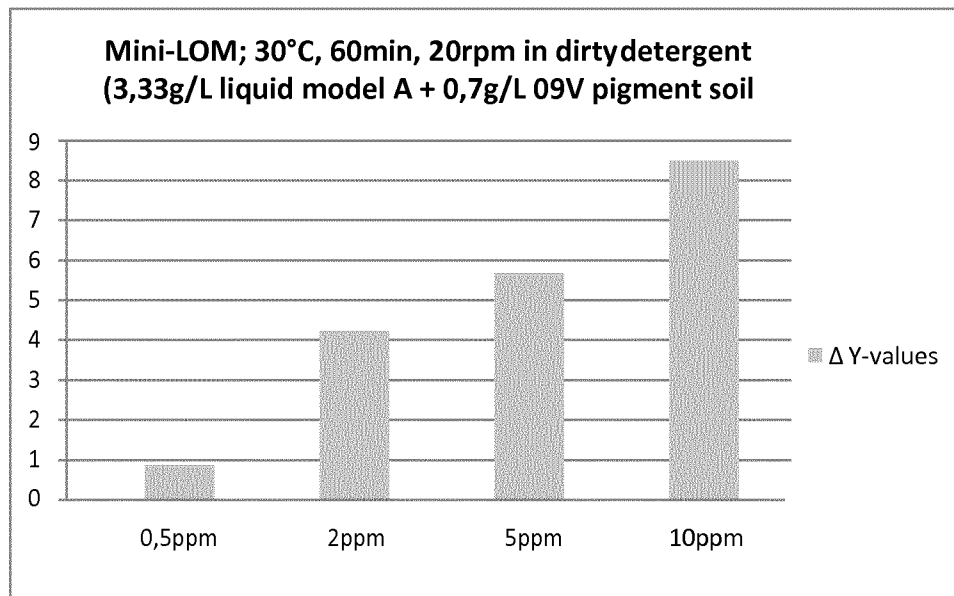


Figure 1

2/2

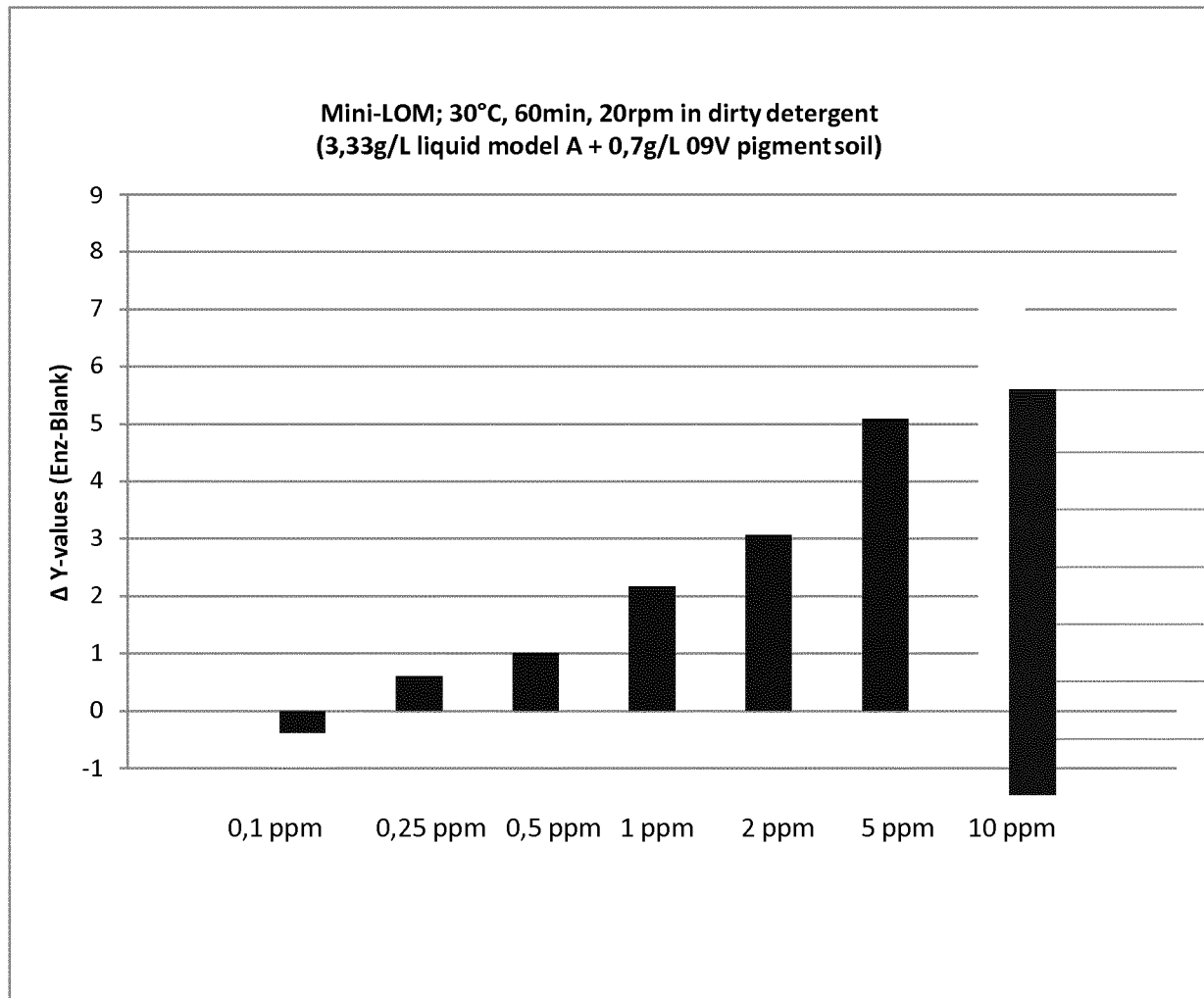


Figure 2

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/067054

A. CLASSIFICATION OF SUBJECT MATTER

INV. C11D3/386 C12N9/38
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)

C11D C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/185689 A1 (NOVOZYMES AS [DK]) 10 December 2015 (2015-12-10) claims 1-21; examples 1-8 -----	1-30
X	DATABASE REfseq [Online] ncbi; 20 August 2015 (2015-08-20), "hypothetical protein [Bacillus sp. SA1-12].", XP002774556, retrieved from NCBI Database accession no. WP_052737847 sequence -----	1-5,8,9, 11
A	WO 00/47711 A2 (NOVO NORDISK AS [DK]) 17 August 2000 (2000-08-17) claims 1-40; figure 1; examples 1-3 -----	1-30



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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"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

17 October 2017

Date of mailing of the international search report

26/10/2017

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/067054

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015185689 A1	10-12-2015	CN 106414698 A	15-02-2017
		EP 3152290 A1	12-04-2017
		WO 2015185689 A1	10-12-2015
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WO 0047711 A2	17-08-2000	AU 2535600 A	29-08-2000
		WO 0047711 A2	17-08-2000
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