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(54) Title: CLEANING COMPOSITIONS AND USES THEREOF

(57) Abstract: The present invention relates to compositions such as cleaning compositions comprising a mix of enzymes. The invention further relates, use of compositions comprising such enzymes in cleaning processes.



**CLEANING COMPOSITIONS AND USES THEREOF****Reference to a Sequence Listing**

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

**Background of the Invention**

The present invention relates to compositions such as cleaning compositions comprising  
a mix of enzymes. The invention further relates, use of compositions comprising such enzymes  
10 in cleaning processes.

**Description of the Related Art**

Enzymes have been used in detergents for decades. Usually a cocktail of various  
enzymes is added to detergent compositions. The enzyme cocktail often comprises various  
15 enzymes, wherein each enzyme targets its specific substrate e.g. amylases are active towards  
starch stains, proteases on protein stains and so forth. Textiles surface and hard surfaces, such  
as dishes or the inner space of a laundry machine enduring a number of wash cycles, become  
soiled with many different types of soiling which may compose of proteins, grease, starch etc.  
One type of soiling such as biofilm, EPS, etc. composes different molecules such as  
20 polysaccharides, extracellular DNA (eDNA), and proteins. Some soiling composes an  
extracellular polymeric matrix, which may be sticky or glueing, which when present on textile,  
attracts soils and may cause redeposition or backstaining of soil resulting in a greying of the  
textile. Additionally, e.g. biofilm often cause malodor issues. There is still a need for cleaning  
compositions, which effectively prevent, reduce or remove components of organic soiling, an  
25 effect described in the present application as "deep cleaning". The present invention provides  
new compositions fulfilling such need.

**Summary of the Invention**

The present invention relates to cleaning compositions in particular to cleaning compositions  
30 comprising at least 0.001 ppm DNase, at least 0.001 ppm haloperoxidase and a cleaning  
component, wherein the cleaning component is selected from the group consisting of:

- a. from about 0.1 to about 60 wt%, e.g. 0.1 to 15 wt% of at least one surfactant;
- b. from about 1 to about 50 wt% e.g. 0.5 to 20 wt% of at least one builder; and
- c. from about 1 to about 20 wt% 0.01 to 10 wt% of at least one bleach component

35 The invention further relates to the use of a composition for deep cleaning of an item, wherein  
the item is a textile or a surface. The invention further relates to a method of formulating a cleaning  
composition comprising adding a DNase, a haloperoxidase and at least one cleaning component.

The invention further relates to a kit intended for deep cleaning, wherein the kit comprises a solution of an enzyme mixture comprising a DNase, haloperoxidase and optionally a protease. The invention further relates to a method of deep cleaning of an item, comprising the steps of: a) contacting the item with a solution comprising an enzyme mixture comprising a DNase and a haloperoxidase, a source of peroxide; and a cleaning component, wherein the cleaning component is selected from about 0.1 to about 60 wt%, e.g. 0.1 to 15 wt% of at least one a surfactant; from about 1 to about 50 wt% e.g. 0.5 to 20 wt% of at least one builder; from about 1 to about 20 wt% 0.01 to 10 wt% of at least one bleach component; and b) and optionally rinsing the item, wherein the item is preferably a textile.

### Detailed Description of the Invention

Various enzymes are applied in cleaning processes each targeting specific types of soiling such as protein, starch and grease soiling. Enzymes are now standard ingredients in detergents for laundry and dish wash. The effectiveness of these commercial enzymes provides detergents which removes much of the soiling. However, components of organic matters such as EPS (extracellular polymeric substance) comprised in much biofilm constitute a challenging type of stains due to the complex nature of such organic matters. None of the commercially available cleaning compositions effectively remove or reduce EPS and/or biofilm related stains. Biofilm may be produced when a group of microorganisms' cells stick to each other or stick to a surface, such as a textile, dishware or hard surface or another kind of surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS), which constitute 50% to 90% of the biofilm's total organic matter. EPS is mostly composed of polysaccharides (exopolysaccharides) and proteins, but include other macro-molecules such as eDNA, lipids and other organic substances. Organic matter like biofilm may be sticky or glueing, which when present on textile, may give rise to redeposition or backstaining of soil resulting in a greying of the textile. Another drawback of organic matter e.g. biofilm is the malodor as various malodor related molecules are often associated with organic matter e.g. biofilm. Further, when dirty laundry items are washed together with less dirty laundry items the dirt present in the wash liquor tend to stick to organic matter e.g. biofilm or biofilm components as a result, hereof the laundry item is more "soiled" after wash than before wash. This is effect may also be termed re-deposition.

The composition of the invention is preferably a cleaning composition; the composition comprises at least one DNase and at least one haloperoxidase. Examples of useful DNases and haloperoxidases are mentioned below in the sections "Polypeptides having DNase activity" and "Polypeptides having haloperoxidase activity" respectively.

The compositions of the invention comprise a blend of a DNase and a haloperoxidase and are effective in reducing or removing organic components and soiling from organic matter.

Polypeptides having DNase activity

The term "DNase" means a polypeptide with DNase activity that catalyzes the hydrolytic cleavage of phosphodiester linkages in a DNA backbone, thus degrading DNA. The term "DNases" and the expression "a polypeptide with DNase activity" are used interchangeably throughout the application. The DNase activity may be determined according to the procedure described in the Assay 1. Preferably the nuclease is selected from any of the enzyme classes E.C. 3.1.21.X, where X = 1, 2, 3, 4, 5, 6, 7, 8 or 9, e.g. Deoxyribonuclease I, Deoxyribonuclease IV, Type I site-specific deoxyribonuclease, Type II site-specific deoxyribonuclease, Type III site-specific deoxyribonuclease, CC-preferring endo-deoxyribonuclease, Deoxyribonuclease V, T(4) deoxyribonuclease II, T(4) deoxyribonuclease IV or E.C. 3.1.22.Y where Y = 1, 2, 4 or 5, e.g. Deoxyribonuclease II, *Aspergillus* deoxyribonuclease K(1), Crossover junction endo-deoxyribonuclease, Deoxyribonuclease X.

The DNase may be obtainable from *Bacillus* e.g. *Bacillus*, such as a *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus* sp-62451, *Bacillus horikoshii*, *Bacillus* sp-62451, *Bacillus* sp-16840, *Bacillus* sp-62668, *Bacillus* sp-13395, *Bacillus horneckiae*, *Bacillus* sp-11238, *Bacillus cibi*, *Bacillus idriensis*, *Bacillus* sp-62520, *Bacillus* sp-16840, *Bacillus* sp-62668, *Bacillus algicola*, *Bacillus vietnamensis*, *Bacillus hwajinpoensis*, *Bacillus indicus*, *Bacillus marisflavi*, *Bacillus luciferensis*, *Bacillus* sp. SA2-6.

The DNase may also be obtained from any of the following *Pyrenochaetopsis* sp., *Vibrissea flavovirens*, *Setosphaeria rostrate*, *Endophragmiella valdina*, *Corynespora cassicola*, *Paraphoma* sp. XZ1965, *Monilinia fructicola*, *Curvularia lunata*, *Penicillium reticulisporum*, *Penicillium quercetorum*, *Setosphaeria* sp., *Alternaria*, *Alternaria* sp. XZ2545, *Trichoderma reesei*, *Chaetomium thermophilum*, *Scytalidium thermophilum*, *Metapochonia suchlasporia*, *Daldinia fissa*, *Acremonium* sp. XZ2007, *Acremonium* sp. XZ2414, *Acremonium dichromosporum*, *Sarocladium* sp. XZ2014, *Metarhizium* sp. HNA15-2, *Isaria tenuipes*, *Scytalidium circinatum*, *Metarhizium lepidiotae*, *Thermobispora bispora*, *Sporormia fimetaria*, *Pycnidophora cf. dispersa*, Environmental sample D, Environmental sample O, *Clavicipitaceae* sp-70249, *Westerdykella* sp. AS85-2, *Humicolopsis cephalosporioides*, *Neosartorya massa*, *Roussoella intermedia*, *Pleosporales*, *Phaeosphaeria* or *Didymosphaeria* *futilis*.

The DNases to be used in a composition of the invention preferable belong to the NUC1 group of DNases. The NUC1 group of DNases comprises polypeptides which in addition to having DNase activity, may comprise one or more of the motifs [T/D/S][G/N]PQL (SEQ ID NO 69), [F/L/Y/I]A[N/R]D[L/I/P/V] (SEQ ID NO: 70), or C[D/N]T[A/R] (SEQ ID NO: 71). One embodiment of the invention relates to a composition comprising polypeptides having DNase activity, wherein the polypeptides comprises one or more of the motifs [T/D/S][G/N]PQL (SEQ ID NO 69), [F/L/Y/I]A[N/R]D[L/I/P/V] (SEQ ID NO: 70) or C[D/N]T[A/R] (SEQ ID NO: 71).

The DNases preferably comprises a NUC1\_A domain [D/Q][IA/DH] (SEQ ID NO 72). In addition to comprising any of the domains [T/D/S][G/N]PQL, [F/L/Y/I]A[N/R]D[L/I/P/V] or C[D/N]T[A/R] the polypeptides having DNase activity, to be used in a composition of the invention, belongs to the NUC1\_A domain and may share the common motif [D/Q][IA/DH] (SEQ ID NO 72). One embodiment the invention relates to compositions comprising polypeptides, which comprises one or more motifs selected from the motifs [T/D/S][G/N]PQL, [F/L/Y/I]A[N/R]D[L/I/P/V], C[D/N]T[A/R] and [D/Q][IA/DH], wherein the polypeptides have DNase activity. The DNases to be added to a composition of the invention preferably belong to the group of DNases comprised in the GYS-clade, which are NUC1 and NUC1\_A DNases further comprising the conservative motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 73) or ASXNRSKG (SEQ ID NO: 74) and which share similar structural and functional properties. The DNases of the GYS-clade are preferably obtained from *Bacillus* genus. One embodiment of the invention relates to a composition comprising a polypeptide of the GYS clade having DNase activity, optionally wherein the polypeptide comprise one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 73), ASXNRSKG (SEQ ID NO: 74) and wherein the polypeptide is selected from the group of polypeptides:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 1,
- b) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 2,
- c) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 3,
- d) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 4,
- e) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 5,
- f) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 6,
- g) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 7,

- 5

- t) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 20,
- u) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 21,
- v) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 22,
- w) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 23,
- x) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 24, and
- y) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 25.

Polypeptides having DNase activity and which comprise the GYS-clade motifs have shown particularly good deep cleaning properties e.g. the DNases are particularly effective in removing or reducing components of organic matter, such as biofilm, from an item such as a textile or a hard surface.

In one embodiment the DNases to be added in a composition of the invention preferably belong to the group of DNases comprised in the NAWK-clade, which are NUC1 and NUC1\_A DNases further comprising the conservative motifs [V/I]PL[S/A]NAWK (SEQ ID NO: 75) or NPQL (SEQ ID NO: 76).

One embodiment of the invention relates to a composition comprising a polypeptide of the NAWK-clade having DNase activity, optionally wherein the polypeptide comprise one or both of the motifs [V/I]PL[S/A]NAWK (SEQ ID NO: 75) or NPQL (SEQ ID NO: 76) and wherein the polypeptide is selected from the group of polypeptides:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 26,
- b) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 27,

- c) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 28,
- d) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 29,
- e) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 30,
- f) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 31,
- g) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 32,
- h) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 33,
- i) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 34,
- j) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 35,
- k) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 36,
- l) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 37, and
- m) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 38.

Polypeptides having DNase activity and which comprise the NAWK-clade motifs have shown particularly good deep cleaning properties e.g. the DNases are particularly effective in removing or reducing components of organic matter, such as biofilm, from an item such as a textile or a hard surface.



The DNases to be added in a composition of the invention preferably belong to the group of DNases comprised in the KNAW-clade, which are NUC1 and NUC1\_A DNases further comprising the conservative motifs P[Q/E]L[W/Y] (SEQ ID NO: 77) or [K/H/E]NAW (SEQ ID NO: 78).

One embodiment of the invention relates to a composition comprising a polypeptide of the KNAW clade having DNase activity, optionally wherein the polypeptide comprise one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 77) or [K/H/E]NAW (SEQ ID NO: 78), and wherein the polypeptide is selected from the group of polypeptides:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 39,
- b) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 40,
- c) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 41,
- d) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 42,
- e) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 43
- f) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 44,
- g) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 45,
- h) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 46,
- i) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 47,

- j) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 48,
- k) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 49,
- l) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 50, and
- m) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 51.

Polypeptides having DNase activity and which comprise the KNAW-clade motifs have shown particularly good deep cleaning properties e.g. the DNases are particularly effective in removing or reducing components of organic matter, such as biofilm, from an item such as a textile or a hard surface.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-62451 and having a sequence identity to the polypeptide shown in SEQ ID NO: 1 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 1.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus horikoshii* and having a sequence identity to the polypeptide shown in SEQ ID NO: 2 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-62520 and having a sequence identity to the polypeptide shown in SEQ ID NO: 3 of at least 60%, e.g., at least 65%,

at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 3.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-62520 and having a sequence identity to the polypeptide shown in SEQ ID NO: 4 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 4.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus horikoshii* and having a sequence identity to the polypeptide shown in SEQ ID NO: 5 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 5.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus horikoshii* and having a sequence identity to the polypeptide shown in SEQ ID NO: 6 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 6.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-16840 and having a sequence identity to the polypeptide shown in SEQ ID NO: 7 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect,

the polypeptides 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: 7.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-16840 and having a sequence identity to the polypeptide shown in SEQ ID NO: 8 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 8.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-62668 and having a sequence identity to the polypeptide shown in SEQ ID NO: 9 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 9.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-13395 and having a sequence identity to the polypeptide shown in SEQ ID NO: 10 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 10.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* *horneckiae* and having a sequence identity to the polypeptide shown in SEQ ID NO: 11 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 11.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-1 1238 and having a sequence identity to the polypeptide shown in SEQ ID NO: 12 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 12.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus cibi* and having a sequence identity to the polypeptide shown in SEQ ID NO: 13 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 13.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp-18318 and having a sequence identity to the polypeptide shown in SEQ ID NO: 14 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 14.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus idriensis* and having a sequence identity to the polypeptide shown in SEQ ID NO: 15 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 15.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus algicola* having a sequence identity to the polypeptide shown in SEQ ID NO: 16 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 16.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from Environmental sample J and having a sequence identity to the polypeptide shown in SEQ ID NO: 17 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 17.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus vietnamensis* and having a

sequence identity to the polypeptide shown in SEQ ID NO: 18 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 18.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus hwajinpoensis* and having a sequence identity to the polypeptide shown in SEQ ID NO: 19 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 19.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Paenibacillus mucilaginosus* and having a sequence identity to the polypeptide shown in SEQ ID NO: 20 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 20.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus indicus* and having a sequence identity to the polypeptide shown in SEQ ID NO: 21 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 21.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus marisflavi* and having a sequence identity to the polypeptide shown in SEQ ID NO: 22 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 22.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus luciferensis* and having a sequence identity to the polypeptide shown in SEQ ID NO: 23 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 95%, at least 96%,

at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 23.

5 In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus marisflavi* and having a sequence identity to the polypeptide shown in SEQ ID NO: 24 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 24.

10 In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus* sp. SA2-6 and having a sequence identity to the polypeptide shown in SEQ ID NO: 25 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 25.

15 In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Pyrenochaetopsis* sp. and having a sequence identity to the polypeptide shown in SEQ ID NO: 26 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 26.

20 In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Vibrissea flavovirens* and having a sequence identity to the polypeptide shown in SEQ ID NO: 27 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 27.

25 In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Setosphaeria rostrate* and having a sequence identity to the polypeptide shown in SEQ ID NO: 28 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 28.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Endophragmiella valdina* and having a sequence identity to the polypeptide shown in SEQ ID NO: 29 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 29.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Corynespora cassicola* and having a sequence identity to the polypeptide shown in SEQ ID NO: 30 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 30.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Paraphoma* sp. XZ1965 and having a sequence identity to the polypeptide shown in SEQ ID NO: 31 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 31.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Monilinia fructicola* and having a sequence identity to the polypeptide shown in SEQ ID NO: 32 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 32.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Curvularia lunata* and having a sequence identity to the polypeptide shown in SEQ ID NO: 33 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 33.



In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Penicillium reticulisporum* and having a sequence identity to the polypeptide shown in SEQ ID NO: 34 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 34.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Penicillium quercetorum* and having a sequence identity to the polypeptide shown in SEQ ID NO: 35 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 35.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Setophaeosphaeria* sp. and having a sequence identity to the polypeptide shown in SEQ ID NO: 36 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 36.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Alternaria* sp. XZ2545 and having a sequence identity to the polypeptide shown in SEQ ID NO: 37 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 37.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Alternaria* and having a sequence identity to the polypeptide shown in SEQ ID NO: 38 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 38.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Trichoderma reesei* and having a sequence identity to the polypeptide shown in SEQ ID NO: 39 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 39.

5 In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Chaetomium thermophilum* and having a sequence identity to the polypeptide shown in SEQ ID NO: 40 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides differ  
10 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: 40.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Scytalidium thermophilum* and having a sequence identity to the polypeptide shown in SEQ ID NO: 41 of at least 60%, e.g., at least 65%, at least 70%, at least  
15 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 41.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Metapochonia suchlasporia* and having a sequence identity to the polypeptide shown in SEQ ID NO: 42 of at least 60%, e.g., at least 65%, at least 70%, at least  
20 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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  
25 in SEQ ID NO: 42.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Daldinia fissa* and having a sequence identity to the polypeptide shown in SEQ ID NO: 43 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least  
30 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 43.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Acremonium* sp. XZ2007 and having a sequence identity to the polypeptide shown in SEQ ID NO: 44 of at least 60%, e.g., at least 65%, at least 70%, at least  
35 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 44.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Acremonium dichromosporum* and having a sequence identity to the polypeptide shown in SEQ ID NO: 45 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 45.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Sarocladium* sp. XZ2014 and having a sequence identity to the polypeptide shown in SEQ ID NO: 46 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 46.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Metarhizium* sp. HNA15-2 and having a sequence identity to the polypeptide shown in SEQ ID NO: 47 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 47.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Acremonium* sp. XZ2414 and having a sequence identity to the polypeptide shown in SEQ ID NO: 48 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 48.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Isaria tenuipes* and having a sequence identity to the polypeptide shown in SEQ ID NO: 49 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 49.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Scytalidium circinatum* and having a sequence identity to the polypeptide shown in SEQ ID NO: 50 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 50.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Metarhizium lepidotae* and having a sequence identity to the polypeptide shown in SEQ ID NO: 51 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 51.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Thermobispora bispora* and having a sequence identity to the polypeptide shown in SEQ ID NO: 52 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 52.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Sporormia fimetaria* and having a sequence identity to the polypeptide shown in SEQ ID NO: 53 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 53.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Pycnidiophora cf. dispersa* and having a sequence identity to the polypeptide shown in SEQ ID NO: 54 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 54.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from Environmental sample D and having a sequence identity to the

polypeptide shown in SEQ ID NO: 55 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 55.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from Environmental sample O and having a sequence identity to the polypeptide shown in SEQ ID NO: 56 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 56.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Clavicipitaceae* sp-70249 and having a sequence identity to the polypeptide shown in SEQ ID NO: 57 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 57.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Westerdykella* sp. AS85-2 and having a sequence identity to the polypeptide shown in SEQ ID NO: 58 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 58.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Humicolopsis cephalosporioides* and having a sequence identity to the polypeptide shown in SEQ ID NO: 59 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 59.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Neosartorya massa* and having a sequence identity to the polypeptide shown in SEQ ID NO: 60 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 95%, at least 96%, at least 97%, at least

98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 60.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Roussoella intermedia* and having a sequence identity to the polypeptide shown in SEQ ID NO: 61 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 61.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Pleosporales* and having a sequence identity to the polypeptide shown in SEQ ID NO: 62 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 62.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Phaeosphaeria* and having a sequence identity to the polypeptide shown in SEQ ID NO: 63 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 63.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Didymosphaeria futilis* and having a sequence identity to the polypeptide shown in SEQ ID NO: 64 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 64.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus licheniformis* having a sequence identity to the polypeptide shown in SEQ ID NO: 65 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect,

the polypeptides 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: 65.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Bacillus* e.g. obtainable from *Bacillus subtilis* having a sequence identity to the polypeptide shown in SEQ ID NO: 66 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 66.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Aspergillus* e.g. obtainable from *Aspergillus oryzae* having a sequence identity to the polypeptide shown in SEQ ID NO: 67 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 67.

In some embodiments, the present invention relates to compositions comprising a polypeptide obtainable from *Trichoderma* e.g. obtainable from *Trichoderma harzianum* having a sequence identity to the polypeptide shown in SEQ ID NO: 68 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity. In one aspect, the polypeptides 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: 68.

The DNases above may be combined with a haloperoxidase as described below to form a blend to be added to a composition according to the invention.

#### Polypeptides having Haloperoxidase activity

The haloperoxidases suitable for being incorporated in the cleaning composition of the invention include chloroperoxidases and bromoperoxidases compounds exhibiting chloroperoxidase or bromoperoxidase activity. Haloperoxidases form a class of enzymes that are capable of oxidizing halides (Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>) in the presence of hydrogen peroxide or a hydrogen peroxide generating system to the corresponding hypohalous acids or hypohalites. For purposes of the present invention, haloperoxidase activity is determined according to the procedure described in the Assay II. Haloperoxidases are classified according to their specificity for halide ions. Chloroperoxidases catalyze formation of hypochlorite from chloride ions, hypobromite from bromide ions and hypoiodite from iodide ions; and bromoperoxidases catalyze formation of hypobromite from bromide ions and hypoiodite from iodide ions. Hypoiodite, however, with iodide

disproportionates to form elemental iodine and thus iodine is the observed product. The hypohalite compounds may subsequently react with other compounds forming halogenated compounds. In one aspect, the haloperoxidase is selected from the group consisting of: a chloride peroxidase (EC 1.11.1.10), a bromide peroxidase (EC 1.11.1.18) and an iodide peroxidase (EC 1.11.1.8). In a preferred embodiment, the haloperoxidase is a chloroperoxidase.

Haloperoxidases have been isolated from various organisms: mammals, marine animals, plants, algae, lichen, fungi and bacteria. It is generally accepted that haloperoxidases are the enzymes responsible for the formation of halogenated compounds in nature, although other enzymes may be involved. 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*. Haloperoxidases have also been isolated from bacteria such as *Pseudomonas*, e.g., *P. pyrrocinia* and *Streptomyces*, e.g., *S. aureofaciens*.

In a preferred embodiment, the haloperoxidase is a vanadium haloperoxidase, i.e. a vanadate-containing haloperoxidase.

In a more preferred embodiment, the haloperoxidase is derivable from *Curvularia* sp., in particular *Curvularia verruculosa*; *C. verruculosa* CBS 147.63 or *C. verruculosa* CBS 444.70 as described in WO 97/04102. In one preferred embodiment, the haloperoxidase is vanadium haloperoxidase hpxl from *Curvularia verruculosa* or a variant hereof.

In an embodiment, the amino acid sequence of the haloperoxidase has 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% identity to the amino acid sequence of a haloperoxidase obtainable from *Curvularia verruculosa* shown in SEQ ID NO 82.

The vanadium chloroperoxidase may also be derivable from *Curvularia inaequalis*, such as *C. inaequalis* CBS 102.42 as described in WO 95/27046; *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.

In one aspect, the haloperoxidase comprises, consists of or consists essentially of the amino acid sequence set forth in SEQ ID NO: 82.

The concentration of the haloperoxidase is preferably in the range of below 2 ppm enzyme protein, such as at from 0.01 ppm-2.0 ppm, 0.05 ppm-2.0 ppm, 0.1 ppm-2.0 ppm, 0.1 ppm-1.5 ppm, 0.1 ppm-1.0 ppm, 0.2 ppm - 2.0 ppm, 0.5 ppm-2.0 ppm, 0.5 ppm-1.5 ppm or preferably at a concentration between 0.5 ppm and 1.0 ppm.

Hydrogen peroxide



The hydrogen peroxide required by the haloperoxidase may be provided as an aqueous solution of hydrogen peroxide or a hydrogen peroxide precursor for in situ production of hydrogen peroxide. Any solid entity which liberates upon dissolution a peroxide, which is useable by haloperoxidase, can serve as a source of hydrogen peroxide. Compounds which yield hydrogen peroxide upon dissolution in water or an appropriate aqueous based medium include but are not limited to metal peroxides, percarbonates, persulphates, perphosphates, peroxyacids, alkylperoxides, acylperoxides, peroxyesters, urea peroxide, perborates and peroxycarboxylic acids or salts thereof.

Another source of hydrogen peroxide is a hydrogen peroxide generating enzyme system, such as an oxidase together with a substrate for the oxidase. Examples of combinations of oxidase and substrate comprise, but are not limited to, amino acid oxidase (see e.g. US 6,248,575) and a suitable amino acid, glucose oxidase (see e.g. WO 95/29996) and glucose, lactate oxidase and lactate, galactose oxidase (see e.g. WO 00/50606) and galactose, and aldose oxidase (see e.g. WO 99/31990) and a suitable aldose.

By studying EC 1.1.3., EC 1.2.3., EC 1.4.3., and EC 1.5.3. or similar classes (under the International Union of Biochemistry), other examples of such combinations of oxidases and substrates are easily recognized by one skilled in the art.

Alternative oxidants which may be applied for haloperoxidases may be oxygen combined with a suitable hydrogen donor like ascorbic acid, dehydroascorbic acid, dihydroxyfumaric acid or cysteine. An example of such oxygen hydrogen donor system is described by Pasta et al., Biotechnology & Bioengineering, (1999) vol. 62, issue 4, pp. 489-493.

Typical amounts of hydrogen peroxide correspond to levels of from 0.001 mM to 25 mM, preferably to levels of from 0.005 mM to 5 mM, and particularly to levels of from 0.01 to 1 mM or 0.02 to 2 mM hydrogen peroxide. Hydrogen peroxide may also be used in an amount corresponding to levels of from 0.1 mM to 25 mM, preferably to levels of from 0.5 mM to 15 mM, more preferably to levels of from 1 mM to 10 mM, and most preferably to levels of from 2 mM to 8 mM hydrogen peroxide.

#### Chloride, Bromide and/or Iodide ions

Chloride ions (Cl<sup>-</sup>), bromide ions (Br<sup>-</sup>) and/or iodide ions (I<sup>-</sup>), for reaction with the haloperoxidase may be provided in many different ways, such as by adding chloride salt(s), bromide salt(s) and/or iodide salt(s) to an aqueous solution. In a preferred embodiment, the chloride salt(s) are sodium chloride (NaCl), potassium chloride (KCl), or ammonium chloride (NH<sub>4</sub>Cl), or mixtures thereof.

In another preferred embodiment, bromide salt(s) are sodium bromide (NaBr), potassium bromide (KBr), or ammonium bromide (NH<sub>4</sub>Br), or mixtures thereof.

In another preferred embodiment, the iodide salt(s) are sodium iodide (NaI), potassium iodide (KI), or ammonium iodide (NH<sub>4</sub>I), or mixtures thereof.

The concentration of chloride ions, bromide ions and/or iodide ions are collectively or individually in the range of from 0.01 mM to 1000 mM, preferably in the range of from 0.05 mM to 500 mM, more preferably in the range of from 0.1 mM to 100 mM, most preferably in the range of from 0.1 mM to 50 mM, and in particular in the range of from 1 mM to 25 mM.

In an embodiment, the collective molar concentration of chloride ions, bromide ions and/or iodide ions is at least the same as the concentration of ammonium ions.

#### Ammonium ions

If desired, ammonium ions (NH<sub>4</sub><sup>+</sup>), for improving the efficacy of the haloperoxidase, may be provided by adding an ammonium salt. In a preferred embodiment, the ammonium salt is ammonium sulphate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), ammonium carbonate ((NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>), ammonium chloride (NH<sub>4</sub>Cl), ammonium bromide (NH<sub>4</sub>Br), ammonium iodide (NH<sub>4</sub>I), or ammonium thiocyanate (NH<sub>4</sub>SCN); or a mixture thereof.

The concentration of ammonium ions is typically in the range of from 0.01 mM to 1000 mM, preferably in the range of from 0.05 mM to 500 mM, more preferably in the range of from 0.1 mM to 100 mM, most preferably in the range of from 0.1 mM to 50 mM, and in particular in the range of from 1 mM to 25 mM.

#### **A composition comprising:**

The invention relates to cleaning compositions comprising a DNase and a haloperoxidase in combination with one or more additional cleaning composition components. One aspect relates to a cleaning composition comprising at least 0.001 ppm DNase, at least 0.001 ppm haloperoxidase and a cleaning component, wherein the cleaning component is selected from the group consisting of:

- a. from about 0.1 to about 60 wt%, e.g. 0.1 to 15 wt% of at least one a surfactant;
- b. from about 1 to about 50 wt% e.g. 0.5 to 20 wt% of at least one builder; and
- c. from about 1 to about 20 wt% 0.01 to 10 wt% of at least one bleach component.

Particularly useful DNases may be those of microbial origin. One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase is microbial, preferably obtained from bacteria or fungi. In one embodiment, the cleaning composition comprise a DNase from bacteria. One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase is obtained from *Bacillus*, preferably *Bacillus cibi*, *Bacillus horikoshii*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus horneckiae*, *Bacillus idriensis*, *Bacillus algicola*, *Bacillus vietnamensis*, *Bacillus hwajinpoensis*, *Bacillus indicus*,

*Bacillus marisflavi* or *Bacillus luciferensis*.

As mentioned above the DNases to be used in a composition of the invention preferable belong to the NUC1 group of DNases. The NUC1 group of DNases may comprise one or more of the motifs [T/D/S][G/N]PQL (SEQ ID NO 69), [F/L/Y/I]A[N/R]D[L/I/P/V] (SEQ ID NO: 70), or C[D/N]T[A/R] (SEQ ID NO: 71). One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase comprises one or more of the motifs [T/D/S][G/N]PQL, [F/L/Y/I]A[N/R]D[L/I/P/V] or C[D/N]T[A/R]. The DNases preferably additionally comprises a NUC1\_A domain [D/Q][IA]/DH (SEQ ID NO 72).

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase comprises one or more motifs selected from the motifs [T/D/S][G/N]PQL, [F/L/Y/I]A[N/R]D[L/I/PA], C[D/N]T[A/R] and [D/Q][IA]/DH.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase comprises one or both of the motif(s) [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 73) or ASXNRSKG (SEQ ID NO: 74). In a particularly preferred embodiment the *Bacillus* DNase comprises one or both of the motif(s) [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 73) or ASXNRSKG (SEQ ID NO: 74). In another particularly preferred embodiment the DNase comprises one or both of the motif(s) [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 73) or ASXNRSKG (SEQ ID NO: 74) and is obtained from *Bacillus cibi*. In yet another preferred embodiment the DNase comprises the amino acid sequence shown in SEQ ID NO 13 or DNases closely related hereto. One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and a cleaning component, wherein the DNase is selected from the group of polypeptides having DNase activity consisting of:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 1,
- b) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 2,
- c) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 3,
- d) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 4,

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- q) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 17,
- r) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 18,
- s) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 19,
- t) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 20,
- u) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 21,
- v) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 22,
- w) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 23,
- x) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 24, and
- y) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 25.

In one embodiment the DNase comprises one or both of the motif(s) [V/I]PL[S/A]NAWK (SEQ ID NO: 75) or NPQL (SEQ ID NO: 76)

One embodiment relates to One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and a cleaning component, wherein the DNase is selected from the group of polypeptides having DNase activity consisting of:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 26,

- 29

In one preferred embodiment the DNase comprises one or both of the motif(s) P[Q/E]L[W/Y] (SEQ ID NO: 77) or [K/H/E]NAW (SEQ ID NO: 78).

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and a cleaning component, wherein the DNase is selected from the group of polypeptides having DNase activity consisting of:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 39,
- b) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 40,
- c) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 41,
- d) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 42,
- e) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 43
- f) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 44,
- g) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 45,
- h) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 46,
- i) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 47,
- j) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 48,

- k) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 49,
- l) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 50, and
- m) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 51.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and a cleaning component, wherein the DNase is selected from the group of polypeptides having DNase activity consisting of:

- a) polypeptide obtainable from *Bacillus licheniformis* having a sequence identity to the polypeptide shown in SEQ ID NO: 65 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,
- b) polypeptide obtainable from *Bacillus subtilis* having a sequence identity to the polypeptide shown in SEQ ID NO: 66 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,
- c) polypeptide obtainable from *Aspergillus oryzae* having a sequence identity to the polypeptide shown in SEQ ID NO: 67 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,
- d) polypeptide obtainable from *Trichoderma harzianum* having a sequence identity to the polypeptide shown in SEQ ID NO: 68 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,

and combinations thereof.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 13.

Other preferred DNases include those comprising the amino acid sequence shown in SEQ ID NO 65 and 66.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at



least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 65.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 66.

The DNase may also preferably be fungal. Particularly preferred are DNases obtained from *Aspergillus* in particular, *Aspergillus oryzae*.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 67.

Other particularly preferred are DNases obtained from *Trichoderma* in particular, *Trichoderma harzianum*.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 68.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 68.

The haloperoxidase is preferably selected from the group consisting of: a chloride peroxidase (EC 1.11.1.10) and a bromide peroxidase (EC 1.11.1.18). One embodiment, relates to a cleaning composition comprising a DNase, a haloperoxidase and a cleaning component, wherein the haloperoxidase is selected from the group consisting of: a chloride peroxidase (EC 1.11.1.10) and a bromide peroxidase (EC 1.11.1.18).

The haloperoxidase is preferably a vanadium haloperoxidase hpxl from *Curvularia veruculosa* or a variant thereof. One embodiment, relates to a cleaning composition comprising a DNase, a haloperoxidase and a cleaning component, wherein the haloperoxidase is vanadium haloperoxidase hpxl from *Curvularia veruculosa* or a variant thereof.

The haloperoxidase is preferably selected from the group consisting of:

(a) a polypeptide having haloperoxidase activity having at least 60%, 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% sequence identity to the amino acid sequence shown in SEQ ID NO: 82;

(b) a polypeptide having haloperoxidase activity, which is a variant of the polypeptide shown in SEQ ID NO: 82, comprising a substitution, deletion, and/or insertion at one or more positions;

(c) a polypeptide having haloperoxidase activity, which is immunologically cross-reactive with the amino acid sequence set forth in SEQ ID NO: 82;

(e) a polypeptide having haloperoxidase activity, which is a fragment of the polypeptide of (a) (b) or (c)

and combinations thereof.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and a cleaning component, wherein the haloperoxidase is selected from the group consisting of:

(a) a polypeptide having haloperoxidase activity having at least 60%, 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% sequence identity to the amino acid sequence shown in SEQ ID NO: 82;

(b) a polypeptide having haloperoxidase activity, which is a variant of the polypeptide shown in SEQ ID NO: 82, comprising a substitution, deletion, and/or insertion at one or more positions;

(c) a polypeptide having haloperoxidase activity, which is immunologically cross-reactive with the amino acid sequence set forth in SEQ ID NO: 82;

(e) a polypeptide having haloperoxidase activity, which is a fragment of the polypeptide of (a) (b) or (c)

and combinations thereof.

The haloperoxidase is preferably a haloperoxidase having 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% sequence identity to the amino acid sequence shown in SEQ ID NO 82

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 13 and wherein haloperoxidase has 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% sequence identity to the amino acid sequence shown in SEQ ID NO 82.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at

least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 65 and wherein haloperoxidase has 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% sequence identity to the amino acid sequence shown in SEQ ID NO 82.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 66 and wherein haloperoxidase has 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% sequence identity to the amino acid sequence shown in SEQ ID NO 82.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 67 and wherein haloperoxidase has 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% sequence identity to the amino acid sequence shown in SEQ ID NO 82.

One embodiment of the invention relates to a cleaning composition comprising a DNase, a haloperoxidase and at least one cleaning component, wherein the DNase has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 68 and wherein haloperoxidase has 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% sequence identity to the amino acid sequence shown in SEQ ID NO 82.

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.

One aspect of the invention relates to a composition comprising:

- a) at least 0.001 ppm e.g. 0.1 ppm or 1 ppm of at least one polypeptide having DNase activity (DNase), wherein the DNase is selected for the group consisting of:
  - i) a DNase comprising one or more of the motifs [T/D/S][G/N]PQL (SEQ ID NO 69), [F/L/Y/I]A[N/R]D[L/I/P/V] (SEQ ID NO: 70), or C[D/N]T[A/R] (SEQ ID NO: 71);

- ii) a DNase comprising the motif [D/Q][I/A]DH (SEQ ID NO 72);
- iii) a DNase comprising one or both of the motifs [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 73) or ASXNRSKG (SEQ ID NO: 74);
- iv) a DNase comprising one or both of the motifs [V/I]PL[S/A]NAWK (SEQ ID NO: 75) or NPQL (SEQ ID NO: 76);
- v) a DNase comprising one or both of the motifs P[Q/E]L[W/Y] (SEQ ID NO: 77) or [K/H/E]NAW (SEQ ID NO: 78);
- vi) a polypeptide having DNase activity selected from: a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 1, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 2, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 3, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 4, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 5, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 6, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 7, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 8, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 9, a polypeptide having

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66, a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 67, and a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 68, and optionally

b) at least 0.01 ppm e.g. 0.1 ppm or e.g. 1 ppm of one haloperoxidase selected from the group consisting of: a chloride peroxidase (EC 1.11.1.10) and a bromide peroxidase (EC 1.11.1.18), preferably a vanadium haloperoxidase hpxl from *Curvularia veruculosa* or a variant thereof; selected from the group consisting of:

- i) a polypeptide having haloperoxidase activity having at least 60%, 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% sequence identity to the amino acid sequence shown in SEQ ID NO: 82;
- ii) a polypeptide having haloperoxidase activity, which is a variant of the polypeptide shown in SEQ ID NO: 82, comprising a substitution, deletion, and/or insertion at one or more positions;
- iii) a polypeptide having haloperoxidase activity, which is immunologically cross-reactive with the amino acid sequence set forth in SEQ ID NO: 82;
- iv) a polypeptide having haloperoxidase activity, which is a fragment of the polypeptide of (a) (b) or (c); and

c) at least one cleaning composition component, preferably selected from surfactants, builders, bleach components, polymers, dispersing agents and additional enzymes.

The choice of cleaning 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. Although components mentioned below are categorized by general header according to a particular functionality, this is not to be construed as a limitation, as a component may comprise additional functionalities as will be appreciated by the skilled artisan.

#### Surfactants

The cleaning 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 wt%, such as about 0.1 to about 15 wt%, or about 3 to about 20 wt%, or about 3 to about 10 wt%. The surfactant(s) is chosen based on the desired cleaning application, and may include any conventional surfactant(s) known in the art. When included therein the detergent will usually contain from about 1% to about 40% by weight of an anionic surfactant, such as from about 5 to about 30 wt%, including from about 5 to about 15 wt%, or from about 15 to about 20 wt%, or from about 20 to about 25 wt% 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, dodeceny/tetradeceny succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or salt of fatty acids (soap), and combinations thereof.

When included therein the detergent will usually contain from about 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 alkylbenzyltrimethylammonium, alkyl quaternary ammonium compounds, alkoxyated quaternary ammonium (AQA) compounds, ester quats, and combinations thereof.

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

When included therein the detergent will usually contain from about 0.01% to about 10 % by weight of a semipolar surfactant. Non-limiting examples of semipolar surfactants include amine oxides (AO) such as alkyldimethylamineoxide, N-(coco alkyl)-N,N-dimethylamine oxide and N-(tallow-alkyl)-N,N-bis(2-hydroxyethyl)amine oxide, , and combinations thereof.

When included therein the detergent will usually contain from about 0.01 % to about 10 % by weight of a zwitterionic surfactant. Non-limiting examples of zwitterionic surfactants include betaines such as alkyldimethylbetaines, sulfobetaines, and combinations thereof.

#### Builders and Co-Builders

The cleaning composition may contain about 0-65% by weight, such as about 1 to about 50 wt%, such as about 5 to about 50 wt%, such as about 0.5 to about 20 wt% of a detergent builder or co-builder, or a mixture thereof. In a dish wash detergent, the level of builder is typically 40-65%, particularly 50-65%. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in cleaning 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), 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 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), 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), oalanine-N,N-diacetic acid (a-ALDA), serine-N,N-diacetic acid (SEDA), isoserine-N,N-diacetic acid

(ISDA), phenylalanine-N,N-diacetic acid (PHDA), anthranilic acid-N,N-diacetic acid (ANDA), sulfanilic acid-N,N-diacetic acid (SLDA), taurine-N,N-diacetic acid (TUDA) and sulfomethyl-N,N-diacetic acid (SMDA), N-(2-hydroxyethyl)ethylenediamine-N,N',N"-triacetic acid (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053

### Bleaching Systems

The detergent may contain 0-30% by weight, such as about 0.01 to 10 wt%, such as about 1 to about 20 wt% 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 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:

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.

a) Suitable preformed peracids include, but are not limited to, peroxycarboxylic acids such as peroxybenzoic acid and its ring-substituted derivatives, peroxy-onaphthoic acid, peroxyphthalic acid, peroxyauric acid, peroxysearic acid,  $\epsilon$ -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; peroxyisilic 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.

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 tetraacetythylenediamine (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 W098/17767. A particular

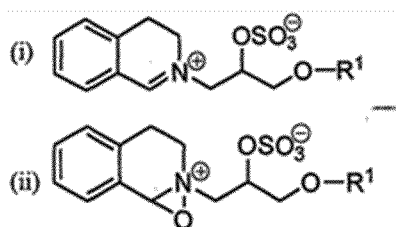
family of bleach activators of interest was disclosed in EP624154 and particularly preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that they are environmentally friendly. Furthermore, acetyl triethyl citrate and triacetin have 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.

#### Bleach catalysts and boosters

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(0)3Mn(Me3-TACN)](PF6)2, and [2,2',2''-nitritotris(ethane-1,2-diylazanylylidene -KN-methanylylidene)triphenolato-K30]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 R<sup>1</sup> is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R<sup>1</sup> is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R<sup>1</sup> 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, EP1 867708 (Vitamin K) and WO2007/087242. Suitable photobleaches may for example be sulfonated zinc or aluminium phthalocyanines.

#### Metal care agents

Metal care agents may prevent or reduce the tarnishing, corrosion or oxidation of metals,

including aluminium, stainless steel and non-ferrous metals, such as silver and copper. Suitable examples include one or more of the following:

(a) benzotriazoles, including benzotriazole or bis-benzotriazole and substituted derivatives thereof. Benzotriazole derivatives are those compounds in which the available substitution sites on the aromatic ring are partially or completely substituted. Suitable substituents include linear or branch-chain C<sub>1</sub>-C<sub>20</sub>- alkyl groups (e.g., C<sub>1</sub>-C<sub>20</sub>- alkyl groups) and hydroxyl, thio, phenyl or halogen such as fluorine, chlorine, bromine and iodine.

(b) metal salts and complexes chosen from the group consisting of zinc, manganese, titanium, zirconium, hafnium, vanadium, cobalt, gallium and cerium salts and/or complexes, the metals being in one of the oxidation states II, III, IV, V or VI. In one aspect, suitable metal salts and/or metal complexes may be chosen from the group consisting of Mn(II) sulphate, Mn(II) citrate, Mn(II) stearate, Mn(II) acetylacetonate, K<sup>+</sup>TiF<sub>6</sub> (e.g., K<sub>2</sub>TiF<sub>6</sub>), K<sup>+</sup>ZrF<sub>6</sub> (e.g., K<sub>2</sub>ZrF<sub>6</sub>), CoSO<sub>4</sub>, Co(NO<sub>3</sub>)<sub>2</sub> and Ce(NO<sub>3</sub>)<sub>3</sub>, zinc salts, for example zinc sulphate, hydrozincite or zinc acetate;

(c) silicates, including sodium or potassium silicate, sodium disilicate, sodium metasilicate, crystalline phyllosilicate and mixtures thereof.

Further suitable organic and inorganic redox-active substances that act as silver/copper corrosion inhibitors are disclosed in WO 94/26860 and WO 94/26859. Preferably the composition of the invention comprises from 0.1 to 5% by weight of the composition of a metal care agent, preferably the metal care agent is a zinc salt.

#### Hydrotropes

The cleaning composition 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 polyglycoethers, sodium hydroxynaphthoate, sodium hydroxynaphthalene sulfonate, sodium ethylhexyl sulfate, and combinations thereof.

#### Polymers

The cleaning composition 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), polyvinyl 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(oxyethylene terephthalate) (PET-POET), PVP, poly(vinylimidazole) (PVI), poly(vinylpyridine-N-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Suitable examples include PVP-K15, PVP-K30, ChromaBond S-400, ChromaBond S-403E and Chromabond S-100 from Ashland Aqualon, and Sokalan® HP 165, Sokalan® HP 50 (Dispersing agent), Sokalan® HP 53 (Dispersing agent), Sokalan® HP 59 (Dispersing agent), Sokalan® HP 56 (dye transfer inhibitor), Sokalan® HP 66 K (dye transfer inhibitor) from BASF. Further exemplary polymers include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated. Particularly preferred polymer is ethoxylated homopolymer Sokalan® HP 20 from BASF, which helps to prevent redeposition of soil in the wash liquor.

#### Fabric hueing agents

The cleaning composition 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 EP1 876226 (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.



Enzymes

The cleaning composition may comprise one or more additional enzymes such as one or more lipase, cutinase, an amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, xylanase, oxidase, e.g., a laccase, and/or peroxidase.

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

**Proteases**

The term "protease" is defined herein as an enzyme that hydrolyzes peptide bonds. It includes any enzyme belonging to the EC 3.4 enzyme group (including each of the thirteen subclasses thereof). The EC number refers to Enzyme Nomenclature 1992 from NC-IUBMB, Academic Press, San Diego, California, including supplements 1-5 published in *Eur. J. Biochem.* 1223: 1-5 (1994); *Eur. J. Biochem.* 232: 1-6 (1995); *Eur. J. Biochem.* 237: 1-5 (1996); *Eur. J. Biochem.* 250: 1-6 (1997); and *Eur. J. Biochem.* 264: 610-650 (1999); respectively. The most widely used proteases in the detergent industry such as laundry and dish wash are the serine proteases. Serine proteases is a subgroup of proteases characterised by having a serine in the active site, which forms a covalent adduct with the substrate. Serine proteases are characterized by having two active site amino acid residues apart from the serine, namely a histidine residue and an aspartic acid residue. Proteases usable in cleaning compositions of the present invention are mainly endopeptidases (EC 3.4.21). There are several protease activity types: The three main activity types are: trypsin-like where there is cleavage of amide substrates following Arg or Lys at P1, chymotrypsin-like where cleavage occurs following one of the hydrophobic amino acids at P1, and elastase-like with cleavage following an Ala at P1.

Subtilase refer to a sub-group of serine protease according to Siezen *et al.*, 1991, *Protein Engng.* 4: 719-737 and Siezen *et al.*, 1997, *Protein Science* 6: 501-523. 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 Pyrolysins family. The term "protease activity" means a proteolytic activity (EC 3.4).

Suitable proteases for the compositions of the invention 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 metalloprotease protease may for example be a thermolysin from e.g. family M4 or other metalloprotease such as those from M5, M7 or M8 families.

Examples of subtilases are those derived from *Bacillus* such as *Bacillus lentus*, *Bacillus alkalophilus*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii* described in; US7262042 and WO09/021867, and subtilisin *lentus*, subtilisin Novo, subtilisin Carlsberg, *Bacillus licheniformis*, subtilisin BPN', subtilisin 309, subtilisin 147 and subtilisin 168 described in WO89/06279 and protease PD138 described in (WO93/18140). Other useful proteases may be those described in W092/175177, 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, W094/25583 and WO05/040372, and the chymotrypsin proteases derived from *Cellulomonas* described in WO05/052161 and WO05/052146.

A further preferred protease is the alkaline protease from *Bacillus lentus* DSM 5483, as described for example in W095/23221, and variants thereof which are described in WO92/21760, W095/23221, EP1921 147 and EP1921 148.

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: W092/1 9729, WO96/034946, WO98/201 15, WO98/201 16, WO99/01 1768, WO01/44452, WO03/006602, WO04/03186, WO04/041979, WO07/006305, W01 1/036263, W01 1/036264, especially protease variants comprising a substitution 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, 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 79. More preferred the protease variants may comprise one or more of the mutations selected from the group consisting of 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, 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, L21 1Q, L21 1D, N212D, N212S, M216S, A226V, K229L, Q230H, Q239R, N246K, N255W, N255D, N255E, L256E, L256D T268A and R269H. The protease variants are preferably variants of the *Bacillus lentus* protease (Savinase®) shown in SEQ ID NO 79 or the *Bacillus amylolichenifaciens* protease (BPN') shown in SEQ ID NO 80. The protease variants preferably have at least 80 % sequence identity to SEQ ID NO 79 or SEQ ID NO 80.

A protease variant comprising a substitution at one or more positions corresponding to positions 171, 173, 175, 179, or 180 of SEQ ID NO: 81, wherein said protease variant has a sequence identity of at least 75% but less than 100% to SEQ ID NO: 81.

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 Eivity® 100T, Blaze Eivity® 125T, Blaze Eivity® 150T, Neutrase®, Everlase® and Esperase® (Novozymes A/S), those sold under the tradename 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 US5352604) and variants hereof (Henkel AG) and KAP (Bacillus alkalophilus subtilisin) from Kao.

### Cellulases

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 96/1 1262, 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), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

### Mannanases

Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. The mannanase may be an alkaline mannanase of Family 5 or 26. It may be a wild-type from Bacillus or Humicola, particularly B. agaradhaerens, B.

licheniformis, *B. halodurans*, *B. clausii*, or *H. insolens*. Suitable mannanases are described in WO 1999/064619. A commercially available mannanase is Mannaway (Novozymes A/S).

### Lipases and Cutinases:

5 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*, 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.*  
 10 *pseudocaligenes* (EP218272), *P. cepacia* (EP331376), *P. sp.* strain SD705 (WO95/06720 & WO96/27002), *P. wisconsinensis* (WO96/12012), GDSL-type *Streptomyces* lipases (WO 10/065455), cutinase from *Magnaporthe grisea* (WO 10/1 07560), cutinase from *Pseudomonas mendocina* (US5,389,536), lipase from *Thermobifida fusca* (WO 11/084412), *Geobacillus stearothermophilus* lipase (WO 11/084417), lipase from *Bacillus subtilis*  
 15 (WO 11/084599), and lipase from *Streptomyces griseus* (WO1 1/150157) and *S. pristinaespiralis* (WO12/137147).

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,  
 20 WO07/87508 and WO09/1 09500.

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  
 25 perhydrolases, e.g. acyltransferases with homology to *Candida antarctica* lipase A (WO 10/1 11143), 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 used in the commercial product Gentle Power Bleach from Huntsman Textile Effects Pte Ltd (WO1 0/1 00028).

### 30 Amylases:

Suitable amylases include alpha-amylases and/or a glucoamylases and may be of bacterial or fungal origin. Chemically modified 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.

35 Suitable amylases include amylases having SEQ ID NO: 2 in WO 95/10603 or variants having 90% sequence identity to SEQ ID NO: 3 thereof. Preferred variants are described in WO 94/02597, WO 94/18314, WO 97/43424 and SEQ ID NO: 4 of WO 99/019467, such as variants

with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and 444.

Different suitable amylases include amylases having SEQ ID NO: 6 in WO 02/010355 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a deletion in positions 181 and 182 and a substitution in position 193.

Other amylases which are suitable are hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of the *B. licheniformis* alpha-amylase shown in SEQ ID NO: 4 of WO 2006/066594 or variants having 90% sequence identity thereof. Preferred variants of this hybrid alpha-amylase are those having a substitution, a deletion or an insertion in one or more of the following positions: G48, T49, G107, H156, A181, N190, M197, I201, A209 and Q264. Most preferred variants of the hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 are those having the substitutions:

M197T;

H156Y+A181T+N190F+A209V+Q264S; or

G48A+T49I+G107A+H156Y+A181T+N190F+I201 F+A209V+Q264S.

Further amylases which are suitable are amylases having SEQ ID NO: 6 in WO 99/019467 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a substitution, a deletion or an insertion in one or more of the following positions: R181, G182, H183, G184, N195, I206, E212, E216 and K269. Particularly preferred amylases are those having deletion in positions R181 and G182, or positions H183 and G184.

Additional amylases which can be used are those having SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873 or variants thereof having 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7 are those having a substitution, a deletion or an insertion in one or more of the following positions: 140, 181, 182, 183, 184, 195, 206, 212, 243, 260, 269, 304 and 476, 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 are those having a deletion in positions 183 and 184 and a substitution in one or more of positions 140, 195, 206, 243, 260, 304 and 476.

Other amylases which can be used are amylases having SEQ ID NO: 2 of WO 08/153815, SEQ ID NO: 10 in WO 01/66712 or variants thereof having 90% sequence identity to SEQ ID NO: 2 of WO 08/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 01/66712.

Preferred variants of SEQ ID NO: 10 in WO 01/66712 are those having a substitution, a deletion or an insertion in one of more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264.

Further suitable amylases are amylases having SEQ ID NO: 2 of WO 09/061380 or variants having 90% sequence identity to SEQ ID NO: 2 thereof. Preferred variants of SEQ ID NO: 2 are those having a truncation of the C-terminus and/or a substitution, a deletion or an insertion in one of more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320, Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one of more of the following positions: Q87E,R, Q98R, S125A, N128C, T131 I, T165I, K178L, T182G, M201 L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:

N128C+K178L+T182G+Y305R+G475K;

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

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

S125A+N128C+T131 I+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.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO 2013/184577 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: K176, R178, G179, T180, G181, E187, N192, M199, I203, S241, R458, T459, D460, G476 and G477. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: K176L, E187P, N192FYH, M199L, I203YF, S241QADN, R458N, T459S, D460T, G476K and G477K and/or deletion in position R178 and/or S179 or of T180 and/or G181. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

E187P+I203Y+G476K

E187P+I203Y+R458N+T459S+D460T+G476K

wherein the variants optionally further comprise a substitution at position 241 and/or a deletion at position 178 and/or position 179.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO 2010/104675 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: N21, D97, V128, K177, R179, S180, I181, G182, M200, L204, E242, G477 and G478. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the

following positions: N21 D, D97N, V128I K177L, M200L, L204YF, E242QA, G477K and G478K and/or deletion in position R179 and/or S180 or of 1181 and/or G182. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

N21 D+D97N+V128I

5 wherein the variants optionally further comprise a substitution at position 200 and/or a deletion at position 180 and/or position 181 .

Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO01/66712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one or more of the following positions  
10 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  
15 the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions.

Other examples are amylase variants such as those described in WO20 11/098531 , WO201 3/001 078 and WO201 3/001 087.

Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™, Stainzyme  
20 ™, Stainzyme Plus™, Natalase™, Liquozyme X and BAN™ (from Novozymes A/S), and Rapidase™, Purastar™/Effectenz™, Powerase, Preferenz S1000, Preferenz S100 and Preferenz S110 (from Genencor International Inc./DuPont).

#### Dispersants

25 The cleaning compositions of the present invention can also contain dispersants. In particular, powdered detergents may comprise dispersants. Suitable water-soluble organic materials include the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms. Suitable dispersants are for example described in Powdered Detergents, Surfactant science  
30 series volume 71, Marcel Dekker, Inc.

#### Dye Transfer Inhibiting Agents

The cleaning compositions of the present invention may also include one or more dye transfer  
35 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.

#### Fluorescent whitening agent

5 The cleaning 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  
10 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-hydroxy-ethylamino)-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-(2H-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  
20 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 %  
25 to upper levels of 0.5 or even 0.75 wt%.

#### Soil release polymers

The cleaning 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  
30 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 is amphiphilic alkoxyated grease cleaning  
35 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 2006/1 13314 (hereby incorporated by reference). Suitable polyethylene glycol polymers include random graft co-polymers comprising: (i) hydrophilic backbone comprising polyethylene glycol; and (ii) side chain(s) selected from the group consisting of: C4-C25 alkyl group, polypropylene, polybutylene, vinyl ester of a saturated C1-C6 mono-carboxylic acid, C1-C 6 alkyl ester of acrylic or methacrylic acid, and mixtures thereof. Suitable polyethylene glycol polymers have a polyethylene glycol backbone with random grafted polyvinyl acetate side chains. The average molecular weight of the polyethylene glycol backbone can be in the range of from 2,000 Da to 20,000 Da, or from 4,000 Da to 8,000 Da. The molecular weight ratio of the polyethylene glycol backbone to the polyvinyl acetate side chains can be in the range of from 1: 1 to 1:5, or from 1: 1.2 to 1:2. The average number of graft sites per ethylene oxide units can be less than 1, or less than 0.8, the average number of graft sites per ethylene oxide units can be in the range of from 0.5 to 0.9, or the average number of graft sites per ethylene oxide units can be in the range of from 0.1 to 0.5, or from 0.2 to 0.4. A suitable polyethylene glycol polymer is Sokalan HP22. 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.

#### Anti-redeposition agents

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

#### Rheology Modifiers

The cleaning 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 cleaning composition components 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.

#### Formulation of cleaning products

The cleaning 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.

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: US2009/001 1970 A 1.

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 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 be non-aqueous.

#### Granular detergent formulations

- 5 The composition(s) of the invention 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.
- 10 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), and the composition additionally comprises from 20 to 80 wt% detergent moisture sink component.
- 15 The multi-enzyme co-granule may comprise one or more enzymes selected from the group of cellulases, xyloglucanases, perhydrolases, peroxidases, lipoxygenases, laccases, hemicellulases, proteases, care cellulases, cellobiose dehydrogenases, xylanases, phospho lipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, tannases, pentosanases, lichenases
- 20 glucanases, arabinosidases, hyaluronidase, chondroitinase, amylases, and mixtures thereof.

#### **Uses**

- The present invention is also directed to methods for using the compositions thereof. Laundry/textile/fabric (House hold laundry washing, Industrial laundry washing). Hard surface
- 25 cleaning (ADW, car wash, Industrial surface)

#### Use of cleaning composition

- The cleaning composition of the present invention may be formulated, for example, as a hand or machine laundry detergent composition including a laundry additive composition suitable for pre-
- 30 treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for hand or machine dishwashing operations. In a specific aspect, the present invention provides a detergent additive comprising one or more enzymes as described herein.

#### 35 **Definitions**

Biofilm is produced by any group of microorganisms in which cells stick to each other or stick to a surface, such as a textile, dishware or hard surface or another kind of 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. Bacteria living in a biofilm usually have significantly different properties from planktonic 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 for the microorganisms is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. On laundry 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.* On hard surfaces biofilm producing bacteria can be found among the following species: *Acinetobacter sp.*, *Aeromicrobium sp.*, *Brevundimonas sp.*, *Microbacterium sp.*, *Micrococcus luteus*, *Pseudomonas sp.*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Stenotrophomonas sp.* In one aspect, the biofilm producing strain is *Brevundimonas sp.* In one aspect, the biofilm producing strain is *Pseudomonas alcaliphila* or *Pseudomonas fluorescens*. In one aspect, the biofilm producing strain is *Staphylococcus aureus*.

By the term "deep cleaning" is meant reduction, disruption or removal of components which may be comprised in organic matter, e.g. biofilm, such as polysaccharides, proteins, DNA, soil or other components present in the organic matter.

Cleaning component: The cleaning component e.g. the detergent adjunct ingredient is different to the DNase and haloperoxidase enzymes. The precise nature of these additional cleaning components e.g. 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 cleaning components e.g. 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 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.

Cleaning composition: The term "cleaning composition" refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles. The cleaning 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 enzymes, the cleaning composition may contain one or more additional enzymes (such as proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases and mannanases, or any mixture thereof), and/or cleaning components e.g. detergent adjunct ingredients 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.

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 catalyze the formation of bleaching components such as hydrogen peroxide or other peroxides. 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 textile to another textile or another part of the same textile (an effect that is also termed dye transfer inhibition or anti-backstaining), removal of protruding or broken fibers from a textile surface to decrease pilling tendencies or remove already existing pills or fuzz (an effect that also is termed anti-pilling), improvement of the textile-softness, colour clarification of the textile and removal of particulate soils which are trapped in the fibers of the textile. Enzymatic

bleaching is a further enzyme detergency benefit where the catalytic activity generally is used to catalyze the formation of bleaching component such as hydrogen peroxide or other peroxides or other bleaching species."

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

The term "wash performance" is used as an enzyme's ability to remove stains present on the object to be cleaned during e.g. wash or hard surface cleaning.

The term "whiteness" is defined herein as a greying, yellowing of a textile. Loss of whiteness may be due to removal of optical brighteners/hueing agents. 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.

The term "laundering" relates to both household laundering and industrial laundering and means the process of treating textiles with a solution containing a cleaning or detergent composition of the present invention. The laundering 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 "malodor" Examples of malodor is compounds with an unpleasant smell, which may be produced by microorganisms. Another example is unpleasant smells can be sweat or body odor adhered to an item which has been in contact with human or animal. Another example of malodor can be the odor from spices, which sticks to items for example curry or other exotic spices which smells strongly.

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.

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 towelling. The textile may be cellulose based such as natural cellulose, including cotton, flax/linen, jute, ramie, sisal or coir or manmade cellulose (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.

The term "variant" means a polypeptide having the activity of the parent or precursor polypeptide and comprising an alteration, i.e., a substitution, insertion, and/or deletion, at one or more (e.g., several) positions compared to the precursor or parent polypeptide. 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.

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 Molecular Biology Open Software Suite, Rice et al., 2000, Trends Genet. 16: 276-277), preferably version 6.6.0 or later. The parameters used are a gap open penalty of 10, a 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:

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

## Examples

### Assays

#### **Assay 1: testing of DNase activity**

DNase activity was determined on DNase Test Agar with Methyl Green (BD, Franklin Lakes, NJ, USA), which was prepared according to the manual from supplier. Briefly, 21 g of agar was dissolved in 500 ml water and then autoclaved for 15 min at 121 °C. Autoclaved agar was tempered to 48°C in water bath, and 20 ml of agar was poured into petridishes with and allowed to solidify by incubation o/n at room temperature. On solidified agar plates, 5 µl of enzyme solutions are added and DNase activity is observed as colorless zones around the spotted enzyme solutions.

**Assay II: Determination of Haloperoxidase Activity**

An assay for determining haloperoxidase activity may be carried out by mixing 100  $\mu\text{L}$  of haloperoxidase sample (containing about 0.2  $\mu\text{g}$  enzyme protein/mL) and 100  $\mu\text{L}$  of a 0.3 M sodium phosphate pH 7 buffer containing 0.5 M potassium bromide and 0.008% phenol red, adding the solution to 10  $\mu\text{L}$  of 0.3%  $\text{H}_2\text{O}_2$ , and measuring the absorption at 595 nm as a function of time.

Another assay using monochlorodimedone (Sigma M4632,  $\epsilon = 20000 \text{ M}^{-1}\text{cm}^{-1}$  at 290 nm) as a substrate may be carried out by measuring the decrease in absorption at 290 nm as a function of time. The assay is performed in an aqueous solution of 0.1 M sodium phosphate or 0.1 M sodium acetate, 50  $\mu\text{M}$  monochlorodimedone, 10 mM KBr/KCl, 1 mM  $\text{H}_2\text{O}_2$  and about 1  $\mu\text{g}/\text{mL}$  haloperoxidase.

**Automatic Mechanical Stress Assay (AMSA) for laundry**

In order to assess the wash performance in laundry, washing experiments are performed using the Automatic Mechanical Stress Assay (AMSA). With the AMSA, the wash performance of many small volume enzyme-detergent solutions can be examined. The AMSA plate has a number of slots for test solutions and a lid that firmly squeezes the textile to be washed against the slot openings. During the wash, the plate, test solutions, textile and lid are vigorously shaken to bring the test solution in contact with the textile and apply mechanical stress in a regular, periodic, oscillating manner. For further description see WO02/42740 especially the paragraph "Special method embodiments" at page 23-24.

The laundry experiments are conducted under the experimental conditions specified below:

Detergent dosage	5 g/L (liquid detergent) 2.5 g/L (powder detergent)
Test solution volume	160 micro L
pH	Adjusted to pH 7 or pH 6 (liquid detergent) As is (powder detergent)
Wash time	20 minutes
Temperature	60°C, 40°C and 20°C or 15°C
Water hardness	15°dH

Model detergents and test materials are as follows:

Laundry liquid model detergent	Sodium alkylethoxy sulfate (C-9-15, 2EO) 6.0% Sodium dodecyl benzene sulfonate 3.0% Sodium toluene sulfonate 3.0% Oleic acid 2.0%
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	Primary alcohol ethoxylate (C12-15, 7EO) 3.0% Primary alcohol ethoxylate (C12-15, 3EO) 2.5% Ethanol 0.5% Monopropylene glycol 2.0% Tri-sodium citrate dihydrate 4.0% Triethanolamine 0.4% De-ionized water ad 100% pH adjusted to 8.5 with NaOH
Laundry powder model detergent	Sodium citrate dihydrate 32.3% Sodium-LAS 24.2% Sodium lauryl sulfate 32.2% Neodol 25-7 (alcohol ethoxylate) 6.4% Sodium sulfate 4.9%

Water hardness was adjusted to 15°dH by addition of CaCl<sub>2</sub>, MgCl<sub>2</sub>, and NaHCO<sub>3</sub> (Ca<sup>2+</sup>:Mg<sup>2+</sup> = 4:1 :7.5) to the test system. After washing the textiles were flushed in tap water and dried.

The wash performance is measured as the brightness of the colour of the textile washed.

5 Brightness can also be expressed as the intensity of the light reflected from the sample when illuminated with white light. When the sample is stained the intensity of the reflected light is lower, than that of a clean sample. Therefore, the intensity of the reflected light can be used to measure wash performance.

10 Colour measurements are made with a professional flatbed scanner (Kodak iQsmart, Kodak, Midtager 29, DK-2605 Brøndby, Denmark), which is used to capture an image of the washed textile.

15 To extract a value for the light intensity from the scanned images, 24-bit pixel values from the image are converted into values for red, green and blue (RGB). The intensity value (Int) is calculated by adding the RGB values together as vectors and then taking the length of the resulting vector:

$$Int = \sqrt{r^2 + g^2 + b^2}$$

#### Mini wash assay

20 Wash performance is assessed in laundry wash experiment using a Mini wash assay, which is a test method where soiled textile is continuously is lifted up and down into the test solution and subsequently rinsed.

The wash experiment is conducted under various experimental conditions one examples specified below:

Detergent	Model A detergent Model detergent A wash liquor (100%) is prepared by dissolving 3.33 g/l of model detergent A containing 12% LAS, 1.1% AEO Biosoft N25-7 (NI), 7% AEOS (SLES), 6% MPG, 3% ethanol, 3% TEA (triethanolamine), 2.75% cocoa soap, 2.75% soya soap, 2% glycerol, 2% sodium hydroxide, 2% sodium citrate, 1% sodium formate, 0.2% DTMPA and 0.2% PCA (all percentages are w/w (weight volume) in water with hardness 15 dH.
Detergent dose	3.33 g/l
pH	Example: "as is" in the current detergent solution and is not adjusted.
Water hardness	15°dH, adjusted by adding $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{NaHCO}_3$ (4:1 :7.5) to milli-Q water.
Enzymes	Enzyme blend according to the invention
Enzyme conc.	Example 2.5 nM, 5 nM, 10 nM, 30 nM, 60 nM
Test material	Example: Biofilm or EPS swatches
Temperature	e.g. 15°C, 20°C, 30°C, 40°C or 60°C
Test system	Soiled textile continuously lifted up and down into the test solutions, 50 times per minute. The test solutions are kept in 125 ml glass beakers. After wash of the textiles are continuously lifted up and down into tap water, approx. 50 times per minute.

Test materials may be obtained from EMPA Testmaterials AG Movenstrasse 12, CH-9015 St. Gallen, Switzerland, from Center for Testmaterials BV, P.O. Box 120, 3133 KT Vlaardingen, the Netherlands, and WFK Testgewebe GmbH, Christenfeld 10, D-41379 Brijggen, Germany.

The textiles are subsequently air-dried and the wash performance is measured as the brightness of the colour of these textiles. Brightness can also be expressed as the Remission (R), which is a measure for the light reflected or emitted from the test material when illuminated with white light. The Remission (R) of the textiles is measured at 460 nm using a Zeiss MCS 521 VIS spectrophotometer. The measurements are done according to the manufacturer's protocol. The performance may be calculated as the relative performance:

$$RP = (R_{\text{ENZYME}} - R_{\text{BLANK}}) / (R_{\text{REFERENCE}} - R_{\text{BLANK}})$$

An enzyme is considered to exhibit improved wash performance, if it performs better than the reference ( $RP > 1$ ) in at least one detergent composition.

**Claims****What is claimed is:**

- 5 1. A cleaning composition comprising at least 0.001 ppm DNase, at least 0.001 ppm haloperoxidase and a cleaning component, wherein the cleaning component is selected from the group consisting of:
  - a. from about 0.1 to about 60 wt%, e.g. 0.1 to 15 wt% of at least one surfactant;
  - b. from about 1 to about 50 wt% e.g. 0.5 to 20 wt% of at least one builder; and
  - 10 c. from about 1 to about 20 wt% 0.01 to 10 wt% of at least one bleach component.
2. The cleaning composition according to claim 1, wherein the DNase comprises one or both of the motif(s) [D/M/L][S/T]GYSR[D/N] (SEQ ID NO: 73) or ASXNRSKG (SEQ ID NO: 74).
- 15 3. The cleaning composition according to claim 1 or 2, wherein the DNase is selected from the group of polypeptides having DNase activity consisting of:
  - a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 1,
  - 20 b) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 2,
  - c) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 3,
  - 25 d) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 4,
  - e) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 5,
  - 30 f) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 6,
  - 35 g) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 7,

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- t) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 20,
- u) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 21,
- v) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 22,
- w) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 23,
- x) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 24, and
- y) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 25.

4. The cleaning composition according to claim 1 wherein the DNase comprises one or both of the motif(s) [V/I]PL[S/A]NAWK (SEQ ID NO: 75) or NPQL (SEQ ID NO: 76)

5. The cleaning composition according to claim 1 or 4, wherein the DNase is selected from the group of polypeptides having DNase activity consisting of:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 26,
- b) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 27,
- c) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 28,
- d) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 29,

- e) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 30,
- f) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 31,
- g) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 32,
- h) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 33,
- i) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 34,
- j) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 35,
- k) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 36,
- l) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 37, and
- m) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 38.

6. The cleaning composition according to claim 1 wherein the DNase comprises one or both of the motif(s) P[Q/E]L[W/Y] (SEQ ID NO: 77) or [K/H/E]NAW (SEQ ID NO: 78).

7. The cleaning composition according to claim 1 or 6, wherein the DNase is selected from the group of polypeptides having DNase activity consisting of:

- a) a polypeptide having 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the polypeptide shown in SEQ ID NO: 39,

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8. The cleaning composition according to claim 1 wherein the DNase is selected from the group consisting of:

- a) polypeptide obtainable from *Bacillus licheniformis* having a sequence identity to the polypeptide shown in SEQ ID NO: 65 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,
  - b) polypeptide obtainable from *Bacillus subtilis* having a sequence identity to the polypeptide shown in SEQ ID NO: 66 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,
  - c) polypeptide obtainable from *Aspergillus oryzae* having a sequence identity to the polypeptide shown in SEQ ID NO: 67 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,
  - d) polypeptide obtainable from *Trichoderma harzianum* having a sequence identity to the polypeptide shown in SEQ ID NO: 68 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 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity,
- and combinations thereof.

9. The cleaning composition according to any of the preceding claims, wherein the haloperoxidase is selected from the group consisting of: a chloride peroxidase (EC 1.11.1.10) and a bromide peroxidase (EC 1.11.1.18).

10. The cleaning composition according to any of the preceding claims, wherein the haloperoxidase is vanadium haloperoxidase hpxl from *Curvularia veruculosa* or a variant thereof.

11. The cleaning composition according to any of the preceding claims, wherein the haloperoxidase is selected from the group consisting of:

(a) a polypeptide having haloperoxidase activity having at least 60%, 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% sequence identity to the amino acid sequence shown in SEQ ID NO: 82;

(b) a polypeptide having haloperoxidase activity, which is a variant of the polypeptide shown in SEQ ID NO: 82, comprising a substitution, deletion, and/or insertion at one or more positions;



(c) a polypeptide having haloperoxidase activity, which is immunologically cross-reactive with the amino acid sequence set forth in SEQ ID NO: 82;

(d) a polypeptide having haloperoxidase activity, which is a fragment of the polypeptide of (a) (b) or (c)

and combinations thereof.

12. The use of a composition according any of the preceding claims for deep cleaning of an item, wherein the item is a textile or a surface.

13. A method of formulating a cleaning composition comprising adding a DNase, a haloperoxidase and at least one cleaning component.

14. A kit intended for deep cleaning, wherein the kit comprises a solution of an enzyme mixture comprising a DNase, haloperoxidase and optionally a protease.

15. A method of deep cleaning of an item, comprising the steps of:

a) contacting the item with a solution comprising an enzyme mixture comprising a DNase and a haloperoxidase, a source of peroxide; and a cleaning component, wherein the cleaning component is selected from about 0.1 to about 60 wt%, e.g. 0.1 to 15 wt% of at least one a surfactant; from about 1 to about 50 wt% e.g. 0.5 to 20 wt% of at least one builder; from about 1 to about 20 wt% 0.01 to 10 wt% of at least one bleach component; and

b) and optionally rinsing the item, wherein the item is preferably a textile.

## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/056736

A. CLASSIFICATION OF SUBJECT MATTER  
 INV. C11D3/386 C11D1/00 C12N9/22  
 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,P	WO 2017/060475 A2 (NOVOZYMES AS [DK]) 13 April 2017 (2017-04-13)  the whole document -----	1-3,9, 10,12, 13,15
X,P	WO 2017/059802 A1 (NOVOZYMES AS [DK] ; SUN TIANQI [CN]) 13 April 2017 (2017-04-13)  the whole document -----	1-3,9, 10,12, 13,15
X	WO 2017/001472 A1 (NOVOZYMES AS [DK]) 5 January 2017 (2017-01-05)	1,9, 12-15
Y	the whole document -----	10,11
X	WO 2017/001471 A1 (NOVOZYMES AS [DK]) 5 January 2017 (2017-01-05)	1,9, 12-15
Y	the whole document -----  -/-	10,11

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

☒ See patent family annex.

\* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

17 May 2018

Date of mailing of the international search report

16/08/2018

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/056736

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2018/056736

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

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

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos. :
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos. :

1-3 , 9-15 (al l parti al ly)

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-3, 9-15 (all partially)

Cleaning composition comprising DNase polypeptide exhibiting at least 80% sequence identity to SEQ.ID.NO: 1, haloperoxidase and a cleaning component selected from a. a surfactant; b. a builder; and c. a bleach component; methods, uses and kits relating thereto.

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2-25. claims: 1-3, 9-15 (all partially)

Cleaning composition etc. as in non unity subject 1, but comprising DNase polypeptides exhibiting at least 80% sequence identity to SEQ.ID.NO: 2-25, respectively

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26-38. claims: 1, 4, 5, 9-15 (all partially)

Cleaning composition etc. as in non unity subject 1, but comprising DNase polypeptides exhibiting at least 80% sequence identity to SEQ.ID.NO: 26-38, respectively

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39-51. claims: 1, 6, 7, 9-15 (all partially)

Cleaning composition etc. as in non unity subject 1, but comprising DNase polypeptides exhibiting at least 80% sequence identity to SEQ.ID.NO: 39-51, respectively

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52-55. claims: 1, 8-15 (all partially)

Cleaning composition etc. as in non unity subject 1, but comprising DNase polypeptides exhibiting at least 60% sequence identity to SEQ.ID.NO: 65-68, respectively

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2018/056736

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2018/056736

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