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(54) Title: POLYPEPTIDES

(57) Abstract: The present invention relates to new polypeptides, nucleotides encoding the polypeptide, as well as methods of producing the polypeptides. The present invention also relates to detergent composition comprising polypeptides, a laundering method and the use of polypeptides.

POLYPEPTIDES

Reference to a Sequence Listing

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

Field of the Invention

The present invention relates to new polypeptides having deoxyribonuclease (DNase) activity, nucleotides encoding the polypeptide, as well as methods of producing the polypeptides. The present invention also relates to detergent composition comprising a DNase, a laundering method and the use of DNase.

Background of invention

Microorganisms generally live attached to surfaces in many natural, industrial, and medical environments, encapsulated by extracellular substances including biopolymers and macromolecules. The resulting layer of slime encapsulated microorganism is termed a biofilm. Biofilms are the predominant mode of growth of bacteria in the natural environment, and bacteria growing in biofilms exhibit distinct physiological properties. Compared to their planktonically grown counterparts, the bacteria in a biofilm are more resistant to antibiotics, UV irradiation, detergents and the host immune response.

A biofilm may include one or more microorganisms, including gram-positive and gram-negative bacteria, algae, protozoa, and/or yeast or filamentous fungi and viruses and/or bacteriophage. Examples of problematic biofilms are dental plaque, infections on medical implants, but also the initial fouling on ship hulls. Biofilms are attributed to the pathogenesis of many infections in humans and are a significant problem in industry in terms of biofouling of exposed surfaces, where biofilm colonisation can form the base component of a localised ecosystem which can disrupt and interfere with industrial processes and components.

When laundry items like T-shirts or sportswear are used, they are exposed to bacteria from the body of the user and from the rest of the environment in which they are used. Some of these bacteria are capable of adhering to the laundry item and form a biofilm on the item. The presence of bacteria implies that the laundry items become sticky and therefore soil adheres to the sticky areas. This soil has shown difficult to remove by commercially available detergent compositions. Further, when very dirty laundry items are washed together with less dirty laundry items the dirt present in the wash liquor tend to stick to the biofilm. As a result hereof the laundry item is more “soiled” after wash than before wash. Further, these bacteria are a source of bad

odour, which develops after use of the laundry item. The bad odour (malodour) is difficult to remove and may remain even after wash. The reason for this bad odour is adhesion of bacteria to the textile surface. Because of the adhesion to the textile, the bacteria may remain even after wash, and continue to be a source of bad odour.

International patent applications WO2011/098579 (University of Newcastle) and WO2014/087011 (Novozymes A/S) relate to deoxyribonuclease compounds and methods for biofilm disruption and prevention.

Summary of the Invention

The invention relates to novel polypeptides having DNase (deoxyribonuclease) activity and the polynucleotides encoding these.

One aspect of the invention relates to a composition comprising a polypeptide having DNase activity, wherein the polypeptide comprises any of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R|Q] (SEQ ID NO 75), and at least on additional component, selected from,

i. a polyol,
ii. an enzyme preferably selected from protease and lipases,
iii. a surfactant, preferably selected from anionic or nonionic surfactants and
iv. one or more polymer.

A second aspect of the invention relates to a detergent composition comprising a polypeptide having DNase activity selected from the group consisting of SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 or DNases having at least 80% sequence identity hereto and a detergent adjunct ingredient.

A third aspect of the invention relates to a polypeptide having DNase activity, wherein the polypeptide comprises one or more of the motifs [G/Y/W/F/A/H] NI[R/Q/D/E/V] (SEQ ID NO 73), SDH [D/H/L] P (SEQ ID NO 74) or GGNI [R|Q] (SEQ ID NO 75) and wherein the polypeptide is selected from the polypeptides:

a) a polypeptide having at least 72% sequence identity to the polypeptide of SEQ ID NO: 6,
b) a polypeptide having at least 90% sequence identity to the polypeptide of SEQ ID NO: 7,
c) a polypeptide having at least 84,5% sequence identity to the polypeptide of SEQ ID NO: 10,
d) a polypeptide having at least 82% sequence identity to the polypeptide of SEQ ID NO: 27,
e) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 30,

f) a polypeptide having at least 60% sequence identity to the polypeptide of SEQ ID NO: 33,
g) a polypeptide having at least 65% sequence identity to the polypeptide of SEQ ID NO: 36,
h) a polypeptide having at least 91% sequence identity to the polypeptide of SEQ ID NO: 39,
i) a polypeptide having at least 99% sequence identity to the polypeptide of SEQ ID NO: 42,
j) a polypeptide having at least 68% sequence identity to the polypeptide of SEQ ID NO: 45,
k) a polypeptide having at least 94% sequence identity to the polypeptide of SEQ ID NO: 48,
l) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 51,
m) a polypeptide having at least 73% sequence identity to the polypeptide of SEQ ID NO: 57,
n) a polypeptide having at least 90% sequence identity to the polypeptide of SEQ ID NO: 60,
o) a polypeptide having at least 84% sequence identity to the polypeptide of SEQ ID NO: 63,
p) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 66,
q) a polypeptide having at least 72% sequence identity to the polypeptide of SEQ ID NO: 69, and
r) a polypeptide having at least 68% sequence identity to the polypeptide of SEQ ID NO: 72.

One aspect of the invention relates to a polypeptide having DNase activity, selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the polypeptide comprising SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10;

(b) a variant of the mature polypeptide of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 comprising a substitution, deletion, and/or insertion at one or more positions; and

(c) a fragment of the polypeptide of (a) or (b), that has DNase activity.

Another aspect the invention relates to detergent compositions comprising a polypeptide having DNase activity and preferably a detergent adjunct ingredient, such as surfactants, builders etc. One aspect of the invention relates to a composition comprising a polypeptide having DNase activity and having at least 60% sequence identity to a polypeptide selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 and a detergent adjunct.

The invention further relates to a cleaning or laundering method for cleaning or laundering an item comprising the steps of:

a. Exposing an item to a wash liquor comprising a polypeptide having DNase activity or a detergent composition comprising a polypeptide having DNase activity;
b. Completing at least one wash cycle; and
c. Optionally rinsing the item,

wherein the item is a textile and wherein the DNase is selected from the group consisting of polypeptides with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or a polypeptide having at least 60 %

sequence identity to any of the polypeptides with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10.

In addition is claimed the use of a DNase selected from the group consisting of polypeptides with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or a polypeptide having at least 60 % sequence identity to any of the polypeptides with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 for preventing, reducing or removing the biofilm of an item.

The present invention further relates to polynucleotides encoding polypeptides having DNase activity selected from the group consisting of polypeptides with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 and methods of producing the polypeptides.

Sequences

SEQ ID NO 1 DNA sequence obtained from *Vibrissea flavovirens*
SEQ ID NO 2 is the polypeptide sequence derived from SEQ ID NO 1
SEQ ID NO 3 mature polypeptide obtained from *Vibrissea flavovirens*
SEQ ID NO 4 mature polypeptide obtained from *Penicillium reticulisporum*
SEQ ID NO 5 mature polypeptide obtained from *Acremonium dichromosporum*
SEQ ID NO 6 mature polypeptide obtained from *Preussia aemulans*
SEQ ID NO 7 mature polypeptide obtained from *Colletotrichum circinans*
SEQ ID NO 8 mature polypeptide obtained from *Clavicipitaceae*
SEQ ID NO 9 mature polypeptide obtained from *Preussia aemulans*
SEQ ID NO 10 mature polypeptide obtained from *Trichurus spiralis*
SEQ ID NO 11 DNA obtained from *Penicillium reticulisporum*
SEQ ID NO 12 polypeptide obtained SEQ ID NO 11
SEQ ID NO 13 DNA obtained from *Acremonium dichromosporum*
SEQ ID NO 14 polypeptide obtained from SEQ ID NO 13
SEQ ID NO 15 DNA obtained from *Preussia aemulans*
SEQ ID NO 16 polypeptide obtained from SEQ ID NO 15
SEQ ID NO 17 DNA obtained from *Colletotrichum circinans*
SEQ ID NO 18 polypeptide obtained from SEQ ID NO 17
SEQ ID NO 19 DNA obtained from *Clavicipitaceae*
SEQ ID NO 20 polypeptide obtained from SEQ ID NO 19
SEQ ID NO 21 DNA obtained from *Preussia aemulans*
SEQ ID NO 22 polypeptide obtained from SEQ ID NO 21
SEQ ID NO 23 DNA obtained from *Trichurus spiralis*
SEQ ID NO 24 polypeptide obtained from SEQ ID NO 23
SEQ ID NO 25 DNA sequence obtained from *Pyrenochaetopsis sp.*

SEQ ID NO 26 is the polypeptide sequence derived from SEQ ID NO 25
SEQ ID NO 27 mature polypeptide obtained from *Pyrenochaetopsis sp.*
SEQ ID NO 28 DNA sequence obtained from *Aspergillus sydowii*
SEQ ID NO 29 is the polypeptide sequence derived from SEQ ID NO 28
SEQ ID NO 30 mature polypeptide obtained from *Aspergillus sydowii*
SEQ ID NO 31 DNA sequence obtained from *Cladosporium cladosporioides*
SEQ ID NO 32 is the polypeptide sequence derived from SEQ ID NO 31
SEQ ID NO 33 mature polypeptide obtained from *Cladosporium cladosporioides*
SEQ ID NO 34 DNA sequence obtained from *Rhinocladiella sp.*
SEQ ID NO 35 is the polypeptide sequence derived from SEQ ID NO 34
SEQ ID NO 36 mature polypeptide obtained from *Rhinocladiella sp.*
SEQ ID NO 37 DNA sequence obtained from *Pyronema domesticum*
SEQ ID NO 38 is the polypeptide sequence derived from SEQ ID NO 37
SEQ ID NO 39 mature polypeptide obtained from *Pyronema domesticum*
SEQ ID NO 40 DNA sequence obtained from *Aspergillus niger*
SEQ ID NO 41 is the polypeptide sequence derived from SEQ ID NO 40
SEQ ID NO 42 mature polypeptide obtained from *Aspergillus niger*
SEQ ID NO 43 DNA sequence obtained from *Phialophora geniculata*
SEQ ID NO 44 is the polypeptide sequence derived from SEQ ID NO 43
SEQ ID NO 45 mature polypeptide obtained from *Phialophora geniculata*
SEQ ID NO 46 DNA sequence obtained from *Paradendryphiella salina*
SEQ ID NO 47 is the polypeptide sequence derived from SEQ ID NO 46
SEQ ID NO 48 mature polypeptide obtained from *Paradendryphiella salina*
SEQ ID NO 49 DNA sequence obtained from *Aspergillus insuetus*
SEQ ID NO 50 is the polypeptide sequence derived from SEQ ID NO 49
SEQ ID NO 51 mature polypeptide obtained from *Aspergillus insuetus*
SEQ ID NO 52 DNA sequence obtained from *Purpureocillium lilacinum*
SEQ ID NO 53 is the polypeptide sequence derived from SEQ ID NO 52
SEQ ID NO 54 mature polypeptide obtained from *Purpureocillium lilacinum*
SEQ ID NO 55 DNA sequence obtained from *Warcupiella spinulosa*
SEQ ID NO 56 is the polypeptide sequence derived from SEQ ID NO 55
SEQ ID NO 57 mature polypeptide obtained from *Warcupiella spinulosa*
SEQ ID NO 58 DNA sequence obtained from *Stenocarpella maydis*
SEQ ID NO 59 is the polypeptide sequence derived from SEQ ID NO 58
SEQ ID NO 60 mature polypeptide obtained from *Stenocarpella maydis*

SEQ ID NO 61 DNA sequence obtained from *Acrophialophora fusispora*
SEQ ID NO 62 is the polypeptide sequence derived from SEQ ID NO 61
SEQ ID NO 63 mature polypeptide obtained from *Acrophialophora fusispora*
SEQ ID NO 64 DNA sequence obtained from *Chaetomium luteum*
SEQ ID NO 65 is the polypeptide sequence derived from SEQ ID NO 64
SEQ ID NO 66 mature polypeptide obtained from *Chaetomium luteum*
SEQ ID NO 67 DNA sequence obtained from *Arthrinium arundinis*
SEQ ID NO 68 is the polypeptide sequence derived from SEQ ID NO 67
SEQ ID NO 69 mature polypeptide obtained from *Arthrinium arundinis*
SEQ ID NO 70 DNA sequence obtained from *Phialophora geniculata*
SEQ ID NO 71 is the polypeptide sequence derived from SEQ ID NO 70
SEQ ID NO 72 mature polypeptide obtained from *Phialophora geniculata*
SEQ ID NO 73 motif [G/Y/W/F/A/H]NI[R/Q/D/E/V]
SEQ ID NO 74 motif SDH[D/H/L]P
SEQ ID NO 75 motif GGNI[R/Q]

Definitions

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

Biofilm: A biofilm is any group of microorganisms in which cells stick to each other on a surface, such as a textile, dishware or hard surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium. Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community.

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.

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

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

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

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

Deep cleaning: By the term “deep cleaning” is meant disruption or removal of a biofilm or components of a biofilm such as polysaccharides, proteins, DNA, soil or other components present in the biofilm.

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

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

Detergent Composition: The term "detergent composition" refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles. The detergent composition may be used to e.g. clean textiles for both household cleaning and industrial cleaning. The terms encompass any materials/compounds selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, gel, powder, granulate, paste, or spray compositions) and includes, but is not limited to, detergent compositions (e.g., liquid and/or solid laundry detergents and fine fabric detergents; fabric fresheners; fabric softeners; and textile and laundry pre-spotters/pretreatment). In addition to containing the DNases of the invention, the detergent formulation may contain one or more additional enzymes (such as proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases and mannanases, or any mixture thereof), and/or 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.

DNase (deoxyribonuclease): The term "DNase" means a polypeptide with DNase activity that catalyzes the hydrolytic cleavage of phosphodiester linkages in the DNA backbone, thus degrading DNA. The term "DNases" and the expression "a polypeptide with DNase activity" are used interchangeably throughout the application. For purposes of the present invention, DNase activity is determined according to the procedure described in the Assay I. In one aspect, the polypeptides of the present invention have at least 20%, e.g., at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the DNase activity of the mature polypeptide shown in SEQ ID NOS: 2, or the mature polypeptide shown in 3, 4, 5, 6, 7, 8, 9 or 10. In one aspect, the polypeptides of the present invention have at least 20%, e.g., at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the DNase activity of the mature polypeptide shown in SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72. In one embodiment, the polypeptides of the present invention have improved DNase activity, e.g. such that the DNase activity of the polypeptide is at

least 105%, e.g., at least 110%, at least 120%, at least 130%, at least 140%, at least 160%, at least 170%, at least 180%, or at least 200% with reference to the DNase activity of the mature polypeptide of SEQ ID NOS: 2, or the mature polypeptide shown in 3, 4, 5, 6, 7, 8, 9 or 10.

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

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

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

Fragment: The term “fragment” means a polypeptide having one or more (*e.g.*, several) amino acids absent from the amino and/or carboxyl terminus of a mature polypeptide or domain; wherein the fragment has DNase activity.

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

Improved wash performance: The term “improved wash performance” is defined herein as an enzyme displaying an increased wash performance in a detergent composition relative to the wash

performance of same detergent composition without the enzyme e.g. by increased stain removal or less redeposition. The term “improved wash performance” includes wash performance in laundry.

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

Laundering: 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.

Malodour: By the term”malodour” is meant an odour which is not desired on clean items. The cleaned item should smell fresh and clean without malodour s adhered to the item. One example of malodour is compounds with an unpleasant smell, which may be produced by microorganisms. Another example is unpleasant smells can be sweat or body odour adhered to an item which has been in contact with human or animal. Another example of malodour can be the odour from spices, which sticks to items for example curry or other exotic spices which smells strongly. One way of measuring the ability of an item to adhere malodour is by using Assay II disclosed herein.

Mature polypeptide: The term “mature polypeptide” means a polypeptide in its final form following translation and any post-translational modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc. In one aspect, the mature polypeptides are SEQ ID NOS: 3, 4, 5, 6, 7, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In one aspect, the mature polypeptides are any of the following SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72. In another aspect the mature polypeptide is amino acid 20 to 209 of SEQ ID NO 2. In another aspect the mature polypeptide is amino acid 1 to 191 of SEQ ID NO 12. In another aspect the mature

polypeptide is amino acid 1 to 182 of SEQ ID NO 14. In another aspect the mature polypeptide is amino acid 1 to 590 of SEQ ID NO 16. In another aspect the mature polypeptide is amino acid 1 to 589 of SEQ ID NO 18. In another aspect the mature polypeptide is amino acid 1 to 186 of SEQ ID NO 20. In another aspect the mature polypeptide is amino acid 1 to 281 of SEQ ID NO 22. In another aspect the mature polypeptide is amino acid 1 to 585 of SEQ ID NO 24. In another aspect the mature polypeptide is amino acid 1 to 592 of SEQ ID NO 26. In another aspect the mature polypeptide is amino acid 1 to 589 of SEQ ID NO 29. In another aspect the mature polypeptide is amino acid 1 to 597 of SEQ ID NO 32. In another aspect the mature polypeptide is amino acid 1 to 600 of SEQ ID NO 35. In another aspect the mature polypeptide is amino acid 1 to 588 of SEQ ID NO 38. In another aspect the mature polypeptide is amino acid 1 to 585 of SEQ ID NO 41. In another aspect the mature polypeptide is amino acid 1 to 587 of SEQ ID NO 44. In another aspect the mature polypeptide is amino acid 1 to 592 of SEQ ID NO 47. In another aspect the mature polypeptide is amino acid 1 to 594 of SEQ ID NO 50. In another aspect the mature polypeptide is amino acid 1 to 588 of SEQ ID NO 53. In another aspect the mature polypeptide is amino acid 1 to 586 of SEQ ID NO 56. In another aspect the mature polypeptide is amino acid 1 to 590 of SEQ ID NO 59. In another aspect the mature polypeptide is amino acid 1 to 596 of SEQ ID NO 62. In another aspect the mature polypeptide is amino acid 1 to 598 of SEQ ID NO 65. In another aspect the mature polypeptide is amino acid 1 to 592 of SEQ ID NO 68. In another aspect the mature polypeptide is amino acid 1 to 766 of SEQ ID NO 71. It is known in the art that a host cell may produce a mixture of two of more different mature polypeptides (*i.e.*, with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide. It is also known in the art that different host cells process polypeptides differently, and thus, one host cell expressing a polynucleotide may produce a different mature polypeptide (*e.g.*, having a different C-terminal and/or N-terminal amino acid) as compared to another host cell expressing the same polynucleotide. The mature polypeptide of SEQ ID NO 2 is SEQ ID NO 3.

Mature polypeptide coding sequence: The term “mature polypeptide coding sequence” means a polynucleotide that encodes a mature polypeptide having DNase activity. In one aspect, the mature polypeptide coding sequence is the nucleotides 60 to 627 of SEQ ID NO 1.

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

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

Pharmaceutical adjunct ingredient means any pharmaceutical excipient suitable for formulating the pharmaceutical compound. Such excipients, carriers, vehicles etc. are well known to those of skill in the art and are described in text books such as Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985. Pharmaceutically acceptable excipients which are suitable for use in tablet formulations include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. Tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. For hard gelatin capsule formulations, the active ingredient can be mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. For soft gelatin capsule formulations the active ingredient can be mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil. Excipients suitable for the manufacture of aqueous suspensions include suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally- occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters obtained from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters obtained from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. Aqueous suspensions may also contain one or more preservatives, for example benzoates, such as ethyl, or n-propyl p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin. Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavouring agents may be added. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Remission value: Wash performance maybe expressed as a Remission value of the stained swatches. After washing and rinsing the swatches are spread out flat and allowed to air dry at room temperature overnight. All washes swatches are evaluated the day after the wash. Light reflectance evaluations of the swatches may be done using a Macbeth Colour Eye 7000 reflectance

spectrophotometer with very small aperture. Measurements are made without UV in the incident light and remission at 460 nm is extracted.

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 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows: (Identical Residues x 100)/ (Length of Alignment – Total Number of Gaps in Alignment). For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, supra) as implemented in the Needle program of the EMBOSS package (EM-BOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, supra), prefer-ably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

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

Stringency conditions: The term "very low stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 45°C.

The term "low stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 50°C.

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

minutes using 2X SSC, 0.2% SDS at 55°C.

The term "medium-high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 35% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 60°C.

The term "high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 50% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 65°C.

The term "very high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 50% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 70°C.

Subsequence: The term "subsequence" means a polynucleotide having one or more (*e.g.*, several) nucleotides absent from the 5' and/or 3' end of a mature polypeptide coding sequence; wherein the subsequence encodes a fragment having DNase activity.

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

context of the present invention, the term "textile" also covers fabrics.

Variant: The term "variant" means a polypeptide having same activity as the parent enzyme comprising an alteration, *i.e.*, a substitution, insertion, and/or deletion, at one or more (*e.g.*, several) positions. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding an amino acid adjacent to and immediately following the amino acid occupying a position. In the context of the present invention, a variant of an identified DNase has the enzymatic activity of the parent, *i.e.* the capacity of catalyzing the hydrolytic cleavage of phosphodiester linkages in the DNA backbone (deoxyribonuclease activity). In one embodiment, the deoxyribonuclease activity of the variant is increased with reference to the parent DNase, e.g. the polypeptides of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10.

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

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

Wash time**:** The term "wash time" is defined herein as the time it takes for the entire washing process; i.e. the time for the wash cycle(s) and rinse cycle(s) together.

Whiteness: The term "Whiteness" is defined herein as a broad term with different meanings in different regions and for different consumers. Loss of whiteness can e.g. be due to greying, yellowing, or 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.

Nomenclature

For purposes of the present invention, the nomenclature [E/Q] means that the amino acid at this position may be a glutamic acid (Glu, E) or a glutamine (Gln, Q). Likewise the nomenclature [V/G/A/I] means that the amino acid at this position may be a valine (Val, V), glycine (Gly, G), alanine (Ala, A) or isoleucine (Ile, I), and so forth for other combinations as described herein. Unless otherwise limited further, the amino acid X is defined such that it may be any of the 20 natural amino acids.

Detailed Description of the Invention

The present invention relates to novel polypeptides having deoxyribonuclease (DNase) activity (DNases). The DNases can be used for preventing, reducing or removing biofilm on items such as textiles and/or fabric. A polypeptide having DNase activity or a deoxyribonuclease (DNase) is any enzyme that catalyzes the hydrolytic cleavage of phosphodiester linkages in the DNA backbone, thus degrading DNA. The two terms polypeptide having DNase activity and DNase are used interchangeably throughout the application.

Polypeptides

Polypeptides having DNase activity have been described previously e.g. in WO2015/155350 (Novozymes A/S) and WO2015/155351 (Novozymes A/S), describing fungal DNases and the use of such e.g. in detergents. The present invention describes novel polypeptides having DNase activity and the use of such polypeptides and compositions for e.g. preventing, reduction or removal of a biofilm. The polypeptides of the present invention comprises not previously described motifs and domains, which are structurally different and distinguishable from the domains of the DNases described in WO2015/155350 (Novozymes A/S) and WO2015/155351 (Novozymes A/S), in fact the domain and motifs described in the present invention have been identified by the inventors and have not previously been described.

Examples of such a domain is a domain termed NUC1 by the inventors, this domain comprises the motifs [E/D/H]H[I/V/L/F/M]X[P/A/S], [T/D/S][G/N]PQL, [G/T]Y[D/S][R/K/L], [F/L]AND[L/I], C[D/N]T[A/R] and [D/Q][I/V]D[H] the amino acids is in one letter code and the brackets indicate that the amino acids within the bracket are alternatives. Polypeptides having DNase activity and comprises these motif(s) effectively prevent, remove or reduce biofilm and the DNases are particularly useful in cleaning processes, such as laundry and dish wash. Examples of DNases of the invention comprising this domain are the polypeptide shown in SEQ ID NO 3 (mature polypeptide from *Vibrissea flavovirens*) and the polypeptide shown in SEQ ID NO 4 (mature polypeptide from *Penicillium reticulisporum*).

An example of another domain is a domain termed NUC2 by the inventors; this domain comprises the motif [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), where amino acids in brackets are alternatives. The polypeptides of the invention having DNase activity preferably comprises the motif [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73). Polypeptides having DNase activity and comprises these motif have shown to effectively prevent, remove or reduce biofilm and the DNases are particularly useful in cleaning processes, such as laundry and dish wash.

Another distinguishable domain also identified by the inventors is the NUC2_B domain. The polypeptides in NUC2 can be separated into at least one distinct sub-clusters, which were denoted NUC2_B and is defined with motif SDH[D/H/L]P (SEQ ID NO 74), corresponding to position 610 to 614 of SEQ ID NO: 36. In one embodiment of the invention the polypeptides having DNase activity comprises the motif SDH[D/H/L]P (SEQ ID NO 74), wherein the positions corresponding to positions 610 to 614 in SEQ ID NO: 36. The polypeptides of the NUC2_B domain may also comprise the motif GGNI[R/Q] (SEQ ID NO 75), corresponding to positions 395 to 399 of SEQ ID NO: 36. In one embodiment of the invention the polypeptides having DNase activity comprises the motif GGNI[R/Q] (SEQ ID NO 75), wherein the position corresponding to positions 610 to 614 in SEQ ID NO: 36.

the NUC2_B domain have a trusted domain cut-off score of at least 100.0, preferably a score of at least 135, preferably a score of at least 150, preferably a score of at least 250 when queried using a Profile Hidden Markov Model prepared as described in Methods.

Polypeptides belonging to the NUC2_B domain group may share this motif, which is thus common to the DNase polypeptides in the NUC2_B domain. In one embodiment the invention relates to polypeptides comprising the motif SDH[D/H/L]P (SEQ ID NO:74), wherein the polypeptides have DNase activity. Polypeptides having DNase activity and which comprises this motif SDH[D/H/L]P (SEQ ID NO:75) effectively remove or reduce biofilm and the DNases are particularly useful in cleaning processes, such as laundry and dish wash.

In one embodiment the invention relates to polypeptides comprising the motif GGNI[R/Q] (SEQ ID NO: 75), wherein the polypeptides have DNase activity. Polypeptides having DNase activity and which comprises this motif GGNI[R/Q] (SEQ ID NO: 76) effectively remove or reduce biofilm and the DNases are particularly useful in cleaning processes, such as laundry and dish wash.

Examples of DNases of the invention of the NUC2_B domain group are the polypeptide shown in SEQ ID NO 6 (mature polypeptide from Preussia aemulans), the polypeptide shown in SEQ ID NO 7 (mature polypeptide from Colletotrichum circinans), the polypeptide shown in SEQ ID NO 10 (mature polypeptide from Trichurus spiralis), the polypeptide shown in SEQ ID NO 27 (mature polypeptide from Pyrenochaetopsis sp.), the polypeptide shown in SEQ ID NO 30 (mature polypeptide from Aspergillus sydowii), the polypeptide shown in SEQ ID NO 33 (mature polypeptide from Cladosporium cladosporioides), the polypeptide shown in SEQ ID NO 36 (mature polypeptide from Rhinocladiella sp.), the polypeptide shown in SEQ ID NO 39 (mature polypeptide from Pyronema domesticum), the polypeptide shown in SEQ ID NO 42 (mature polypeptide from Aspergillus niger), the polypeptide shown in SEQ ID NO 45 (mature polypeptide from Phialophora geniculata), the polypeptide shown in SEQ ID NO 48 (mature polypeptide from Paradendryphiella

salina), the polypeptide shown in SEQ ID NO 51 (mature polypeptide from Aspergillus insuetus), the polypeptide shown in SEQ ID NO 54 (mature polypeptide from Purpureocillium lilacinum), the polypeptide shown in SEQ ID NO 57 (mature polypeptide from Warcupiella spinulosa), the polypeptide shown in SEQ ID NO 60 (mature polypeptide from Stenocarpella maydis), the polypeptide shown in SEQ ID NO 63 (mature polypeptide from Acrophialophora fusispora), the polypeptide shown in SEQ ID NO 66 (mature polypeptide from Chaetomium luteum), the polypeptide shown in SEQ ID NO 69 (mature polypeptide from Arthrinium arundinis) and the polypeptide shown in SEQ ID NO 72 (mature polypeptide from Phialophora geniculata). The DNases listed above preferably further comprise one or more of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R/Q] (SEQ ID NO 75).

One embodiment of the invention relates to a polypeptide having DNase activity, wherein the polypeptide preferably comprises one or more of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R/Q] (SEQ ID NO 75), wherein the polypeptide is selected from the polypeptides:

a) a polypeptide having at least 72% sequence identity to the polypeptide of SEQ ID NO: 6,
b) a polypeptide having at least 90% sequence identity to the polypeptide of SEQ ID NO: 7,
c) a polypeptide having at least 84,5% sequence identity to the polypeptide of SEQ ID NO: 10,
d) a polypeptide having at least 82% sequence identity to the polypeptide of SEQ ID NO: 27,
e) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 30,
f) a polypeptide having at least 60% sequence identity to the polypeptide of SEQ ID NO: 33,
g) a polypeptide having at least 65% sequence identity to the polypeptide of SEQ ID NO: 36,
h) a polypeptide having at least 91% sequence identity to the polypeptide of SEQ ID NO: 39,
i) a polypeptide having at least 99% sequence identity to the polypeptide of SEQ ID NO: 42,
j) a polypeptide having at least 68% sequence identity to the polypeptide of SEQ ID NO: 45,
k) a polypeptide having at least 94% sequence identity to the polypeptide of SEQ ID NO: 48,
l) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 51,
m) a polypeptide having at least 73% sequence identity to the polypeptide of SEQ ID NO: 57,
n) a polypeptide having at least 90% sequence identity to the polypeptide of SEQ ID NO: 60,
o) a polypeptide having at least 84% sequence identity to the polypeptide of SEQ ID NO: 63,
p) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 66,
q) a polypeptide having at least 72% sequence identity to the polypeptide of SEQ ID NO: 69, and
r) a polypeptide having at least 68% sequence identity to the polypeptide of SEQ ID NO: 72.

A DNase of the present invention may be obtained from *Vibrissea flavovirens* (SEQ ID NO 3 or the mature polypeptide of SEQ ID NO 2), *Penicillium reticulisporum* (SEQ ID NO 4), *Acremonium dichromosporum* (SEQ ID NO 5), *Preussia aemulans* (SEQ ID NO 6), *Colletotrichum circinans* (SEQ ID NO 7), *Clavicipitaceae* (SEQ ID NO 8), *Preussia aemulans* (SEQ ID NO 9) or *Trichurus spiralis* (SEQ ID NO 10). A DNase of the invention may further be obtained from Pyrenochaetopsis sp (SEQ ID NO 27), Aspergillus sydowii (SEQ ID NO 30), Cladosporium cladosporioides (SEQ ID NO 33), Rhinocladiella sp. (SEQ ID NO 36), Pyronema domesticum (SEQ ID NO 39), Aspergillus niger (SEQ ID NO 42), Phialophora geniculata (SEQ ID NO 45), Paradendryphiella salina (SEQ ID NO 48), Aspergillus insuetus (SEQ ID NO 51), Purpureocillium lilacinum (SEQ ID NO 54), Warcupiella spinulosa (SEQ ID NO 57), Stenocarpella maydis (SEQ ID NO 60), Acrophialophora fusispora (SEQ ID NO 63), Chaetomium luteum (SEQ ID NO 66), Arthrinium arundinis (SEQ ID NO 69) and Phialophora geniculata (SEQ ID NO 72).

The DNase of the present invention includes the polypeptides of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or polypeptides having a sequence identity to the mature polypeptide of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 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 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. The DNases of the present invention have all shown to have deep cleaning performance i.e. they have demonstrated to effectively prevent, reduce or limit the presence of biofilm on or in e.g. fabrics/textiles.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 3, or polypeptides 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 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% and which have DNase activity.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 5, or polypeptides 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 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% and which have DNase activity.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 6, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 7, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 8, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 9, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 10, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 27, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 30, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 33, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 36, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 39, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 42, or polypeptides 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 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% and which have DNase activity.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 45, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 48, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 51, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 54, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 57, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 60, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 63, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 66, or polypeptides 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 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.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 69, or polypeptides having a sequence identity to the polypeptide shown in SEQ ID NO 69 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity.

A DNase of the present invention include the polypeptide shown in SEQ ID NO 72, or polypeptides having a sequence identity to the polypeptide shown in SEQ ID NO 72 of at least 60%, e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% and which have DNase activity.

The DNases of the present invention have all shown to have deep cleaning performance i.e. they have demonstrated to effectively prevent, reduce or limit the presence of biofilm on or in e.g. fabrics/textiles.

The DNase may be obtained from *Vibrissea* preferably *Vibrissea flavovirens.* The DNase of the invention may be a polypeptide comprising the polypeptide of SEQ ID NO 3 or a polypeptide

closely related hereto. The DNase of the invention may be obtained from *Vibrissea flavovirens* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 3. The DNase may be obtained from *Penicillium* preferably *Penicillium reticulisporum.* The DNase of the invention may be a polypeptide comprising the polypeptide of SEQ ID NO 4 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Penicillium reticulisporum* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 4. The DNase may be obtained from *Acremonium* preferably *Acremonium dichromosporum.* The DNase of the invention may be a polypeptide comprising the polypeptide of SEQ ID NO 5 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Penicillium reticulisporum* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 5. The DNase may be obtained from *Preussia* preferably *Preussia aemulans.* The DNase of the invention may be a polypeptide comprising the polypeptide of SEQ ID NO 6 or a polypeptide closely related hereto. The DNase of the invention may be a polypeptide comprising the polypeptide of SEQ ID NO 9 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Preussia aemulans* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 5. The DNase of the invention may be obtained from *Preussia aemulans* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 9. The DNase may be obtained from *Colletotrichum* preferably *Colletotrichum circinans.* The DNase of the invention may be a polypeptide comprising the polypeptide of SEQ ID NO 7 or a polypeptide closely related hereto.

The DNase of the invention may be obtained from *Colletotrichum circinans* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 7.

The DNase may be obtained from *Clavicipitaceae.* The DNase of the invention may be a

polypeptide comprising the polypeptide of SEQ ID NO 8 or a polypeptide closely related hereto.

The DNase of the invention may be obtained from *Clavicipitaceae* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 8.

The DNase may be obtained from *Trichurus* preferably *Trichurus spiralis*. The DNase of the invention may be a polypeptide comprising the polypeptide of SEQ ID NO 10 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Trichurus spiralis* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 10.

The DNase may be obtained from *Pyrenochaetopsis* preferably *Pyrenochaetopsis sp.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 27 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Pyrenochaetopsis sp.* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 27.

The DNase may be obtained from *Aspergillus* preferably *Aspergillus sydowii.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 30 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Aspergillus sydowii* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 30.

The DNase may be obtained from *Cladosporium* preferably *Cladosporium cladosporioides.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 33 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Cladosporium cladosporioides* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 33.

The DNase may be obtained from *Rhinocladiella* preferably *Rhinocladiella sp.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 36 or a

polypeptide closely related hereto. The DNase of the invention may be obtained from *Rhinocladiella sp.* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 36.

The DNase may be obtained from *Pyronema* preferably *Pyronema domesticum.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 39 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Pyronema domesticum* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 39.

The DNase may be obtained from *Aspergillus* preferably *Aspergillus niger.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 42 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Aspergillus niger* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 42.

The DNase may be obtained from *Phialophora* preferably *Phialophora geniculata.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 45 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Phialophora geniculata* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 45.

The DNase may be obtained from *Paradendryphiella* preferably *Paradendryphiella salina.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 48 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Paradendryphiella salina* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 48.

The DNase may be obtained from *Aspergillus* preferably *Aspergillus insuetus.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 51 or a

polypeptide closely related hereto. The DNase of the invention may be obtained from *Aspergillus insuetus* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 51.

The DNase may be obtained from *Warcupiella* preferably *Warcupiella spinulosa.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 57 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Warcupiella spinulosa* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 57.

The DNase may be obtained from *Stenocarpella* preferably *Stenocarpella maydis.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 60 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Stenocarpella maydis* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 60.

The DNase may be obtained from *Acrophialophora* preferably *Acrophialophora fusispora.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 63 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Acrophialophora fusispora* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 63.

The DNase may be obtained from *Chaetomium* preferably *Chaetomium luteum.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 66 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Chaetomium luteum* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 66.

The DNase may be obtained from *Arthrinium* preferably *Arthrinium arundinis.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 69 or a

polypeptide closely related hereto. The DNase of the invention may be obtained from *Arthrinium arundinis* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 69.

The DNase may be obtained from *Phialophora* preferably *Phialophora geniculata.* The DNase of the invention may be a polypeptide comprising the polypeptide shown in SEQ ID NO 69 or a polypeptide closely related hereto. The DNase of the invention may be obtained from *Phialophora geniculata* and comprise 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO 72.

In one aspect of the invention, the polypeptide having DNase activity is obtained from *Vibrissea*, in particular from *Vibrissea flavovirens*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Penicillium*, in particular from *Penicillium reticulisporum*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Acremonium*, in particular from *Acremonium dichromosporum*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Preussia*, in particular from *Preussia aemulans*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Colletotrichum*, in particular from *Colletotrichum circinans*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Clavicipitaceae*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Trichurus*, in particular from *Trichurus spiralis*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Pyrenochaetopsis* sp. In one aspect of the invention the polypeptide having DNase activity is obtained from *Aspergillus* in particular from *Aspergillus sydowii*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Cladosporium*, in particular *Cladosporium cladosporioides*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Rhinocladiella* sp. In one aspect of the invention the polypeptide having DNase activity is obtained from *Pyronema*, in particular *Pyronema domesticum*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Aspergillus*, in particular *Aspergillus niger*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Phialophora*, in particular *Phialophora geniculata*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Paradendryphiella*, in particular *Paradendryphiella salina*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Aspergillus*, in particular *Aspergillus insuetus*. In one aspect of the invention the polypeptide having DNase activity is

obtained from *Warcupiella*, in particular *Warcupiella* spinulosa. In one aspect of the invention the polypeptide having DNase activity is obtained from *Stenocarpella maydis*, in particular *Stenocarpella maydis*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Acrophialophora*, in particular *Acrophialophora* fusispora. In one aspect of the invention the polypeptide having DNase activity is obtained from *Chaetomium*, in particular *Chaetomium luteum*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Arthrinium*, in particular *Arthrinium arundinis*. In one aspect of the invention the polypeptide having DNase activity is obtained from *Phialophora*, in particular *Phialophora geniculata*.

In one aspect of the invention, the polypeptide having DNase activity is obtained from *Vibrissea flavovirens* and comprises the mature polypeptide of SEQ ID NO 2 i.e. the polypeptide with SEQ ID NO 3. In a preferred aspect of the invention the DNase is obtained from *Penicillium reticulisporum* and comprises the polypeptide sequence with SEQ ID NO 4. In a preferred aspect of the invention the DNase is obtained from *Acremonium dichromosporum* and comprises the polypeptide sequence with SEQ ID NO 5. In a preferred aspect of the invention the DNase is obtained from *Preussia aemulans* and comprises any of the polypeptide sequence with SEQ ID NO 6 or SEQ ID NO 9. In a preferred aspect of the invention the DNase is obtained from *Colletotrichum circinans* and comprises the polypeptide sequence with SEQ ID NO 7. In a preferred aspect of the invention the DNase is obtained from *Clavicipitaceae* and comprises the polypeptide sequence with SEQ ID NO 8. In a preferred aspect of the invention the DNase is obtained from *Trichurus spiralis* and comprises the polypeptide sequence with SEQ ID NO 10.

In one aspect of the invention, the polypeptide having DNase activity is obtained from *Vibrissea flavovirens* and consist the mature polypeptide of SEQ ID NO 2 i.e. the polypeptide with SEQ ID NO 3. In a preferred aspect of the invention the DNase is obtained from *Penicillium reticulisporum* and consist the polypeptide sequence with SEQ ID NO 4. In a preferred aspect of the invention the DNase is obtained from *Acremonium dichromosporum* and consist the polypeptide sequence with SEQ ID NO 5. In a preferred aspect of the invention the DNase is obtained from *Preussia aemulans* and consists any of the polypeptide sequence with SEQ ID NO 6 or SEQ ID NO 9. In a preferred aspect of the invention the DNase is obtained from *Colletotrichum circinans* and consist the polypeptide sequence with SEQ ID NO 7. In a preferred aspect of the invention the DNase is obtained from *Clavicipitaceae* and consist the polypeptide sequence with SEQ ID NO 8. In a preferred aspect of the invention the DNase is obtained from *Trichurus spiralis* and consist the polypeptide sequence with SEQ ID NO 10.

In a preferred aspect of the invention the DNase is obtained from *Pyrenochaetopsis sp.* and consist the polypeptide sequence with SEQ ID NO 27. In a preferred aspect of the invention

the DNase is obtained from *Pyrenochaetopsis sp.* and comprises the polypeptide sequence with SEQ ID NO 27.

In a preferred aspect of the invention the DNase is obtained from *Aspergillus sydowii* and consist the polypeptide sequence with SEQ ID NO 30. In a preferred aspect of the invention the DNase is obtained from *Aspergillus sydowii* and comprises the polypeptide sequence with SEQ ID NO 30.

In a preferred aspect of the invention the DNase is obtained from *Cladosporium cladosporioides* and consist the polypeptide sequence with SEQ ID NO 33. In a preferred aspect of the invention the DNase is obtained from *Cladosporium cladosporioides* and comprises the polypeptide sequence with SEQ ID NO 33.

In a preferred aspect of the invention the DNase is obtained from *Rhinocladiella sp.* and consist the polypeptide sequence with SEQ ID NO 36. In a preferred aspect of the invention the DNase is obtained from *Rhinocladiella sp.* and comprises the polypeptide sequence with SEQ ID NO 36.

In a preferred aspect of the invention the DNase is obtained from *Pyronema domesticum* and consist the polypeptide sequence with SEQ ID NO 39. In a preferred aspect of the invention the DNase is obtained from *Pyronema domesticum* and comprises the polypeptide sequence with SEQ ID NO 39.

In a preferred aspect of the invention the DNase is obtained from *Aspergillus niger* and consist the polypeptide sequence with SEQ ID NO 42. In a preferred aspect of the invention the DNase is obtained from *Aspergillus niger* and comprises the polypeptide sequence with SEQ ID NO 42.

In a preferred aspect of the invention the DNase is obtained from *Phialophora geniculata* and consist the polypeptide sequence with SEQ ID NO 45. In a preferred aspect of the invention the DNase is obtained from *Phialophora geniculata* and comprises the polypeptide sequence with SEQ ID NO 45.

In a preferred aspect of the invention the DNase is obtained from *Paradendryphiella salina* and consist the polypeptide sequence with SEQ ID NO 48. In a preferred aspect of the invention the DNase is obtained from *Paradendryphiella salina* and comprises the polypeptide sequence with SEQ ID NO 48.

In a preferred aspect of the invention the DNase is obtained from *Aspergillus insuetus* and consist the polypeptide sequence with SEQ ID NO 51. In a preferred aspect of the invention the DNase is obtained from *Aspergillus insuetus* and comprises the polypeptide sequence with SEQ ID NO 51.

In a preferred aspect of the invention the DNase is obtained from *Warcupiella spinulosa*

and consist the polypeptide sequence with SEQ ID NO 57. In a preferred aspect of the invention the DNase is obtained from *Warcupiella spinulosa* and comprises the polypeptide sequence with SEQ ID NO 57.

In a preferred aspect of the invention the DNase is obtained from *Stenocarpella maydis* and consist the polypeptide sequence with SEQ ID NO 60. In a preferred aspect of the invention the DNase is obtained from *Stenocarpella maydis* and comprises the polypeptide sequence with SEQ ID NO 60.

In a preferred aspect of the invention the DNase is obtained from *Acrophialophora fusispora* and consist the polypeptide sequence with SEQ ID NO 63. In a preferred aspect of the invention the DNase is obtained from *Acrophialophora fusispora* and comprises the polypeptide sequence with SEQ ID NO 63.

In a preferred aspect of the invention the DNase is obtained from *Chaetomium luteum* and consist the polypeptide sequence with SEQ ID NO 66. In a preferred aspect of the invention the DNase is obtained from *Chaetomium luteum* and comprises the polypeptide sequence with SEQ ID NO 66.

In a preferred aspect of the invention the DNase is obtained from *Arthrinium arundinis* and consist the polypeptide sequence with SEQ ID NO 69. In a preferred aspect of the invention the DNase is obtained from *Arthrinium arundinis* and comprises the polypeptide sequence with SEQ ID NO 69.

In a preferred aspect of the invention the DNase is obtained from *Phialophora geniculata* and consist the polypeptide sequence with SEQ ID NO 72. In a preferred aspect of the invention the DNase is obtained from *Phialophora geniculata* and comprises the polypeptide sequence with SEQ ID NO 72.

Biofilm can develop on textile when microorganisms are present on an item and stick together on the item. Some microorganisms tend to adhere to the surface of items such as textiles. Some microorganisms adhere to such surfaces and form a biofilm on the surface. The biofilm may be sticky and the adhered microorganisms and/or the biofilm may be difficult to remove. Furthermore, biofilm adhere soil due to the sticky nature of the biofilm. The commercial laundry detergent compositions available on the marked do not remove such adhered microorganisms or biofilm.

The present invention relates to polypeptides having DNase activity and the use of such polypeptides for preventing, reducing or removing a biofilm from an item, such as textiles. In one embodiment of the invention the polypeptide having DNase activity is used for preventing, reducing or removing the stickiness of an item. In one embodiment of the invention, the polypeptide having DNase activity improves whiteness of an item, such as a textile. In one embodiment the polypeptide

of the invention having DNase activity helps maintaining the colour on textiles. When textiles are repeatedly washed the colours tend to be less bright. In one embodiment a polypeptide of the invention having DNase has an improved effect of maintaining the colour of coloured textiles even after repeated washes. In one embodiment the polypeptide of the invention also reduced the colouring of non-coloured part of the same or additional textile present in the wash.

The polypeptide having DNase activity can further be used for pretreating stains on textile such as textile with a pronounced amount of biofilm adhered to the textile.

Additionally, the invention relates to the use of a polypeptide having DNase activity for preventing, reducing or removing redeposition of soil during a wash cycle. When the polypeptide is used for example in the laundering of textile, the polypeptides hinders deposition of soil present in the wash liquor to deposit on the textile.

Further, the invention relates to the use of a polypeptide having DNase activity for preventing, reducing or removing the adherence of soil to an item. In one embodiment, the item is textile. When the soil does not adhere to the item, the item appears cleaner. Thus, the invention further concerns the use of a polypeptide having DNase activity for maintaining or improving the whiteness of the item.

When items like T-shirts or sportswear are used, they are exposed to bacteria from the body of the user and from the rest of the environment in which they are used. This may cause malodour on the item even after the item is washed. The present invention therefore also concerns removal or reduction of malodour on textile. The malodour may be caused by bacteria producing compounds with an unpleasant smell. One example of such unpleasant smelling compounds is E-2-nonenal. The malodour can be present on newly washed textile which is still wet. Or the malodour can be present on newly washed textile, which has subsequently been dried. The malodour may also be present on textile, which has been stored for some time after wash. The present invention relates to the use of DNases of the invention for reduction or removal of malodour such as E-2-nonenal from wet or dry textile.

The polypeptides of the invention having DNase activity i.e. the DNases of the invention have very good cleaning performance in detergents. Examples of beneficial effects of the DNases with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 are the deep-cleaning effect as shown in example 2, e.g. preventing laundry in becoming grey. Examples of beneficial effects of the DNases comprising any of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R|Q] (SEQ ID NO 75 e.g. the polypeptides having DNase activity and comprising the polypeptides shown in SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO

66, SEQ ID NO 69 and SEQ ID NO 72 are the deep-cleaning effect as shown in examples, such effects includes preventing laundry in becoming grey e.g. anti-redeposition effect. Another effect is prevention of static electricity. The polypeptides of the invention having DNase activity can further be used for preventing, reducing or removing static electricity from an item on which static electricity may accumulate, such item maybe a textile or a hard surface. The polypeptide having DNase activity can further be used for preventing, reducing and/or removing a biofilm from an item, such item may be a hard surface e.g. dishes, cutlery, porcelain, china, crockery etc. Thus in some aspect the polypeptide having DNase activity may be used in an ADW (Automatic dishwash) process.

The polypeptides comprising SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 are novel polypeptides having DNase activity and having deep cleaning effect in detergents particular in liquid detergents. The DNase comprising SEQ ID NO 3 also shows deep cleaning effect when tested in powder detergent as shown in example 3.

The polypeptides having DNase activity and comprising the amino acid sequence selected from the sequences shown in SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 72 are novel polypeptides having DNase activity and having deep cleaning effect in detergents such as liquid or powder detergents.

The polypeptide comprising SEQ ID NO 3 is preferably obtained from *Vibrissea flavovirens.* The polypeptide comprising SEQ ID NO 4 is preferably obtained from *Penicillium reticulisporum.* The polypeptide comprising SEQ ID NO 5 is preferably obtained from *Acremonium dichromosporum.* The polypeptide comprising SEQ ID NO 6 is preferably obtained from *Preussia aemulans.* The polypeptide comprising SEQ ID NO 7 is preferably obtained from *Colletotrichum circinans.* The polypeptide comprising SEQ ID NO 8 is preferably obtained from *Clavicipitaceae.* The polypeptide comprising SEQ ID NO 9 is preferably obtained from *Preussia aemulans.* The polypeptide comprising SEQ ID NO 3 is preferably obtained from *Trichurus spiralis*. The invention relates to polypeptides having a sequence identity to any of the polypeptides with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 of at least 60% which have DNase activity and wherein the polypeptides are used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 3 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence

identity to the polypeptide of SEQ ID NO: 4 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 5 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 7 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 8 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 9 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 10 of at least 60%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 33 of at least 60%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 3 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 4 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 5 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 7 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 8 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 9 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 10 of at least 70%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 33 of at least 70%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 36 of at least 70%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 45 of at least 70%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 72 of at least 70%, which have DNase activity and

wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 3 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 4 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 5 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 7 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 8 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 9 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 10 of at least 80%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 33 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence

identity to the polypeptide of SEQ ID NO: 36 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 45 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 72 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 30 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 57 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence

identity to the polypeptide of SEQ ID NO: 69 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 51 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 66 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 3 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 4 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 5 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 7 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 8 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 9 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 10 of at least 90%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 33 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 36 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 45 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 72 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 30 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 57 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 69 of at least 80%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 51 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 66 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 7 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 10 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 63 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 27 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 60 of at least 90%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 3 of at least 95%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 4 of at least 95%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 5 of at least 95%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 95%, which have DNase activity and wherein

the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 7 of at least 95%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 8 of at least 95%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 9 of at least 95%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In an embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 10 of at least 95%, which have DNase activity and wherein the polypeptide is used for preventing, reducing or removing a biofilm from an item.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 33 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 36 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 45 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 72 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least

one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 6 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 30 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 57 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 69 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 51 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 66 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least

one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 7 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 10 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 63 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 27 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 39 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 60 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least

one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 48 of at least 95%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

In one embodiment, the present invention relates to a polypeptide having a sequence identity to the polypeptide of SEQ ID NO: 42 of at least 99%, which have DNase activity and wherein the polypeptide is capable of reducing at least 10%, such as at least 20%, at least 30%, at least 40%, at last 50%, at least 60%, at least 70%, at last 80%, at least 90% or 100% of at least one biofilm from an item when the biofilm reduction is measured e.g. as described in example 2 or 3.

The deep cleaning effect of the polypeptides comprising SEQ ID NO 2, 3, 4, 5, 6, 7, 8, 9 and 10 is shown in example 2. As described above the term “deep cleaning” mean disruption or removal of a biofilm or components of a biofilm such as polysaccharides, proteins, DNA, soil or other components present in the biofilm. A polypeptide of the present invention preferably comprises or consists of the amino acid sequence of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or an allelic variant thereof; or is a fragment thereof having DNase activity. A polypeptide of the present invention preferably comprises or consists of the amino acid sequence of SEQ ID NO: SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 or an allelic variant thereof; or is a fragment thereof having DNase activity In one aspect, the polypeptide of the invention comprises or consists of a polypeptide selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10. In one aspect, the polypeptide of the invention comprises or consists of a polypeptide selected from the group consisting of SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72.

In one embodiment, the present invention relates to an isolated polypeptide with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or a polypeptide with SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO

51, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72 having DNase activity and which is encoded by a polynucleotide that hybridizes under low stringency conditions with (i) the mature polypeptide coding sequence, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii) (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d edition, Cold Spring Harbor, New York). In an embodiment, the polypeptide has been isolated.

In another embodiment, the present invention relates to an isolated polypeptide having DNase activity which is encoded by a polynucleotide that hybridizes under low-medium stringency conditions with (i) the mature polypeptide coding sequence, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii). In an embodiment, the polypeptide has been isolated.

In another embodiment, the present invention relates to an isolated polypeptide having DNase activity which is encoded by a polynucleotide that hybridizes under medium stringency conditions with (i) the mature polypeptide coding sequence, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii). In an embodiment, the polypeptide has been isolated.

In another embodiment, the present invention relates to an isolated polypeptide having DNase activity which is encoded by a polynucleotide that hybridizes under medium-high stringency conditions with (i) the mature polypeptide coding sequence, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii). In an embodiment, the polypeptide has been isolated.

In another embodiment, the present invention relates to an isolated polypeptide having DNase activity which is encoded by a polynucleotide that hybridizes under high stringency conditions with (i) the mature polypeptide coding sequence, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii). In an embodiment, the polypeptide has been isolated.

In another embodiment, the present invention relates to an isolated polypeptide having DNase activity which is encoded by a polynucleotide that hybridizes under very high stringency conditions with (i) the mature polypeptide coding sequence, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii). In an embodiment, the polypeptide has been isolated.

The polynucleotide or a subsequence thereof, as well as the polypeptides of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or the polypeptides comprising an amino acid sequence shown in any of SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72 or a fragment thereof, may be used to design nucleic acid probes to identify and clone DNA encoding polypeptides having DNase activity from strains of different genera or species according to methods well known in the art. In particular, such probes can be used for hybridization with the genomic DNA or cDNA of a cell of interest, following standard Southern blotting procedures, in order to identify and isolate the corresponding gene

therein. Such probes can be considerably shorter than the entire sequence, but should be at least 15, *e.g.*, at least 25, at least 35, or at least 70 nucleotides in length. Preferably, the nucleic acid probe is at least 100 nucleotides in length, *e.g.*, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides or at least 600 nucleotides in length. Both DNA and RNA probes can be used. The probes are typically labeled for detecting the corresponding gene (for example, with ^{32}P, ^{3}H, ^{35}S, biotin, or avidin). Such probes are encompassed by the present invention.

A genomic DNA or cDNA library prepared from such other strains may be screened for DNA that hybridizes with the probes described above and encodes a polypeptide having DNase activity. Genomic or other DNA from such other strains may be separated by agarose or polyacrylamide gel electrophoresis, or other separation techniques. DNA from the libraries or the separated DNA may be transferred to and immobilized on nitrocellulose or other suitable carrier material. In order to identify a clone or DNA that hybridizes with the DNA sequences that encodes SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 or a subsequence thereof, the carrier material is used in a Southern blot.

For purposes of the present invention, hybridization indicates that the polynucleotide hybridizes to a labeled nucleic acid probe corresponding to (i) the mature polypeptide coding sequence; (ii) the cDNA sequence thereof; (iii) the full-length complement thereof; or (iv) a subsequence thereof; under very low, low stringency conditions, low-medium stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions to very high stringency conditions. Molecules to which the nucleic acid probe hybridizes under these conditions can be detected using, for example, X-ray film or any other detection means known in the art.

In another embodiment, the present invention relates to variants of the polypeptide of SEQ ID NOS: 3, 4, 5, 6, 7, 8, 9 or 10 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the mature polypeptide of SEQ ID NOS: 3, 4, 5, 6, 7, 8, 9 or 10 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 3 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide with SEQ ID NO: 3 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide

of SEQ ID NO: 4 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 4 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 5 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 5 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 6 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 6 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 7 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 7 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 8 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 8 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 9 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 9 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 10 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 10 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 27 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 27 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 30 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 30 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 33 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 33 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 36 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 36 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 39 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 39 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 42 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 42 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 45 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 45 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 48 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 48 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 51 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 51 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 54 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 54 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 57 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions

introduced into the polypeptide of SEQ ID NO: 57 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 60 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 60 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 63 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 63 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 66 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 66 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 69 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 69 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In another embodiment, the present invention relates to variants of the mature polypeptide of SEQ ID NO: 72 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the polypeptide of SEQ ID NO: 72 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

The amino acid changes may be of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of 1-30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

Examples of conservative substitutions are within the groups of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In*, *The Proteins*, Academic Press, New York. Common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn,

Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly. Alternatively, the amino acid changes are of such a nature that the physico-chemical properties of the polypeptides are altered. For example, amino acid changes may improve the thermal stability of the polypeptide, alter the substrate specificity, change the pH optimum, and the like. Essential amino acids in a polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for DNase activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identity of essential amino acids can also be inferred from an alignment with a related polypeptide.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer, 1988, *Science* 241: 53-57; Bowie and Sauer, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2152-2156; WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (*e.g.*, Lowman *et al.*, 1991, *Biochemistry* 30: 10832-10837; U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis (Derbyshire *et al.*, 1986, *Gene* 46: 145; Ner *et al.*, 1988, *DNA* 7: 127).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides expressed by host cells (Ness *et al.*, 1999, *Nature Biotechnology* 17: 893-896). Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using standard methods in the art. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide.

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

The polypeptide may be a fusion polypeptide or cleavable fusion polypeptide in which another polypeptide is fused at the N-terminus or the C-terminus of the polypeptide of the present invention. A fusion polypeptide is produced by fusing a polynucleotide encoding another polypeptide to a polynucleotide of the present invention. Techniques for producing fusion

polypeptides are known in the art, and include ligating the coding sequences encoding the polypeptides so that they are in frame and that expression of the fusion polypeptide is under control of the same promoter(s) and terminator. Fusion polypeptides may also be constructed using intein technology in which fusion polypeptides are created post-translationally (Cooper *et al.*, 1993, *EMBO J.* 12: 2575-2583; Dawson *et al.*, 1994, *Science* 266: 776-779).

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

Polynucleotides

The present invention also relates to polynucleotides encoding a polypeptide, as described herein. In an embodiment, the polynucleotide encoding the polypeptide of the present invention has been isolated. In one another embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 1.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 11.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 13.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 15.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 17.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 19.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 21.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 23.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 25.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 28.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 31.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 33.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 17.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 19.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 21.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 23.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 25.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 28.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 31.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 34.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 37.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 40.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 43.

. In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 46.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 49.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 52.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention

comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 55.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 58.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 61.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 64.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 67.

In one embodiment, the polynucleotide encoding a polypeptide of the present invention comprises or consists of the polynucleotide sequence set forth in SEQ ID NO: 70.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 11 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 13 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 15 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 17 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 19 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 21 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 23 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 25 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 28 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at

least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 31 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 34 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 37 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 40 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 43 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 46 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 49 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 52 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 55 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 58 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 61 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 64 or the cDNA sequence thereof of at least

60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 67 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having DNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 70 or the cDNA sequence thereof of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

The techniques used to isolate or clone a polynucleotide are known in the art and include isolation from genomic DNA or cDNA, or a combination thereof. The cloning of the polynucleotides from genomic DNA can be effected, *e.g.*, by using the well-known polymerase chain reaction (PCR) or antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. See, *e.g.*, Innis *et al.*, 1990, *PCR: A Guide to Methods and Application*, Academic Press, New York. Other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligation activated transcription (LAT) and polynucleotide-based amplification (NASBA) may be used.

Modification of a polynucleotide encoding a polypeptide of the present invention may be necessary for synthesizing polypeptides substantially similar to the polypeptide. The term "substantially similar" to the polypeptide refers to non-naturally occurring forms of the polypeptide. These polypeptides may differ in some engineered way from the polypeptide isolated from its native source, *e.g.*, variants that differ in specific activity, thermostability, pH optimum, or the like. The variants may be constructed on the basis of the polynucleotide presented as the mature polypeptide coding sequence or the cDNA sequence thereof, *e.g.*, a subsequence thereof, and/or by introduction of nucleotide substitutions that do not result in a change in the amino acid sequence of the polypeptide, but which correspond to the codon usage of the host organism intended for production of the enzyme, or by introduction of nucleotide substitutions that may give rise to a different amino acid sequence. For a general description of nucleotide substitution, see *e.g.*, Ford *et al.*, 1991, *Protein Expression and Purification* 2: 95-107.

Nucleic Acid Constructs

The present invention also relates to nucleic acid constructs comprising a polynucleotide of the present invention operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

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

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

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

Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*), *Aspergillus oryzae* TAKA amylase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Fusarium oxysporum* trypsin-like protease (WO 96/00787), *Fusarium venenatum* amyloglucosidase (WO 00/56900), *Fusarium venenatum* Daria (WO 00/56900), *Fusarium venenatum* Quinn (WO 00/56900), *Rhizomucor miehei* lipase, *Rhizomucor miehei* aspartic

proteinase, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei* cellobiohydrolase I, *Trichoderma reesei* cellobiohydrolase II, *Trichoderma reesei* endoglucanase I, *Trichoderma reesei* endoglucanase II, *Trichoderma reesei* endoglucanase III, *Trichoderma reesei* endoglucanase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* xylanase III, *Trichoderma reesei* beta-xylosidase, and *Trichoderma reesei* translation elongation factor, as well as the NA2-tpi promoter (a modified promoter from an *Aspergillus* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus* triose phosphate isomerase gene; non-limiting examples include modified promoters from an *Aspergillus niger* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus nidulans* or *Aspergillus oryzae* triose phosphate isomerase gene); and mutant, truncated, and hybrid promoters thereof. Other promoters are described in U.S. Patent No. 6,011,147.

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

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

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

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

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

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

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

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

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

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

The control sequence may also be a polyadenylation sequence; a sequence operably linked to the 3'-terminus of the polynucleotide and, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence that is functional in the host cell may be used.

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

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

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

contain a signal peptide coding sequence. Alternatively, a foreign signal peptide coding sequence may simply replace the natural signal peptide coding sequence in order to enhance secretion of the polypeptide. However, any signal peptide coding sequence that directs the expressed polypeptide into the secretory pathway of a host cell may be used.

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

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

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

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

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

It may also be desirable to add regulatory sequences that regulate expression of the polypeptide relative to the growth of the host cell. Examples of regulatory sequences are those that cause expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory sequences in prokaryotic systems include the *lac*, *tac*, and *trp* operator systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the *Aspergillus niger* glucoamylase promoter, *Aspergillus oryzae* TAKA

alpha-amylase promoter, and *Aspergillus oryzae* glucoamylase promoter, *Trichoderma reesei* cellobiohydrolase I promoter, and *Trichoderma reesei* cellobiohydrolase II promoter may be used. Other examples of regulatory sequences are those that allow for gene amplification. In eukaryotic systems, these regulatory sequences include the dihydrofolate reductase gene that is amplified in the presence of methotrexate, and the metallothionein genes that are amplified with heavy metals. In these cases, the polynucleotide encoding the polypeptide would be operably linked to the regulatory sequence.

Expression Vectors

The present invention also relates to recombinant expression vectors comprising a polynucleotide of the present invention, a promoter, and transcriptional and translational stop signals. The various nucleotide and control sequences may be joined together to produce a recombinant expression vector that may include one or more convenient restriction sites to allow for insertion or substitution of the polynucleotide encoding the polypeptide at such sites. Alternatively, the polynucleotide may be expressed by inserting the polynucleotide or a nucleic acid construct comprising the polynucleotide into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression. The recombinant expression vector may be any vector (*e.g.*, a plasmid or virus) that can be conveniently subjected to recombinant DNA procedures and can bring about expression of the polynucleotide. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may be a linear or closed circular plasmid.

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

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

Examples of bacterial selectable markers are *Bacillus licheniformis* or *Bacillus subtilis dal*

genes, or markers that confer antibiotic resistance such as ampicillin, chloramphenicol, kanamycin, neomycin, spectinomycin, or tetracycline resistance. Suitable markers for yeast host cells include, but are not limited to, ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *adeA* (phosphoribosylaminoimidazole-succinocarboxamide synthase), *adeB* (phosphoribosyl-aminoimidazole synthase), *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus* cell are *Aspergillus nidulans* or *Aspergillus oryzae amdS* and *pyrG* genes and a *Streptomyces hygroscopicus bar* gene. Preferred for use in a *Trichoderma* cell are *adeA*, *adeB*, *amdS*, *hph*, and *pyrG* genes. The selectable marker may be a dual selectable marker system as described in WO 2010/039889. In one aspect, the dual selectable marker is an *hph-tk* dual selectable marker system.

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

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

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

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

pTA1060, and pAMß1 permitting replication in *Bacillus*.

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

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

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

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

Host Cells

The present invention also relates to recombinant host cells, comprising a polynucleotide of the present invention operably linked to one or more control sequences that direct the production of a polypeptide of the present invention. A construct or vector comprising a polynucleotide is introduced into a host cell so that the construct or vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector as described earlier. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication. The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source.

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

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

The bacterial host cell may be any Bacillus cell including, but not limited to, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, and Bacillus thuringiensis cells.

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

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

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

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

The host cell may be a fungal cell. “Fungi” as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota as well as the Oomycota and all mitosporic fungi (as defined by Hawksworth *et al.*, *In*, *Ainsworth and Bisby’s Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK).

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

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

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

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

For example, the filamentous fungal host cell may be an Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanugiNOS:a, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete

chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolour , Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, or Trichoderma viride cell.

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

Methods of Production

The present invention also relates to methods of producing a polypeptide of the present invention, comprising (a) cultivating a cell, which in its wild-type form produces the polypeptide, under conditions conducive for production of the polypeptide; and optionally, (b) recovering the polypeptide.

The present invention also relates to methods of producing a polypeptide of the present invention, comprising (a) cultivating a recombinant host cell of the present invention under conditions conducive for production of the polypeptide; and optionally, (b) recovering the polypeptide.

One aspect of the invention relates to a method of producing a polypeptide, wherein the polypeptide is selected from the group consisting of polypeptides in shown in SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and polypeptide having at least 80% sequence identity hereto, wherein the polypeptide has DNase activity

(a) cultivating the recombinant host cell under conditions conducive for production of the polypeptide; and

(b) recovering the polypeptide.

In one aspect, the cell is Aspergillus, however the host cell may be any of those described under the heading “host cells”

The host cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods known in the art. For example, the cells may be cultivated by shake flask cultivation, or small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

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

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

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

In an alternative aspect, the polypeptide is not recovered, but rather a host cell of the present invention expressing the polypeptide is used as a source of the polypeptide.

In one embodiment, the invention further comprises producing the polypeptide by cultivating the recombinant host cell further comprising a polynucleotide encoding a second polypeptide of interest; preferably an enzyme of interest; more preferably a secreted enzyme of interest; even more preferably a hydrolase, isomerase, ligase, lyase, oxidoreductase, or a transferase; and most preferably the secreted enzyme is an alpha-galactosidase, alpha-glucosidase, aminopeptidase, amylase, asparaginase, beta-galactosidase, beta-glucosidase, beta-xylosidase, carbohydrase, carboxypeptidase, catalase, cellobiohydrolase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, endoglucanase, esterase, green fluorescent protein, glucano-transferase, glucoamylase, invertase, laccase, lipase, manNOS:idase,

mutanase, oxidase, pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or a xylanase.

In one embodiment, the second polypeptide of interest is heterologous or homologous to the host cell.

In one embodiment, the recombinant host cell is a fungal host cell; preferably a filamentous fungal host cell; more preferably an Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes, or Trichoderma cell; most preferably an Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanugiNOS:a, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolour , Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, or Trichoderma viride cell.

In one embodiment, a method of producing the second polypeptide of interest comprises cultivating the host cell under conditions conducive for production of the second polypeptide of interest.

In one embodiment, the method further comprises recovering the second polypeptide of interest.

Fermentation Broth Formulations or Cell Compositions

The present invention also relates to a fermentation broth formulation or a cell composition comprising a polypeptide of the present invention. The fermentation broth product further comprises

additional ingredients used in the fermentation process, such as, for example, cells (including, the host cells containing the gene encoding the polypeptide of the present invention which are used to produce the polypeptide of interest), cell debris, biomass, fermentation media and/or fermentation products. In some embodiments, the composition is a cell-killed whole broth containing organic acid(s), killed cells and/or cell debris, and culture medium.

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

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

In one aspect, the composition contains an organic acid(s), and optionally further contains killed cells and/or cell debris. In one embodiment, the killed cells and/or cell debris are removed from a cell-killed whole broth to provide a composition that is free of these components.

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

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

be permeabilized and/or lysed using methods known in the art.

A whole broth or cell composition as described herein is typically a liquid, but may contain insoluble components, such as killed cells, cell debris, culture media components, and/or insoluble enzyme(s). In some embodiments, insoluble components may be removed to provide a clarified liquid composition.

The whole broth formulations and cell compositions of the present invention may be produced by a method described in WO 90/15861 or WO 2010/096673.

Compositions

The present invention relates to compositions comprising a DNase according to the invention.

Some aspect of the invention relates to a composition comprising at least 0.002 ppm of a polypeptide having DNase activity, wherein the polypeptide comprises any of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R/Q] (SEQ ID NO 75).

The amount of DNase is preferably at least 0.02 ppm but may be at least 0.00008, at least 0.0001, at least 0.0002 at least 0.0005, at least 0.0008, at least 0.001, at least 0.002, at least 0.005, at least 0.008, at least 0.01, at least 0.02, at least 0.05, at least 0.08, at least 0.1, at least 0.2, at least 0.5 or at least 0.5 ppm enzyme protein per gram composition. The amount of DNase is preferably at least 0.02 ppm but may be from 0.00008 to 100, in the range of 0.0001-100, in the range of 0.0002-100, in the range of 0.0004-100, in the range of 0.0008-100, in the range of 0.001-100 ppm enzyme protein, 0.01-100 ppm enzyme protein, 0.01-50 ppm enzyme protein, preferably 0.05-50 ppm enzyme protein, preferably 0.02-50 ppm enzyme protein, 0.015-50 ppm enzyme protein, preferably 0.01-50 ppm enzyme protein, preferably 0.1-50 ppm enzyme protein, preferably 0.2-50 ppm enzyme protein, preferably 0.1-30 ppm enzyme protein, preferably 0.5-20 ppm enzyme protein or preferably 0.5-10 ppm enzyme protein per gram composition.

Some aspect of the invention relates to a composition comprising a polypeptide having DNase activity, wherein the polypeptide having DNase activity comprises the NUC2_B domain, comprises one or both of the motifs SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R/Q] (SEQ ID NO 75). Preferably the amount of DNase is at least 0.02 ppm but may be at least 0.00008, at least 0.0001, at least 0.0002 at least 0.0005, at least 0.0008, at least 0.001, at least 0.002, at least 0.005, at least 0.008, at least 0.01, at least 0.02, at least 0.05, at least 0.08, at least 0.1, at least 0.2, at least 0.5 or at least 0.5 ppm enzyme protein per gram composition

Some aspect of the invention relates to a composition comprising a polypeptide having DNase activity, wherein the polypeptide having DNase activity comprises one or both of the motifs

SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R/Q] (SEQ ID NO 75). Preferably the amount of DNase is at least 0.02 ppm but may be at least 0.00008, at least 0.0001, at least 0.0002 at least 0.0005, at least 0.0008, at least 0.001, at least 0.002, at least 0.005, at least 0.008, at least 0.01, at least 0,02, at least 0.05, at least 0.08, at least 0.1, at least 0.2, at least 0.5 or at least 0.5 ppm enzyme protein per gram composition.

Some aspects of the invention relates to a composition comprising a polypeptide having DNase activity, wherein the polypeptide comprises one or more of the motifs selected from the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R/Q] (SEQ ID NO 75), wherein the polypeptide having DNase activity is selected from the group consisting SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and polypeptides having at least 80 % sequence identity hereto.

Some aspects of the invention relates to a composition comprising a polypeptide having DNase activity, wherein the polypeptide having DNase activity is selected from the group consisting of:

a) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 3,

b) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 4,

c) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 5,

d) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 6,

e) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 7,

f) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 8,

g) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 9,

h) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 10,

i) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 27,

j) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least

95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 30,

k) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 33,

l) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 36,

m) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 39,

n) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 42,

o) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 45,

p) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 48,

q) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 51,

r) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 54,

s) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 57,

t) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 60,

u) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 63,

v) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 66,

x) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 69, and

y) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 72.

The present invention further concerns a detergent composition comprising a polypeptide having DNase activity and preferably a detergent adjunct ingredient. The detergent composition can be used for preventing, reducing or removing biofilm from an item, for preventing, reducing or removing the stickiness of an item, for pretreating stains on the item, for preventing, reducing or removing redeposition of soil during a wash cycle, for reducing or removing adherence of soil to an

item, for maintaining or improving the whiteness of an item and for preventing, reducing or removing malodour from an item, such as E-2-nonenal as described in Assay II. The detergent compositions comprising the polypeptides of the present invention overcomes the problems of the prior art.

The polypeptides of the invention having DNase activity are useful in powder and liquid detergent. In one embodiment of the invention, the detergent composition comprises a DNase, selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and a detergent adjunct. In one embodiment of the invention, the detergent adjunct ingredient is selected from the group consisting of surfactants, builders, flocculating aid, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, builders and co-builders, fabric hueing agents, anti-foaming agents, dispersants, processing aids, and/or pigments.

The detergent adjunct ingredient may be a surfactant. One advantage of including a surfactant in a detergent composition comprising a DNase is that the wash performance may be improved. In one embodiment, the detergent adjunct ingredient is a builder or a clay soil removal/anti-redeposition agent. In one embodiment, detergent adjunct ingredient is an enzyme.

The detergent composition may comprise one or more enzymes, as specified below. The one or more enzymes may be selected from the group consisting of proteases, lipases, cutinases, amylases, carbohydrases, cellulases, pectinases, mannanases, arabinases, galactanases, xylanases and oxidases. Specific enzymes suitable for the detergent compositions of the invention are described below.

In one embodiment, the detergent composition is capable of reducing adhesion of bacteria selected from the group consisting of *Acinetobacter* sp., *Aeromicrobium* sp., *Brevundimonas* sp., *Microbacterium* sp., *Micrococcus luteus*, *Pseudomonas* sp., *Staphylococcus epidermidis,* and *Stenotrophomonas* sp. to a surface, or releasing the bacteria from a surface to which they adhere.

Biofilm which growth in laundry items may originate from many organisms as described previously. One particular abundant bacterium in biofilm originates from *Brevundimonas*. As shown in the examples the DNases of the invention are particularly effective in reducing the growth of the bacterium and reducing the malodour, stickiness and/or re-deposition coursed by these bacteria. One embodiment of the invention relates to the use of a DNase, selected from the group consisting

of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72 in reduction of malodour, reducing stickiness and/or re-deposition. One embodiment relates to the use in laundering of a DNase, selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72, wherein the DNase reducing adhesion of bacteria from *Brevundimonas.*

In one embodiment of the invention, the surface is a textile surface. The textile can be made of cotton, Cotton/Polyester, Polyester, Polyamide, Polyacryl and/or silk.

The detergent composition may be formulated as 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. The detergent composition can be a liquid detergent, a powder detergent or a granule detergent.

The DNases of the invention are suitable for use in cleaning such as laundry. The invention further relates a method for laundering an item, which method comprises the steps of:

a. Exposing an item to a wash liquor comprising a polypeptide having DNase activity selected from the group consisting of polypeptides comprising SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or a detergent composition comprising the polypeptide;
b. Completing at least one wash cycle; and
c. Optionally rinsing the item,

wherein the item is a textile.

The invention further relates a method for laundering an item, which method comprises the steps of:

a. Exposing an item to a wash liquor comprising a polypeptide having DNase activity selected from the group consisting of polypeptides comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72. or a detergent composition comprising the polypeptide;
b. Completing at least one wash cycle; and
c. Optionally rinsing the item,

wherein the item is a textile.

The pH of the liquid solution is in the range of 1 to 11, such as in the range 5.5 to 11, such as in the range of 7 to 9, in the range of 7 to 8 or in the range of 7 to 8.5.

The wash liquor may have a temperature in the range of 5ºC to 95ºC, or in the range of 10ºC to 80ºC, in the range of 10ºC to 70ºC, in the range of 10ºC to 60ºC, in the range of 10ºC to 50ºC, in the range of 15ºC to 40ºC or in the range of 20ºC to 30ºC. In one embodiment the temperature of the wash liquor is 30ºC.

In one embodiment of the invention, the method for laundering an item further comprises draining of the wash liquor or part of the wash liquor after completion of a wash cycle. The wash liquor can then be re-used in a subsequent wash cycle or in a subsequent rinse cycle. The item may be exposed to the wash liquor during a first and optionally a second or a third wash cycle. In one embodiment the item is rinsed after being exposed to the wash liquor. The item can be rinsed with water or with water comprising a conditioner.

The invention further concerns an item washed according to the inventive method.

The detergent composition comprising a polypeptide selected from the group consisting of polypeptides comprising SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 having DNase activity can be used for releasing or removing a biofilm or preventing biofilm formation.

The detergent composition comprising a polypeptide selected from the group consisting of polypeptides comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and having DNase activity can be used for releasing or removing a biofilm or preventing biofilm formation.

The DNases of the invention may be added to a wash liquor.

Thus, one embodiment of the invention relates to a detergent composition comprising one or more anionic surfactants; an enzyme selected from the group consisting of: a protease, a lipase, a cutinase, an amylase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, and an oxidase; and a DNase, selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10.

Thus, one embodiment of the invention relates to a detergent composition comprising one or more anionic surfactants; an enzyme selected from the group consisting of: a protease, a lipase, a cutinase, an amylase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, and an oxidase; and a DNase, selected from the group consisting of SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9,

SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 72.

One embodiment further relates to a washing method for textile comprising:

a. exposing a textile to a wash liquor comprising a DNase or a detergent composition comprising at least one of the DNases,

b. completing at least one wash cycle; and

c. optionally rinsing the textile,

wherein the DNases are selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10.

One embodiment further relates to a washing method for textile comprising:

a. exposing a textile to a wash liquor comprising a DNase or a detergent composition comprising at least one of the DNases,

b. completing at least one wash cycle; and

c. optionally rinsing the textile,

wherein the DNases are selected from the group consisting of SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72.

Another embodiment relates to a textile washed according to the inventive method.

The concentration of the DNase in the wash liquor is typically in the range of 0.00004-100 ppm enzyme protein, such as in the range of 0.00008-100, in the range of 0.0001-100, in the range of 0.0002-100, in the range of 0.0004-100, in the range of 0.0008-100, in the range of 0.001-100 ppm enzyme protein, 0.01-100 ppm enzyme protein, preferably 0.05-50 ppm enzyme protein, more preferably 0.1-50 ppm enzyme protein, more preferably 0.1-30 ppm enzyme protein, more preferably 0.5-20 ppm enzyme protein, and most preferably 0.5-10 ppm enzyme protein.

The DNase of the present invention may be added to a detergent composition in an amount corresponding to at least 0.002 mg of DNase protein, such as at least 0.004 mg of DNase protein, at least 0.006 mg of DNase protein, at least 0.008 mg of DNase protein, at least 0.01 mg of DNase protein, at least 0.1 mg of protein, preferably at least 1 mg of protein, more preferably at least 10 mg of protein, even more preferably at least 15 mg of protein, most preferably at least 20 mg of protein, and even most preferably at least 25 mg of protein. Thus, the detergent composition may comprise at least 0.00008% DNase protein, preferably at least 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.008%, 0.01%, 0.02%, 0.03%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.6%, 0.7%, 0.8%, 0.9% or 1.0% of

DNase protein.

A enzymes present in a detergent of the invention may be stabilized using conventional stabilizing agents, e.g. a polyols such as propylene glycol or glycerol, a sugar or sugar alcohol, different salts such as NaCl or KCl. The proteases of the invention may be stabilized by lactic acid, formic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, or a peptide aldehyde such as di-, tri- or tetrapeptide aldehydes or aldehyde analogues (either of the form B1-B0-R wherein, R is H, CH3, CX3, CHX2, or CH2X (X=halogen), B0 is a single amino acid residue (preferably with an optionally substituted aliphatic or aromatic side chain); and B1 consists of one or more amino acid residues (preferably one, two or three), optionally comprising an N-terminal protection group, or as described in WO09118375, WO98/13459) or a protease inhibitor of the protein type such as RASI, BASI, WASI (bifunctional alpha-amylase/subtilisin inhibitors of rice, barley and wheat) or CI2 or SSI. The composition may be formulated as described in e.g. WO 92/19709, WO 92/19708 and US6,472,364. In some embodiments, the enzymes employed herein are stabilized by the presence of water-soluble sources of zinc (II), calcium (II) and/or magnesium (II) ions in the finished compositions that provide such ions to the enzymes, as well as other metal ions (e.g., barium (II), scandium (II), iron (II), manganese (II), aluminum (III), Tin (II), cobalt (II), copper (II), Nickel (II), and oxovanadium (IV)).

In one embodiment, the polypeptides are stabilized using peptide aldehydes or ketones Suitable peptide aldehydes are described in WO94/04651, WO95/25791, WO98/13458, WO98/13459, WO98/13460, WO98/13461, WO98/13462, WO07/141736, WO07/145963, WO09/118375, WO10/055052 and WO11/036153. A polypeptide of the present invention may also be incorporated in the detergent formulations disclosed in WO 97/07202, which is hereby incorporated by reference.

In another embodiment, the polypeptides are stabilized using a phenyl boronic acid derivative is 4-formylphenylboronic acid (4-FPBA) with the following formula:

The detergent compositions may comprise two or more stabilizing agents e.g. such as those selected from the group consisting of propylene glycol, glycerol, 4-formylphenyl boronic acid and borate.

The detergent compositions may comprise two or more stabilizing agents e.g. such as those selected from the group consisting of propylene glycol, glycerol, 4-formylphenyl boronic acid and borate.

The stabilizing agent(s) is preferably present in the detergent composition in a quantity of from 0.001 to about 5.0 wt%, from 0.01 to about 2.0 wt%, from 0.1 to about 3 wt% or from 0.5% to

about 1.5 wt%.

Liquid detergent composition

The DNases of the invention may also be formulated in liquid laundry compositions such as a liquid laundry compositions composition comprising:

a) at least 0.005 mg of active DNase protein per litre detergent wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10 or a DNase having at least 80 % sequence identity hereto,

b) 2 wt% to 60 wt% of at least one surfactant, and

c) 5 wt% to 50 wt% of at least one builder

The DNases of the invention may also be formulated in liquid laundry compositions such as a liquid laundry compositions composition comprising:

a) at least 0.002 ppm DNase polypeptide, wherein the DNase polypeptide is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 or a DNase having at least 80 % sequence identity hereto,

b) 2 wt% to 60 wt% of at least one surfactant, and

c) 5 wt% to 50 wt% of at least one builder

The surfactant may be selected among nonionic, anionic and/or amphoteric surfactants as described above, preferably anionic or nonionic surfactants but also amphoteric surfactants may be used. In general, bleach-stable surfactants are preferred. Preferred anionic surfactants are sulphate surfactants and in particular alkyl ether sulphates, especially C-9-15 alcohol ethersulfates , C12-15 primary alcohol ethoxylate, C8-C16 ester sulphates and C10-C14 ester sulphates, such as mono dodecyl ester sulphates Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, linear alkylbenzenesulfonates (LAS), isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS), primary alcohol sulfates (PAS), alcohol ethersulfates (AES or AEOS or FES, also known as alcohol ethoxysulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenyl/tetradecenyl succinic acid (DTSA),

fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or salt of fatty acids (soap), and combinations thereof.

The anionic surfactants are preferably added to the detergent in the form of salts. Suitable cations in these salts are alkali metal ions, such as sodium, potassium and lithium and ammonium salts, for example (2-hydroxyethyl)ammonium, bis(2-hydroxyethyl)ammonium and tris(2-hydroxyethyl)ammonium salts. Non-limiting examples of nonionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxylated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxylated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxyalkyl fatty acid amides, or N-acyl N-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamides, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof. Commercially available nonionic surfactants includes Plurafac ™, Lutensol™ and Pluronic™ range from BASF, Dehypon™ series from Cognis and Genapol™ series from Clariant.

The builder is preferably selected among phosphates, sodium citrate builders, sodium carbonate, sodium silicate, sodium aluminosilicate (zeolite). Suitable builders are alkali metal or ammonium phosphates, polyphosphates, phosphonates, polyphosphonates, carbonates, bicarbonates, borates, citrates, and polycarboxylates. Citrate builders, e.g., citric acid and soluble salts thereof (particularly sodium salt), are polycarboxylate builders. Citrates can be used in combination with zeolite, silicates like the BRITESIL types, and/or layered silicate builders. The builder is preferably added in an amount of about 0-65% by weight, such as about 5% to about 50% by weight. In a laundry detergent, the level of builder is typically about 40-65% by weight, particularly about 50-65% by weight, particularly from 20% to 50% by weight. 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), and (carboxymethyl)inulin (CMI), and combinations thereof. Further non-limiting examples of builders 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), methylglycine-N,N- diacetic acid (MGDA), glutamic acid-N,N-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid, 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-(sulfomethyl)aspartic acid (SMAS), N-(2-sulfoethyl)-aspartic acid (SEAS), N-(sulfomethylglutamic acid (SMGL), N-(2-sulfoethyl)-glutamic acid (SEGL), N-methyliminodiacetic acid (MIDA), 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 N´-(2-hydroxyethyl)ethylenediamine-N,N,N'-triacetic acid (HEDTA), diethanolglycine (DEG), and combinations and salts thereof. Phosphonates suitable for use herein include 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetrakis(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis(methylenephosphonic acid) (DTMPA or DTPMPA or DTPMP), nitrilotris(methylenephosphonic acid) (ATMP or NTMP), 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTC), hexamethylenediaminetetrakis(methylenephosphonic acid) (HDTMP).

The 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) or polyaspartic acid. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053.

In one preferred embodiment, the builder is a non-phosphorus based builder such as citric acid and/or methylglycine-N,N-diacetic acid (MGDA) and/or glutamic-N,N-diacetic acid (GLDA) and / or salts thereof. The laundry composition may also be phosphate free in the instance the preferred builders includes citrate and/or methylglycine-N,N-diacetic acid (MGDA) and/or glutamic-N,N-diacetic acid (GLDA) and / or salts thereof.

One embodiment of the invention concerns a liquid laundry compositions composition comprising:

a) at least 0.005 mg of active DNase per litre of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10 or DNases having at least 80% sequence identity hereto,

b) 1% to 15% by weight of at least one surfactant wherein the surfactant is LAS, AEOS and/or SLES, and

c) 5% to 50% by weight of at least one builder selected from HEDP, DTMPA or DTPMPA.

One embodiment of the invention concerns a liquid laundry compositions composition comprising:

a) at least 0.005 mg of active DNase per litre of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 or DNases having at least 80% sequence identity hereto,

b) 1% to 15% by weight of at least one surfactant wherein the surfactant is LAS, AEOS and/or SLES, and

c) 5% to 50% by weight of at least one builder selected from HEDP, DTMPA or DTPMPA.

The liquid detergent composition may typically containing at least 20% by weight and up to 95% water, such as up to 70% water, up to 50% water, up to 40% water, up to 30% water, or up to 20% water. Other types of liquids, including without limitation, alkanols, amines, diols, ethers and polyols may be included in an aqueous liquid detergent. An aqueous liquid detergent may contain from 0-30% organic solvent. A liquid detergent may even be non-aqueous, wherein the water content is below 10%, preferably below 5%.

Powder compositions

The detergent composition may also be formulated into a granular detergent for laundry or dish wash. One embodiment of the invention concerns a granular detergent composition comprising

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, or a DNase having at least 80 % sequence identity hereto,

b) 5 wt % to 50 wt % anionic surfactant

c) 1 wt % to 8 wt % nonionic surfactant, and

d) 5 wt % to 40 wt % builder such as carbonates, zeolites, phosphate builder, calcium sequestering builders or complexing agents

One embodiment of the invention concerns a granular detergent composition comprising

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69,

SEQ ID NO 72 and a DNase having at least 80 % sequence identity hereto,

b) 5 wt % to 50 wt % anionic surfactant

c) 1 wt % to 8 wt % nonionic surfactant, and

d) 5 wt % to 40 wt % builder such as carbonates, zeolites, phosphate builder, calcium sequestering builders or complexing agents

The surfactant may be selected among nonionic, anionic and/or amphoteric surfactants as described above, preferably anionic or nonionic surfactants but also amphoteric surfactants may be used. In general, bleach-stable surfactants are preferred. Preferred anionic surfactants are sulphate surfactants and in particular alkyl ether sulphates, especially C-9-15 alcohol ethersulfates , C12-15 primary alcohol ethoxylate, C8-C16 ester sulphates and C10-C14 ester sulphates, such as mono dodecyl ester sulphates Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, linear alkylbenzenesulfonates (LAS), isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS), primary alcohol sulfates (PAS), alcohol ethersulfates (AES or AEOS or FES, also known as alcohol ethoxysulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenyl/tetradecenyl succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or salt of fatty acids (soap), and combinations thereof.

The anionic surfactants are preferably added to the detergent in the form of salts. Suitable cations in these salts are alkali metal ions, such as sodium, potassium and lithium and ammonium salts, for example (2-hydroxyethyl)ammonium, bis(2-hydroxyethyl)ammonium and tris(2-hydroxyethyl)ammonium salts.

Non-limiting examples of nonionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxylated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxylated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxyalkyl fatty acid amides, or N-acyl N-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamides, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof.

Commercially available nonionic surfactants includes Plurafac ™, Lutensol™ and Pluronic™ range from BASF, Dehypon™ series from Cognis and Genapol™ series from Clariant.

The builder is may be non-phosphate such as citrate preferably as a sodium salt and/or zeolites. Phosphonate builder may be any of those described above.

The builder is preferably selected among phosphates and sodium citrate builders, sodium carbonate, sodium silicate, sodium aluminiumsilicate (zeolite) as described above under the compositions. Suitable builders are described above and include alkali metal or ammonium phosphates, polyphosphates, phosphonates, polyphosphonates, carbonates, bicarbonates, borates, polyhydroxysulfonates, polyacetates, carboxylates, citrates, and polycarboxylates. Citrate builders, e.g., citric acid and soluble salts thereof (particularly sodium salt), are polycarboxylate builders. The builder is preferably added in an amount of about 0-65% by weight, such as about 5% to about 50% by weight, such as 5 to 40 % by weight, such as 10 to 40 % by weight, such as 10 to 30 % by weight, such as 15 to 20 % by weight or such as 20 to 40 % by weight. The builder may be a phosphonate builder including 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra (methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis (methylenephosphonic acid) (DTMPA or DTPMPA), diethylenetriamine penta (methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTC) and hexamethylenediaminetetra (methylenephosphonic acid) (HDTMP).

Preferred phosphonates includes 1-hydroxyethane-1,1-diphosphonic acid (HEDP) and/or diethylenetriaminepentakis (methylenephosphonic acid) (DTMPA or DTPMPA). The phosphonate is preferably added in an amount of about in a level of from about 0.01 % to about 10 % by weight, preferably from 0.1 % to about 5 % by weight, more preferably from 0.5 % to 3 % by weight of the composition.

The laundry composition may also be phosphate free in the instance the preferred builders includes citrate, carbonates and/or sodium aluminiumsilicate (zeolite).

The detergent may contain 0-30% by weight, such as about 1% to about 20%, of a bleaching system. Any bleaching system comprising components known in the art for use in cleaning detergents may be utilized. Suitable bleaching system components include sources of hydrogen 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).

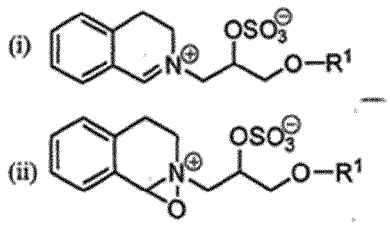
Sources of peracids: Peracids may be (a) incorporated directly as preformed peracids or (b) formed in situ in the wash liquor from hydrogen peroxide and a bleach activator (perhydrolysis) or (c) formed in situ in the wash liquor from hydrogen peroxide and a perhydrolase and a suitable substrate for the 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-α-naphthoic acid, peroxyphthalic acid, peroxylauric acid, peroxystearic acid, ε-phthalimidoperoxycaproic acid [phthalimidoperoxyhexanoic acid (PAP)], and o-carboxybenzamidoperoxycaproic acid; aliphatic and aromatic diperoxydicarboxylic acids such as diperoxydodecanedioic acid, diperoxyazelaic acid, diperoxysebacic acid, diperoxybrassylic acid, 2-decyldiperoxybutanedioic acid, and diperoxyphthalic, -isophthalic and -terephthalic acids; perimidic acids; peroxymoNOS:ulfuric acid; peroxydisulfuric acid; peroxyphosphoric acid; peroxysilicic 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 tetraacetylethylenediamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene-1-sulfonate (ISONOBS), sodium 4-(dodecanoyloxy)benzene-1-sulfonate (LOBS), sodium 4-(decanoyloxy)benzene-1-sulfonate, 4-(decanoyloxy)benzoic acid (DOBA), sodium 4-(nonanoyloxy)benzene-1-sulfonate (NOBS), and/or those disclosed in WO98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particularly preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that they are environmentally friendly. Furthermore, acetyl triethyl citrate and triacetin have good hydrolytical stability in the product upon storage and are efficient bleach activators. Finally, ATC is multifunctional, as the citrate released in the perhydrolysis reaction may function as a builder.

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(O)3Mn(Me3-TACN)](PF6)2, and [2,2',2''-nitrilotris(ethane-1,2-diylazanylylidene-κN-methanylylidene)triphenolato-κ3O]manganese(III). The bleach catalysts may also be other metal compounds, such as iron or cobalt complexes.

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



(iii) and mixtures thereof; wherein each R1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R1 is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, dodecyl, tetradecyl, hexadecyl, octadecyl, isononyl, isodecyl, isotridecyl and isopentadecyl.

Other exemplary bleaching systems are described, e.g. in WO2007/087258, WO2007/087244, WO2007/087259, EP1867708 (Vitamin K) and WO2007/087242. Suitable photobleaches may for example be sulfonated zinc or aluminium phthalocyanines.

According to one embodiment and any of the previous embodiments, the invention also relates to a cleaning composition comprising;

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9 and SEQ ID NO 10 or a DNase having at least 80 % sequence identity hereto,

b) 10-50 wt % builder and

c) at least one bleach component, wherein the bleach is a peroxide and the bleach catalyst is a manganese compound.

According to one embodiment and any of the previous embodiments, the invention also relates to a cleaning composition comprising;

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and a DNase having at least 80 % sequence identity hereto,

b) 10-50 wt % builder and

c) at least one bleach component, wherein the bleach is a peroxide and the bleach catalyst is a manganese compound.

The oxygen bleach is preferably percarbonate and the manganese catalyst preferably 1,4,7-trimethyl-1,4,7-triazacyclononane or manganese (III) acetate tetrahydrate.

According to one embodiment and any of the previous embodiments the invention also relates to a cleaning composition comprising;

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9 and SEQ ID NO 10,

b) 10-50 wt % builder selected from citric acid, methyl glycine-N,N-diacetic acid (MGDA) and/or glutamic-N,N-diacetic acid (GLDA) and mixtures thereof, and

c) at least one bleach component, wherein the bleach is an oxygen bleach and the bleach catalyst is a manganese compound.

According to one embodiment and any of the previous embodiments the invention also relates to a cleaning composition comprising;

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and a DNase having at least 80 % sequence identity hereto,

b) 10-50 wt % builder selected from citric acid, methyl glycine-N,N-diacetic acid (MGDA) and/or glutamic-N,N-diacetic acid (GLDA) and mixtures thereof, and

c) at least one bleach component, wherein the bleach is an oxygen bleach and the bleach catalyst is a manganese compound. The oxygen bleach is preferably percarbonate and the manganese catalyst preferably 1,4,7-trimethyl-1,4,7-triazacyclo-nonane or manganese (II) acetate tetrahydrate

According to one embodiment and any of the previous embodiments the invention also relates to a cleaning composition comprising;

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9 and SEQ ID NO 10 or a DNase having at least 80 % sequence identity hereto,

b) 10-50 wt % builder selected from citric acid, methyl glycine-N,N-diacetic acid (MGDA) and/or glutamic-N,N-diacetic acid (GLDA) and mixtures thereof, and

c) 0.1-40 wt%, preferably from 0.5-30 wt%, of bleaching components, wherein the bleach

components are a peroxide, preferably percabonate and a metal-containing bleach catalyst preferably 1,4,7-trimethyl-1,4,7-triazacyclononane or manganese (II) acetate tetrahydrate (MnTACN).

According to one embodiment and any of the previous embodiments the invention also relates to a cleaning composition comprising;

a) at least 0.005 mg of active DNase per gram of composition wherein the DNase is selected from a polypeptide comprising SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and a DNase having at least 80 % sequence identity hereto,

b) 10-50 wt % builder selected from citric acid, methyl glycine-N,N-diacetic acid (MGDA) and/or glutamic-N,N-diacetic acid (GLDA) and mixtures thereof, and

c) 0.1-40 wt%, preferably from 0.5-30 wt%, of bleaching components, wherein the bleach components are a peroxide, preferably percabonate and a metal-containing bleach catalyst preferably 1,4,7-trimethyl-1,4,7-triazacyclononane or manganese (II) acetate tetrahydrate (MnTACN).

The choice of detergent components may include, for textile care, the consideration of the type of textile to be cleaned, the type and/or degree of soiling, the temperature at which cleaning is to take place, and the formulation of the detergent product. 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.

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 polyglycolethers, 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), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) or poly(ethylene oxide) (PEG), ethoxylated poly(ethyleneimine), carboxymethyl inulin (CMI), and polycarboxylates such as PAA, PAA/PMA, poly-aspartic acid, and lauryl methacrylate/acrylic acid copolymers , hydrophobically modified CMC (HM-CMC) and silicones, copolymers of terephthalic acid and oligomeric glycols, copolymers of poly(ethylene terephthalate) and poly(oxyethene terephthalate) (PET-POET), PVP, poly(vinylimidazole) (PVI), poly(vinylpyridine-*N*-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Further exemplary polymers include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated.

Fabric hueing agents

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

Enzymes

The cleaning compositions of the invention may comprise one or more additional enzymes

such as a protease, lipase, cutinase, an amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, xylanase, oxidase, *e.g.*, a laccase, and/or peroxidase.

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

Cellulases

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

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and WO99/001544.

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

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

Proteases

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

The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. Serine

proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 sub-divisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family. Examples of subtilases are those obtained from *Bacillus* such as *Bacillus lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii* described in; US7262042 and WO09/021867, and *subtilisin lentus*, *subtilisin Novo*, *subtilisin Carlsberg*, *Bacillus licheniformis*, *subtilisin BPN'*, *subtilisin 309*, *subtilisin 147* and *subtilisin 168* described in WO89/06279 and protease PD138 described in (WO93/18140). Other useful proteases may be those described in WO92/175177, WO01/016285, WO02/026024 and WO02/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO89/06270, WO94/25583 and WO05/040372, and the chymotrypsin proteases obtained from *Cellumonas* described in WO05/052161 and WO05/052146.

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

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

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

Specific examples of useful proteases are the variants described in: WO92/19729, WO96/034946, WO98/20115, WO98/20116, WO99/011768, WO01/44452, WO03/006602, WO04/03186, WO04/041979, WO07/006305, WO11/036263, WO11/036264, especially the variants with substitutions in one or more of the following positions: 3, 4, 9, 15, 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 1 of WO 2016/001449. More preferred the subtilase variants may comprise the mutations: S3T, V4I, S9R, S9E, A15T, S24G, S24R, K27R, N42R, S55P, G59E, G59D, N60D, N60E, V66A, N74D, N85S, N85R, , G96S, G96A, S97G, S97D, S97A, S97SD, S99E, S99D, S99G, S99M, S99N, S99R, S99H, S101A, V102I, V102Y, V102N, S104A, G116V, G116R, H118D, H118N, N120S, S126L, P127Q, S128A, S154D, A156E, G157D, G157P, S158E, Y161A, R164S, Q176E, N179E, S182E, Q185N, A188P, G189E, V193M, N198D, V199I, Y203W, S206G, L211Q, L211D, N212D, N212S, M216S, A226V, K229L, Q230H, Q239R, N246K, N255W, N255D, N255E, L256E, L256D T268A, R269H. The protease variants are preferably variants of the Bacillus Lentus protease (Savinase®) shown in SEQ ID NO 1 of WO 2016/001449, the Bacillus amylolichenifaciens protease (BPN') shown in SEQ ID NO 2 of WO 2016/001449. The protease variants preferably have at least 80 % sequence identity to SEQ ID NO 1 or SEQ ID NO 2 of WO 2016/001449.

Or a protease selected from a protease variant comprising a substitution at one or more positions corresponding to positions 171, 173, 175, 179, or 180 of SEQ ID NO: 1 of WO 2004/067737, wherein said protease variant has a sequence identity of at least 75% but less than 100% to SEQ ID NO: 1 of WO 2004/067737.

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, Ovozyme®, Coronase®, Coronase® Ultra, Blaze, Blaze Evity® 100T, Blaze Evity® 125T, Blaze Evity® 150T, Neutrase®, Everlase® and Esperase® (Novozymes A/S), those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Purafect®, Purafect Prime®, Preferenz™, Purafect MA®, Purafect Ox®, Purafect OxP®, Puramax®, Properase®, Effectenz™, 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-Brocases N.V.), BLAP (sequence shown in Figure 29 of US5352604) and variants hereof (Henkel AG) and KAP (*Bacillus alkalophilus* subtilisin) from Kao.

Some aspect of the invention relates to a composition, such as a detergent e.g. cleaning composition comprising a polypeptide having DNase activity, wherein the polypeptide having DNase activity is selected from the group consisting of the polypeptides shown in: SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and polypeptides having at

least 60%, e.g. 70% e.g. 80% e.g. at least 90% sequence identity hereto, wherein the composition further comprises: at least 0.01 ppm of one or more protease variant 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 protease shown in SEQ ID NO 1 of WO 2011/036263.

Lipases and Cutinases

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. lanugiNOS:us* (previously named *Humicola lanugiNOS:a*) as described in EP258068 and EP305216, cutinase from *Humicola*, e.g. *H. insolens* (WO96/13580), lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g. *P. alcaligenes* or *P. pseudoalcaligenes* (EP218272), *P. cepacia* (EP331376), *P. sp.* strain SD705 (WO95/06720 & WO96/27002), *P. wisconsinensis* (WO96/12012), GDSL-type *Streptomyces* lipases (WO10/065455), cutinase from *Magnaporthe grisea* (WO10/107560), cutinase from *Pseudomonas mendocina* (US5,389,536), lipase from *Thermobifida fusca* (WO11/084412), *Geobacillus stearothermophilus* lipase (WO11/084417), lipase from *Bacillus subtilis* (WO11/084599), and lipase from *Streptomyces griseus* (WO11/150157) and *S. pristinaespiralis* (WO12/137147).

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

Preferred commercial lipase products include Lipolase™, Lipex™; Lipolex™ and Lipoclean™ (Novozymes A/S), Lumafast (originally from Genencor) and Lipomax (originally from Gist-Brocades). Still other examples are lipases sometimes referred to as acyltransferases or perhydrolases, e.g. acyltransferases with homology to *Candida antarctica* lipase A (WO10/111143), acyltransferase from *Mycobacterium smegmatis* (WO05/56782), perhydrolases from the CE 7 family (WO09/67279), and variants of the *M. smegmatis* perhydrolase in particular the S54V variant used in the commercial product Gentle Power Bleach from Huntsman Textile Effects Pte Ltd (WO10/100028).

Some aspect of the invention relates to a composition, such as a detergent e.g. cleaning composition comprising a polypeptide having DNase activity, wherein the polypeptide having DNase activity is selected from the group consisting of the polypeptides shown in: SEQ ID NO 3,

SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and polypeptides having at least 60%, e.g. 70% e.g. 80% e.g. at least 90% sequence identity hereto, wherein the composition further comprises:

a) at least 0.01 ppm one or more lipase.

Amylases

Suitable amylases which can be used together with the DNases of the invention may be an alpha-amylase or a glucoamylase 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.

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

M197T;

H156Y+A181T+N190F+A209V+Q264S; or

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

Further amylases which are suitable are amylases having SEQ ID NO: 6 in WO 99/019467

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

Additional amylases which can be used are those having SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873 or variants thereof having 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7 are those having a substitution, a deletion or an insertion in one or more of the following positions: 140, 181, 182, 183, 184, 195, 206, 212, 243, 260, 269, 304 and 476, 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, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:

N128C+K178L+T182G+Y305R+G475K;

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

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

S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K, wherein the variants are C-terminally truncated and optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO01/66712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one of more of the following positions of SEQ ID NO: 12 in WO01/66712: R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, N484. Particular preferred amylases include variants having a deletion of D183 and G184 and having the substitutions R118K, N195F, R320K and R458K, and a variant additionally having substitutions in one or more position selected from the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions.

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

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

Some aspect of the invention relates to a composition, such as a detergent e.g. cleaning composition comprising a polypeptide having DNase activity, wherein the polypeptide having DNase activity is selected from the group consisting of the polypeptides shown in: SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and polypeptides having at least 60%, e.g. 70% e.g. 80% e.g. at least 90% sequence identity hereto, wherein the composition further comprises:

a) at least 0.01 ppm of one or more amylase variant, wherein the variant comprises:

(i) one or more substitutions in the following positions: 9, 26, 30, 33, 82, 37, 106, 118, 128, 133, 149, 150, 160, 178, 182, 186, 193, 195, 202, 203, 214, 231, 256, 257, 258, 269, 270, 272, 283, 295, 296, 298, 299, 303, 304, 305, 311, 314, 315, 318, 319, 320, 323, 339, 345, 361, 378, 383, 419, 421, 437, 441, 444, 445, 446, 447, 450, 458, 461, 471, 482, 484, wherein the positions corresponds to positions of SEQ ID NO 2 of WO2000/060060;

(ii) exhibiting at least 90 percent identity with SEQ ID NO 2 of WO96/023873, with deletions in the 183 and 184 positions; or

(iii) variants exhibiting at least 95 percent identity with SEQ ID NO:3 of WO2008/112459, comprising mutations in one or more of the following positions M202, M208, S255, R172 and/or

M261.

Peroxidases/Oxidases

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

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

A peroxidase according to the invention also includes a haloperoxidase enzyme, such as chloroperoxidase, bromoperoxidase and compounds exhibiting chloroperoxidase or bromoperoxidase activity. Haloperoxidases are classified according to their specificity for halide ions. Chloroperoxidases (E.C. 1.11.1.10) catalyze formation of hypochlorite from chloride ions. In an embodiment, the haloperoxidase of the invention is a chloroperoxidase. Preferably, the haloperoxidase is a vanadium haloperoxidase, *i.e.*, a vanadate-containing haloperoxidase. In a preferred method of the present invention the vanadate-containing haloperoxidase is combined with a source of chloride ion.

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

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

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

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

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

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

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

Non-dusting granulates may be produced, *e.g.* as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

Other materials

Any detergent components known in the art for use in the cleaning composition of the invention may also be utilized. Other optional detergent components include anti-corrosion agents, anti-shrink agents, anti-soil redeposition agents, anti-wrinkling agents, bactericides, binders, corrosion inhibitors, disintegrants/disintegration agents, dyes, enzyme stabilizers (including boric acid, borates, CMC, and/or polyols such as propylene glycol), fabric conditioners including clays, fillers/processing aids, fluorescent whitening agents/optical brighteners, foam boosters, foam (suds) regulators, perfumes, soil-suspending agents, softeners, suds suppressors, tarnish inhibitors, and

wicking agents, either alone or in combination. Any ingredient known in the art for use in detergents may be utilized. The choice of such ingredients is well within the skill of the artisan.

Dispersants

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 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 inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine *N*-oxide polymers, copolymers of *N*-vinylpyrrolidone and *N*-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. 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

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 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-(2*H*-naphtho[1,2-*d*][1,2,3]triazol-2-yl)-2-[(*E*)-2-phenylvinyl]benzenesulfonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt

of 4,4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenyl-styryl)-disulfonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Tinopal CBS-X is a 4.4'-bis-(sulfostyryl)-biphenyl disodium salt also known as Disodium Distyrylbiphenyl Disulfonate. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins.

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

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 polymers may for example be nonionic or anionic terephthalte based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides see for example Chapter 7 in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc. Another type of soil release polymers are amphiphilic alkoxylated grease cleaning polymers comprising a core structure and a plurality of alkoxylate groups attached to that core structure. The core structure may comprise a polyalkylenimine structure or a polyalkanolamine structure as described in detail in WO 2009/087523 (hereby incorporated by reference). Furthermore random graft co-polymers are suitable soil release polymers. Suitable graft co-polymers are described in more detail in WO 2007/138054, WO 2006/108856 and WO 2006/113314 (hereby incorporated by reference). Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose deriviatives such as those described in EP 1867808 or WO 2003/040279 (both are hereby incorporated by reference). Suitable cellulosic polymers include cellulose, cellulose ethers, cellulose esters, cellulose amides and mixtures thereof. Suitable cellulosic polymers include anionically modified cellulose, nonionically modified cellulose, cationically modified cellulose, zwitterionically modified cellulose, and mixtures thereof. Suitable cellulosic polymers include methyl cellulose, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, ester carboxy methyl cellulose, and mixtures thereof.

Anti-redeposition agents

The 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 adjunct materials include, but are not limited to, anti-shrink agents, anti-wrinkling agents, bactericides, binders, carriers, dyes, enzyme stabilizers, fabric softeners, fillers, foam regulators, hydrotropes, perfumes, pigments, sod suppressors, solvents, and structurants for liquid detergents and/or structure elasticizing agents.

Anti-parasitic/viral compounds

The cleaning composition may further comprise an antiparasitic compound can be one or more of a benzazole, such as albendazole, mebendazole and tiabendazole; an azole, such as metronidazole and tinidazole; a macrocycle, such as amphotericin B, rifampin and ivermectin; pyrantel pamoate; diethylcarbamazine; niclosamide; praziquantel; melarsopro; and eflornithine.

The antiviral compound can be one or more of a nucleoside analog reverse transcriptase inhibitor, such as acyclovir, didaNOS:ine, stavudine, zidovudine, lamivudine, abacavir, emtricitabine and entecavir; an uncoating inhibitor such as amantadine, rimantadine and pleconaril; a protease inhibitor such as saquinavir, ritonavir, indinavir, nelfinavir and amprenavir; zanamivir; oseltamivir; and rifampin . The antibacterial compound can be one or more of an aminoglycoside such as gentamicin, kanamycin and streptomycin; a beta-lactam such as penicillin, ampicillin and imipenem; a cephalosporin such as ceftazidime, a quinolone such as ciprofloxacin; a macrolide such as azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin and telithromycin; an oxazolidinone such as linezolid ; an ansamycin such as rifamycin; a sulphonamide; a tetracycline such as doxycycline; a glycopeptide such as vancomycin; sulfisoxazole, trimethoprim, novobiocin, daptomycin and linezolid.

The antifungal compound can be one or more of an azole, such as miconazole, ketoconazole, clotrimazole, econazole, omoconazole, bifonazole, butoconazole, fenticonazole,

isoconazole, sertaconazole, sulconazole, tioconazole, fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazole, terconazole and abafungin; a macrocycle, such as natamycin, rimocidin, filipin, nystatin, amphotericin B, candicin, hamycin; an allyl amine such as terbinafine, naftifine and butenafine; an echinocandin such as andidulafungin, caspofungin and micafungin; or others such as polygodial, ciclopirox, tolnaftate, benzoic acid, undecylenic acid, flucytosine and griseofulvin.

Formulation of DNases in microcapsule

The DNases of the invention may be formulated in microcapsules or in liquid detergents comprising microcapsules. A liquid cleaning composition of the invention may comprise a surfactant and a detergent builder in a total concentration of at least 3% by weight, and an enzyme, which may be a DNase, containing microcapsule, wherein the membrane of the microcapsule is produced by cross-linking of a polybranched polyamine having a molecular weight of more than 1 kDa. Encapsulating of enzymes such as DNases in a microcapsule with a semipermeable membrane having a water activity inside these capsules (prior to addition to the liquid detergent) higher than in the liquid detergent, the capsules will undergo a (partly) collapse when added to the detergent (water is oozing out), thus leaving a more concentrated and more viscous enzyme containing interior in the capsules. The collapse of the membrane may also result in a reduced permeability. This can be further utilized by addition of stabilizers/polymers, especially ones that are not permeable through the membrane. The collapse and resulting increase in viscosity will reduce/hinder the diffusion of hostile components (e.g., surfactants or sequestrants) into the capsules, and thus increase the storage stability of enzymes such as DNases in the liquid detergent. Components in the liquid detergent that are sensitive to the enzyme (e.g., components that act as substrate for the enzyme) are also protected against degradation by the enzyme. During wash the liquid detergent is diluted by water, thus increasing the water activity. Water will now diffuse into the capsules (osmosis). The capsules will swell and the membrane will either become permeable to the enzyme so they can leave the capsules, or simply burst and in this way releasing the enzyme. The concept is very efficient in stabilizing the enzymes such as the DNases of the invention against hostile components in liquid detergent, and vice versa also protects enzyme sensitive components in the liquid detergent from enzymes.

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

Also, sensitive detergent ingredients can be encapsulated, and thus stabilized, in the microcapsules of the invention. Sensitive detergent ingredients are prone to degradation during storage. Such detergent ingredients include bleaching compounds, bleach activators, perfumes, polymers, builder, surfactants, etc.

Generally, the microcapsules can be used to separate incompatible components/compounds in detergents.

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

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

Unless otherwise indicated, all percentages are indicated as percent by weight (% w/w) throughout the application.

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

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

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

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

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

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

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

The resulting capsules can be further formulated, e.g., by addition of surfactants to give the product the desired properties for storage, transport and later handling and addition to the detergent. Other microcapsule formulation agents include rheology modifiers, biocides (e.g., Proxel), acid/base for adjustment of pH (which will also adjust inside the microcapsules), and water for adjustment of water activity.

The capsule forming process may include the following steps:

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

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

A microcapsule may be a small aqueous sphere with a uniform membrane around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the membrane is sometimes called a shell, coating, or wall. The microcapsules typically have diameters between 0.5 µm and 2 millimeters. Preferably, the mean diameter of the microcapsules is in the range of 1 µm to 1000 µm, more preferably in the range of 5 µm to 500 µm, even more preferably in the range of 10 µm to 500 µm, even more preferably in the range of 50 µm to 500 µm, and most preferably in the range of 50 µm to 200 µm. Alternatively, the diameter of the microcapsules is in the range of 0.5 µm to 30 µm; or in the range of 1 µm to 25 µm. The diameter of the microcapsule is measured in the oil phase after polymerization is complete. The diameter of the capsule may change depending on the water activity of the surrounding chemical environment.

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

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

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

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

Emulsions can be further classified as either simple emulsions, wherein the dispersed liquid phase is a simple homogeneous liquid, or a more complex emulsion, wherein the dispersed liquid phase is a heterogeneous combination of liquid or solid phases, such as a double emulsion or a multiple-emulsion. For example, a water-in-oil double emulsion or multiple emulsion may be formed wherein the water phase itself further contains an emulsified oil phase; this type of emulsion may be specified as an oil-in-water-in oil (o/w/o) emulsion. Alternatively, a water-in-oil emulsion may be formed wherein the water phase contains a dispersed solid phase often referred to as a suspension- emulsion. Other more complex emulsions can be described. Because of the inherent difficulty in describing such systems, the term emulsion is used to describe both simple and more complex emulsions without necessarily limiting the form of the emulsion or the type and number of phases present

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

material.

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

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

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

The polybranched polyamine may be a polyethyleneimine (PEI), and modifications thereof, having more than two branching points and more than two reactive amino groups; wherein the reactive amino groups constitute at least 15% of the molecular weight of the PEI, such as more than 20%, or more than 25%. Preferably, the molecular weight of the PEI is at least 1 kDa.

Combinations of different polybranched polyamines may be used for preparing the microcapsule.

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

Crosslinking agent: The crosslinking agent as used in the present invention is a molecule with at least two groups/sites capable of reacting with amines to form covalent bonds.

The crosslinking agent is preferably oil soluble and can be in the form of an acid anhydride or acid halide, preferably an acid chloride. For example, it can be adipoyl chloride, sebacoyl chloride, dodecanedioc acid chloride, phthaloyl chloride, terephthaloyl chloride, isophthaloyl chloride, or trimesoyl chloride; but preferably, the crosslinking agent is terephthaloyl chloride or trimesoyl chloride.

The liquid detergent composition may comprise a microcapsule, and thus form part of, any detergent composition in any form, such as liquid and powder detergents, and soap and detergent bars.

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

The liquid detergent composition has a physical form, which is not solid (or gas). It may be a pourable liquid, a paste, a pourable gel or a non-pourable gel. It may be isotropic or structured, preferably isotropic. It may be a formulation useful for washing in automatic washing machines or for hand washing. It may also be a personal care product, such as a shampoo, toothpaste, or hand soap.

The microcaplsule is further described in WO 2014/177709 which is incorporated by referecence.

Formulation of enzyme in co-granule

Non-dusting granulates may be produced, e.g. as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The DNase 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-granulate for the detergent industry is disclosed in the IP.com disclosure IPCOM000200739D.

Another example of formulation of enzymes by the use of co-granulates are disclosed in WO 2013/188331, which relates to a detergent composition comprising (a) a multi-enzyme co- granule; (b) less than 10 wt zeolite (anhydrous basis); and (c) less than 10 wt phosphate salt (anhydrous basis), wherein said enzyme co-granule comprises from 10 to 98 wt% moisture sink component and the composition additionally comprises from 20 to 80 wt% detergent moisture sink component. WO 2013/188331 also relates to a method of treating and/or cleaning a surface, preferably a fabric surface comprising the steps of (i) contacting said surface with the detergent composition as claimed and described herein in aqueous wash liquor, (ii) rinsing and/or drying the surface.

An embodiment of the invention relates to an enzyme granule/particle comprising the DNase of the invention. The granule is composed of a core, and optionally one or more coatings (outer layers) surrounding the core. Typically the granule/particle size, measured as equivalent spherical diameter (volume based average particle size), of the granule is 20-2000 µm, particularly 50-1500 µm, 100-1500 µm or 250-1200 µm.

The core may include additional materials such as fillers, fibre materials (cellulose or synthetic fibres), stabilizing agents, solubilising agents, suspension agents, viscosity regulating agents, light spheres, plasticizers, salts, lubricants and fragrances.

The core may include binders, such as synthetic polymer, wax, fat, or carbohydrate.

The core may comprise a salt of a multivalent cation, a reducing agent, an antioxidant, a peroxide decomposing catalyst and/or an acidic buffer component, typically as a homogenous blend.

The core may consist of an inert particle with the enzyme absorbed into it, or applied onto the surface, *e.g.*, by fluid bed coating.

The core may have a diameter of 20-2000 µm, particularly 50-1500 µm, 100-1500 µm or 250-1200 µm.

The core can be prepared by granulating a blend of the ingredients, *e.g.*, by a method comprising granulation techniques such as crystallization, precipitation, pan-coating, fluid bed coating, fluid bed agglomeration, rotary atomization, extrusion, prilling, spheronization, size reduction methods, drum granulation, and/or high shear granulation.

Methods for preparing the core can be found in Handbook of Powder Technology; Particle size enlargement by C. E. Capes; Volume 1; 1980; Elsevier.

The core of the enzyme granule/particle may be surrounded by at least one coating, *e.g.*, to improve the storage stability, to reduce dust formation during handling, or for coloring the granule. The optional coating(s) may include a salt coating, or other suitable coating materials, such as polyethylene

glycol (PEG), methyl hydroxy-propyl cellulose (MHPC) and polyvinyl alcohol (PVA). Examples of enzyme granules with multiple coatings are shown in WO 93/07263 and WO 97/23606.

The coating may be applied in an amount of at least 0.1% by weight of the core, *e.g.*, at least 0.5%, 1% or 5%. The amount may be at most 100%, 70%, 50%, 40% or 30%.

The coating is preferably at least 0.1 µm thick, particularly at least 0.5 µm, at least 1 µm or at least 5 µm. In a particular embodiment the thickness of the coating is below 100 µm. In a more particular embodiment the thickness of the coating is below 60 µm. In an even more particular embodiment the total thickness of the coating is below 40 µm.

The coating should encapsulate the core unit by forming a substantially continuous layer. A substantially continuous layer is to be understood as a coating having few or no holes, so that the core unit it is encapsulating/enclosing has few or none uncoated areas. The layer or coating should in particular be homogeneous in thickness.

The coating can further contain other materials as known in the art, *e.g.*, fillers, antisticking agents, pigments, dyes, plasticizers and/or binders, such as titanium dioxide, kaolin, calcium carbonate or talc.

A salt coating may comprise at least 60% by weight w/w of a salt, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% by weight w/w.

The salt may be added from a salt solution where the salt is completely dissolved or from a salt suspension wherein the fine particles is less than 50 µm, such as less than 10 µm or less than 5 µm.

The salt coating may comprise a single salt or a mixture of two or more salts. The salt may be water soluble, in particular having a solubility at least 0.1 grams in 100 g of water at 20°C, preferably at least 0.5 g per 100 g water, *e.g.*, at least 1 g per 100 g water, *e.g.*, at least 5 g per 100 g water.

The salt may be an inorganic salt, *e.g.*, salts of sulfate, sulfite, phosphate, phosphonate, nitrate, chloride or carbonate or salts of simple organic acids (less than 10 carbon atoms, *e.g.*, 6 or less carbon atoms) such as citrate, malonate or acetate. Examples of cations in these salts are alkali or earth alkali metal ions, the ammonium ion or metal ions of the first transition series, such as sodium, potassium, magnesium, calcium, zinc or aluminium. Examples of anions include chloride, bromide, iodide, sulfate, sulfite, bisulfite, thiosulfate, phosphate, monobasic phosphate, dibasic phosphate, hypophosphite, dihydrogen pyrophosphate, tetraborate, borate, carbonate, bicarbonate, metasilicate, citrate, malate, maleate, malonate, succinate, lactate, formate, acetate, butyrate, propionate, benzoate, tartrate, ascorbate or gluconate. In particular alkali- or earth alkali metal salts of sulfate, sulfite, phosphate, phosphonate, nitrate, chloride or carbonate or salts of simple organic acids such as citrate, malonate or acetate may be used.

The salt in the coating may have a constant humidity at 20°C above 60%, particularly above

70%, above 80% or above 85%, or it may be another hydrate form of such a salt (*e.g.*, anhydrate). The salt coating may be as described in WO 00/01793 or WO 2006/034710.

Specific examples of suitable salts are NaCl ($CH_{20°C}$=76%), Na_2CO_3 ($CH_{20°C}$=92%), $NaNO_3$ ($CH_{20°C}$=73%), Na_2HPO_4 ($CH_{20°C}$=95%), Na_3PO_4 ($CH_{25°C}$=92%), NH_4Cl ($CH_{20°C}$ = 79.5%), $(NH_4)_2HPO_4$ ($CH_{20°C}$ = 93,0%), $NH_4H_2PO_4$ ($CH_{20°C}$ = 93.1%), $(NH_4)_2SO_4$ ($CH_{20°C}$=81.1%), KCl ($CH_{20°C}$=85%), K_2HPO_4 ($CH_{20°C}$=92%), KH_2PO_4 ($CH_{20°C}$=96.5%), KNO_3 ($CH_{20°C}$=93.5%), Na_2SO_4 ($CH_{20°C}$=93%), K_2SO_4 ($CH_{20°C}$=98%), $KHSO_4$ ($CH_{20°C}$=86%), $MgSO_4$ ($CH_{20°C}$=90%), $ZnSO_4$ ($CH_{20°C}$=90%) and sodium citrate ($CH_{25°C}$=86%). Other examples include NaH_2PO_4, $(NH_4)H_2PO_4$, $CuSO_4$, $Mg(NO_3)_2$ and magnesium acetate.

The salt may be in anhydrous form, or it may be a hydrated salt, i.e. a crystalline salt hydrate with bound water(s) of crystallization, such as described in WO 99/32595. Specific examples include anhydrous sodium sulfate (Na_2SO_4), anhydrous magnesium sulfate ($MgSO_4$), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), magnesium nitrate hexahydrate ($Mg(NO_3)_2(6H_2O)$), sodium citrate dihydrate and magnesium acetate tetrahydrate.

Preferably the salt is applied as a solution of the salt, *e.g.*, using a fluid bed.

Thus, in a further aspect, the present invention provides a granule, which comprises:

(a) a core comprising a DNase according to the invention, and

(b) optionally a coating consisting of one or more layer(s) surrounding the core.Some aspect of the invention relates to a granule, which comprises:

(a) a core comprising a polypeptide having DNase activity wherein the polypeptide is selected from the group consisting of polypeptides shown in SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 or polypeptides having at least 60%, e.g. 70% e.g. 80%, e.g. at least 90%, e.g. at least 95%, e.g. at least 98%, e.g. at least 69% sequence identity hereto, and

(b) optionally a coating consisting of one or more layer(s) surrounding the core.

Formulation of detergent

Detergent ingredients can be separated physically from each other by compartments in water dissolvable pouches. 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.

The detergent composition may take the form of a unit dose product. A unit dose product is

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

Pouches can be of any form, shape and material which is suitable for holding the composition, *e.g.*, without allowing the 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 derivates 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 a blend compositions comprising hydrolytically degradable and water soluble polymer blends such as polyactide and polyvinyl alcohol (known under the Trade reference M8630 as sold by Chris Craft In. Prod. Of Gary, Ind., US) plus plasticizers 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 (see *e.g.*, US 2009/0011970).

Laundry soap bars

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

The laundry soap bar may contain one or more additional enzymes, protease inhibitors such as peptide aldehydes (or hydrosulfite adduct or hemiacetal adduct), boric acid, borate, borax and/or phenylboronic acid derivatives such as 4-formylphenylboronic acid, one or more soaps or synthetic surfactants, polyols such as glycerine, pH controlling compounds such as fatty acids, citric acid, acetic

acid and/or formic acid, and/or a salt of a monovalent cation and an organic anion wherein the monovalent cation may be for example Na^+, K^+ or NH_4^+ and the organic anion may be for example formate, acetate, citrate or lactate such that the salt of a monovalent cation and an organic anion may be, for example, sodium formate.

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

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

Pharmaceutical compositions and uses

The invention further concerns a pharmaceutical composition comprising a polypeptide having DNase activity and a pharmaceutical adjunct ingredient, wherein the polypeptide having DNase activity. The adjunct ingredient may be any excipient suitable for pharmaceutical compositions. The adjunct/expient is within the choice of the skilled artisan. The pharmaceutical composition further comprises a polypeptide selected from the group consisting of polypeptides comprising SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or DNases having at least 80% sequence identity hereto. The pharmaceutical compositions can be used for releasing or removing a biofilm or preventing biofilm formation on surfaces such as medical devices.

The use may be indwelling medical device characterised in that at least a portion of a patient-contactable surface of said device is coated with the pharmaceutical composition comprising the DNases of the invention.

The device can be a catheter such as a central venous catheter, intravascular catheter, urinary catheter, Hickman catheter, peritoneal dialysis catheter, endrotracheal catheter, or wherein

the device is a mechanical heart valve, a cardiac pacemaker, an arteriovenous shunt, a scleral buckle, a prosthetic joint, a tympanostomy tube, a tracheostomy tube, a voice prosthetic, a penile prosthetic, an artificial urinary sphincter, a synthetic pubovaginal sling, a surgical suture, a bone anchor, a bone screw, an intraocular lens, a contact lens, an intrauterine device, an aortofemoral graft, a vascular graft, a needle, a Luer-Lok connector, a needleless connector or a surgical instrument. The pharmaceutical composition can be formulated as a liquid, lotion, cream, spray, gel or ointment. The pharmaceutical composition can be for administration to an animal patient. The animal patient can be a mammalian patient. The mammalian patient can be a human

The invention is further summarized in the following paragraphs:

1. Use of a polypeptide having DNase activity, wherein the polypeptide is selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or selected from the group of polypeptides shown in SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 72, or a DNase having at least 80%sequence identity hereto for preventing, reducing or removing a biofilm from an item, wherein the item is a textile.
2. Use according to paragraph 1 for preventing, reducing or removing stickiness of the item.
3. Use according to any of paragraphs 1 or 2 for pretreating stains on the item.
4. Use according to any of paragraphs 1-3 for preventing, reducing or removing redeposition of soil during a wash cycle.
5. Use according to any of paragraphs 1-4 for preventing, reducing or removing adherence of soil to the item.
6. Use according to any of the preceding paragraphs for maintaining or improving the whiteness of the item.
7. Use according to any of the preceding paragraphs, wherein the polypeptide is the polypeptide of paragraphs 45-50.
8. Use according to any of the preceding paragraphs, wherein a malodour is reduced or removed from the item.
9. Use according to any of the preceding paragraphs, wherein the malodour is caused by E-2-nonenal.
10. Use according to any of the preceding paragraphs, wherein the amount of E-2-nonenal present on a wet textile is reduced or removed.
11. Use according to any of the preceding paragraphs, wherein the amount of E-2-nonenal present on a dry textile is reduced or removed.

12. A detergent composition comprising a polypeptide having deoxyribonuclease (DNase) activity selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or selected from the group of polypeptides shown in SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 72 or DNases having at least 80% sequence identity hereto and a detergent adjunct ingredient.
13. Detergent composition according to paragraph 12, wherein the polypeptide is obtained from *Vibrissea flavovirens, Penicillium reticulisporum, Acremonium dichromosporum, Preussia aemulans, Colletotrichum circinans, Clavicipitaceae, Preussia aemulans or Trichurus spiralis*.
14. Detergent composition according to any of the preceding composition paragraphs, wherein the polypeptide having DNase activity is selected from the group consisting of polypeptides with; SEQ ID NOS: 2 and 3 obtained from *Vibrissea flavovirens*, SEQ ID NO 4 obtained from *Penicillium reticulisporum*, SEQ ID NO 5 obtained from *Acremonium dichromosporum*, SEQ ID NO 6 and SEQ ID NO 9 obtained from *Preussia aemulans*, SEQ ID NO 7 obtained from *Colletotrichum circinans*, SEQ ID NO 8 obtained from *Clavicipitaceae* and SEQ ID NO 10 obtained from *Trichurus spiralis*.
15. Detergent composition according to any of the preceding paragraphs, wherein the polypeptide is the polypeptide of paragraphs 45-50.
16. Detergent composition according to any of the preceding composition paragraphs, wherein the detergent adjunct ingredient is selected from the group consisting of surfactants, builders, flocculating aid, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, builders and co-builders, fabric huing agents, anti-foaming agents, dispersants, processing aids, and/or pigments.
17. Detergent composition according to any of the preceding composition paragraphs, wherein the composition further comprises one or more enzymes selected from the group consisting of proteases, lipases, cutinases, amylases, carbohydrases, cellulases, pectinases, mannanases, arabinases, galactanases, xylanases and oxidases.
18. Detergent composition according to any of the preceding composition paragraphs, wherein the enzyme is a protease, which is of animal, vegetable or microbial origin.
19. Detergent composition according to any of the preceding composition paragraphs, wherein the protease is chemically modified or protein engineered.

20. Detergent composition according to any of the preceding composition paragraphs, wherein the protease is a serine protease or a metalloprotease, preferably an alkaline microbial protease or a trypsin-like protease.

21. Detergent composition according to any of the preceding composition paragraphs, wherein the protease is selected from the group consisting of *Bacillus*, *e.g.*, subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147, subtilisin 168, trypsin of bovine origin, trypsin of porcine origin and *Fusarium* protease.

22. Detergent composition according to any of the preceding composition paragraphs, wherein the detergent composition is capable of reducing adhesion of bacteria selected from the group consisting of *Acinetobacter* sp., *Aeromicrobium* sp., *Brevundimonas* sp., *Microbacterium* sp., *Micrococcus luteus*, *Pseudomonas* sp., *Staphylococcus epidermidis,* and *Stenotrophomonas* sp. to a surface, or releasing the bacteria from a surface to which they adhere.

23. Detergent composition according to any of the preceding composition paragraphs, wherein the surface is a textile surface.

24. Detergent composition according to any of the preceding composition paragraphs, wherein the textile is made of cotton, Cotton/Polyester, Polyester, Polyamide, Polyacryl and/or silk.

25. Detergent composition according to any of the preceding composition paragraphs, wherein the composition is 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.

26. Detergent composition according to any of the preceding composition paragraphs, wherein the composition is a liquid detergent, a powder detergent or a granule detergent.

27. A laundering method for laundering an item comprising the steps of:
    a. Exposing an item to a wash liquor comprising a polypeptide of paragraphs 45-50 or a detergent composition according to any of paragraphs 12-26;
    b. Completing at least one wash cycle; and
    c. Optionally rinsing the item,

    wherein the item is a textile.

28. Method according to paragraph 27, wherein the pH of the wash liquor is in the range of 1 to 11.

29. Method according to any of the preceding method paragraphs, wherein the pH of the wash liquor is in the range 5.5 to 11, such as in the range of 7 to 9, in the range of 7 to 8 or in the range of 7 to 8.5.

30. Method according to any of the preceding method paragraphs, wherein the temperature of

the wash liquor is in the range of 5ºC to 95ºC, or in the range of 10ºC to 80ºC, in the range of 10ºC to 70ºC, in the range of 10ºC to 60ºC, in the range of 10ºC to 50ºC, in the range of 15ºC to 40ºC or in the range of 20ºC to 30ºC.

31. Method according to any of the preceding method paragraphs, wherein the temperature of the wash liquor is 30ºC.

32. Method according to any of the preceding method paragraphs, wherein the method further comprises draining of the wash liquor or part of the wash liquor after completion of a wash cycle.

33. Method according to any of the preceding method paragraphs, wherein the item is exposed to the wash liquor during a first and optionally a second or a third wash cycle.

34. Method according to any of the preceding method paragraphs, wherein the item is rinsed after being exposed to the wash liquor.

35. Method according to any of the preceding method paragraphs, wherein the item is rinsed with water or with water comprising a conditioner.

36. Method according to any of the preceding method paragraphs, wherein stickiness of the item is reduced.

37. Method according to any of the preceding method paragraphs, wherein stains present on the item is pretreated with a polypeptide of paragraphs 45-50 or a detergent composition according to any of paragraphs 12-26.

38. Method according to any of the preceding method paragraphs, wherein redeposition of soil is reduced.

39. Method according to any of the preceding method paragraphs, wherein adherence of soil to the item is reduced or removed.

40. Method according to any of the preceding method paragraphs, wherein whiteness of the item is maintained or improved.

41. Method according to any of the preceding method paragraphs, wherein malodour is reduced or removed from the item.

42. Method according to any of the preceding method paragraphs, wherein the malodour is caused by E-2-nonenal.

43. Method according to any of the preceding method paragraphs, wherein the amount of E-2-nonenal present on a wet or dry textile is reduced or removed.

44. Method according to any of the preceding method paragraphs, wherein the concentration of the polypeptide in the wash liquor is at least 1 mg of DNase protein, such as at least 5 mg of protein, preferably at least 10 mg of protein, more preferably at least 15 mg of protein, even more preferably at least 20 mg of protein, most preferably at least 30 mg of protein, and even

most preferably at least 40 mg of protein per liter of wash liquor.

45. A polypeptide having DNase activity, selected from the group consisting of:
    a. a polypeptide having at least 60% sequence identity to the polypeptide of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or selected from the group of polypeptides shown in SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 72 or a polypeptide having at least 60% sequence identity hereto;
    b. a polypeptide encoded by a polynucleotide that hybridizes under low stringency conditions with
        i. the mature polypeptide coding sequence,
        ii. the cDNA sequence thereof, or
        iii. the full-length complement of (i) or (ii);
    c. a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence or the cDNA sequence thereof;
    d. a variant of the mature polypeptide of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or aby of the polypeptides shown in SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72 comprising a substitution, deletion, and/or insertion at one or more positions; and
    e. a fragment of the polypeptide of (a), (b), (c), or (d) that has DNase activity;

46. The polypeptide of paragraph 45, having at least 60%, 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 polypeptide with SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or polypeptides shown in SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 72.

47. The polypeptide according to paragraph 45 or 46, which is encoded by a polynucleotide that hybridizes under low stringency conditions, low-medium stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions, or very high stringency conditions with
    i. the mature polypeptide coding sequence,

ii. the cDNA sequence thereof, or

iii. the full-length complement of (i) or (ii).

48. The polypeptide according to any of paragraphs 45-47, comprising or consisting of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9 or 10 or polypeptides shown in SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 7.

49. The polypeptide according to any of paragraphs 45-48, which is a variant of the polypeptides selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 and 10 or polypeptides shown in SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 and SEQ ID NO 7 comprising a substitution, deletion, and/or insertion at one or more positions

50. The polypeptide according to paragraph 49, which is a fragment of SEQ ID NOS: 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein the fragment has DNase activity.

51. A polynucleotide encoding the polypeptide according to any of paragraphs 45-50.

52. A nucleic acid construct or expression vector comprising the polynucleotide of paragraph 51 operably linked to one or more control sequences that direct the production of the polypeptide in an expression host.

53. A recombinant host cell comprising the polynucleotide of paragraph 51-52 operably linked to one or more control sequences that direct the production of the polypeptide.

54. A method of producing the polypeptide of any of paragraphs 45-50, comprising cultivating a cell, which in its wild-type form produces the polypeptide, under conditions conducive for production of the polypeptide.

55. The method of paragraph 54, further comprising recovering the polypeptide.

56. A method of producing a polypeptide having DNase activity, comprising cultivating the host cell of paragraph 53 under conditions conducive for production of the polypeptide.

57. The method of paragraph 56, further comprising recovering the polypeptide.

58. A method of producing a protein, comprising cultivating the recombinant host cell comprising a gene encoding a protein operably linked to the polynucleotide of paragraph 51, wherein the gene is foreign to the polynucleotide encoding the propeptide, under conditions conducive for production of the protein.

59. The method of paragraph 58, further comprising recovering the protein.

60. A whole broth formulation or cell culture composition comprising a polypeptide of any of paragraphs 45-50.

61. An Item laundered according to the method of any of paragraphs 27-44.

62. A pharmaceutical composition comprising a polypeptide having DNase activity and a pharmaceutical adjunct ingredient, wherein the polypeptide is obtained from a fungal source.

63. Pharmaceutical composition according to paragraph 62, wherein the polypeptide having DNase activity is obtained from *Vibrissea flavovirens, Penicillium reticulisporum, Acremonium dichromosporum, Preussia aemulans, Colletotrichum circinans, Clavicipitaceae, Preussia aemulans or Trichurus spiralis*.

64. Pharmaceutical composition according to any of paragraphs 62-63, wherein the polypeptide having DNase activity is selected from the group consisting of polypeptides with; SEQ ID NOS: 2 and 3 obtained from *Vibrissea flavovirens*, SEQ ID NO 4 obtained from *Penicillium reticulisporum*, SEQ ID NO 5 obtained from *Acremonium dichromosporum*, SEQ ID NO 6 and SEQ ID NO 9 obtained from *Preussia aemulans*, SEQ ID NO 7 obtained from *Colletotrichum circinans*, SEQ ID NO 8 obtained from *Clavicipitaceae* and SEQ ID NO 10 obtained from *Trichurus spiralis*.

65. Pharmaceutical composition according to any of paragraphs 62-64, wherein the polypeptide is the polypeptide of paragraphs 45-50.

66. Pharmaceutical composition according to any of paragraphs 62-65, wherein the composition is formulated as a dental paste, a liquid dentifrice, a mouthwash, a troche or a gingival massage ointment.

67. Pharmaceutical composition according to any of paragraphs 62-66, further comprising one or more of an antimicrobial compound, such as an antibacterial compound, an antiparasitic compound, an antifungal compound and an antiviral compound.

68. An indwelling medical device characterised in that at least a portion of a patient-contactable surface of said device is coated with the pharmaceutical composition of any of paragraphs 62-67.

69. The device according to paragraph 68 wherein said device is a catheter such as a central venous catheter, intravascular catheter, urinary catheter, Hickman catheter, peritoneal dialysis catheter, endrotracheal catheter, or wherein the device is a mechanical heart valve, a cardiac pacemaker, an arteriovenous shunt, a schleral buckle, a prosthetic joint, a tympaNOS:tomy tube, a tracheostomy tube, a voice prosthetic, a penile prosthetic, an artificial urinary sphincter, a synthetic pubovaginal sling, a surgical suture, a bone anchor, a bone screw, an intraocular lens, a contact lens, an intrauterine device, an aortofemoral graft, a vascular graft, a needle, a Luer-Lok connector, a needleless connector or a surgical instrument.

70. A method of producing the polypeptide of any of paragraphs 45-50, comprising cultivating the

host cell of paragraph 53 under conditions conducive for production of the polypeptide.

71. The method of paragraph 70, further comprising recovering the polypeptide.

72. The recombinant host cell of paragraph 53 further comprising a polynucleotide encoding a second polypeptide of interest; preferably an enzyme of interest; more preferably a secreted enzyme of interest; even more preferably a hydrolase, isomerase, ligase, lyase, oxidoreductase, or a transferase; and most preferably the secreted enzyme is an alpha-galactosidase, alpha-glucosidase, aminopeptidase, amylase, asparaginase, beta-galactosidase, beta-glucosidase, beta-xylosidase, carbohydrase, carboxypeptidase, catalase, cellobiohydrolase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, endoglucanase, esterase, green fluorescent protein, glucano-transferase, glucoamylase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or a xylanase.

73. The recombinant host cell of paragraph 72, wherein the second polypeptide of interest is heterologous or homologous to the host cell.

74. The recombinant host cell of paragraph 72 or 73, which is a fungal host cell; preferably a filamentous fungal host cell; more preferably an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Bjerkandera*, *Ceriporiopsis*, *Chrysosporium*, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trametes*, or *Trichoderma* cell; most preferably an *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Bjerkandera adusta*, *Ceriporiopsis aneirina*, *Ceriporiopsis caregiea*, *Ceriporiopsis gilvescens*, *Ceriporiopsis pannocinta*, *Ceriporiopsis rivulosa*, *Ceriporiopsis subrufa*, *Ceriporiopsis subvermispora*, *Chrysosporium inops*, *Chrysosporium keratinophilum*, *Chrysosporium lucknowense*, *Chrysosporium merdarium*, *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium tropicum*, *Chrysosporium zonatum*, *Coprinus cinereus*, *Coriolus hirsutus*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum*, *Humicola insolens*, *Humicola lanugiNOS:a*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Phlebia radiata*, *Pleurotus eryngii*, *Thielavia*

terrestris, *Trametes villosa*, *Trametes versicolour* , *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride* cell.

75. A method of producing the second polypeptide of interest as defined in any of paragraphs 70-71, comprising cultivating the host cell of any of paragraphs 72-74 under conditions conducive for production of the second polypeptide of interest.
76. The method of paragraph 75, further comprising recovering the second polypeptide of interest.
77. Detergent composition according to any of the preceding composition paragraphs, wherein the detergent adjunct ingredient is a surfactant.
78. Detergent composition according to any of the preceding composition paragraphs, wherein the detergent adjunct ingredient is a builder.
79. Detergent composition according to any of the preceding composition paragraphs, wherein the detergent adjunct ingredient is a clay soil removal/anti-redeposition agents.
80. Detergent composition according to paragraphs 12-26, wherein the composition is a liquid detergent composition, comprising a surfactant and a detergent builder in a total concentration of at least 3% by weight, and a detergent enzyme containing microcapsule, wherein the membrane of the microcapsule is produced by cross-linking of a polybranched polyamine having a molecular weight of more than 1 kDa.
81. Detergent composition according to paragraphs 80, wherein the reactive amino groups of the polybranched polyamine constitute at least 15% of the molecular weight.
82. Detergent composition according to any of paragraphs 80-81, wherein the microcapsule is produced by using an acid chloride as crosslinking agent.
83. Detergent composition according to any of paragraphs 80-82, wherein the diameter of the microcapsule is at least, or above, 50 micrometers.
84. Detergent composition according to any of paragraphs 80-83, wherein the microcapsule contains at least 1% by weight of active enzyme.
85. Detergent composition according to any of paragraphs 80-84, which further includes an alcohol, such as a polyol.
86. Detergent composition according to any of paragraphs 80-85, wherein the surfactant is an anionic surfactant.
87. Detergent composition according to any of paragraphs 80-86, which is a liquid laundrycomposition.
88. Detergent composition according to any of paragraphs 80-87, which contains less than 90% by weight of water.
89. Detergent composition according to any of paragraphs 80-88, wherein the detergent enzyme

is a polypeptide having DNase activity, protease, amylase, lipase, cellulase, mannanase, pectinase, or oxidoreductase.

90. Detergent composition according to any of paragraphs 80-89, wherein the protease is a metalloprotease or an alkaline serine protease, such as a subtilisin.

91. Detergent composition according to any of paragraphs 80-90, wherein the polypeptide having DNase activity is the polypeptide according to any of claims 45-50.

92. Detergent composition according to any of paragraphs 80-91, wherein the microcapsule is produced by interfacial polymerization using an acid chloride as crosslinking agent.

93. Detergent composition according to any of paragraphs 80-92, wherein the polybranched polyamine is a polyethyleneimine.

94. Detergent composition according to any of paragraphs 80-93, wherein the microcapsule comprises a source of Mg2+, Ca2+, or Zn2+ ions, such as a poorly soluble salt of Mg2+, Ca2+, or Zn2+.

Assays and detergent compositions

Detergent compositions

The below mentioned detergent composition can be used in combination with the enzyme of the invention.

Biotex black (liquid)

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

Composition of Ariel Sensitive White & Colour , liquid detergent composition

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

Composition of WFK IEC-A model detergent (powder)

Ingredients: Linear sodium alkyl benzene sulfonate 8,8 %, Ethoxylated fatty alcohol C12-18 (7 EO) 4,7 %, Sodium soap 3,2 %, Anti foam DC2-4248S 3,9 %, Sodium aluminium silicate zeolite 4A 28,3 %, Sodium carbonate 11,6 %, Sodium salt of a copolymer from acrylic and maleic acid (Sokalan CP5) 2,4 %, Sodium silicate 3,0 %, Carboxymethylcellulose 1,2 %, Dequest 2066 2,8 %, Optical whitener 0,2 %, Sodium sulfate6,5 %, Protease 0,4 %.

Composition of model detergent A (liquid)

Ingredients: 12% LAS, 11% AEO Biosoft N25-7 (NI), 7% AEOS (SLES), 6% MPG (monopropylene glycol), 3% ethanol, 3% TEA, 2.75% cocoa soap, 2.75% soya soap, 2% glycerol, 2% sodium hydroxide, 2% sodium citrate, 1% sodium formiate, 0.2% DTMPA and 0.2% PCA (all percentages are w/w)

Composition of Ariel Actilift (liquid)

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

Composition of Ariel Actilift Colour&Style (liquid)

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

Composition of Persil Small & Mighty (liquid)

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

Persil 2 in1 with Comfort Passion Flower Powder

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

Persil Biological Powder

Sucrose, Sorbitol, Aluminum Silicate, Polyoxymethylene Melamine, Sodium Polyaryl Sulphonate, CI 61585, CI 45100, Lipase, Amylase, Xanthan gum, Hydroxypropyl methyl cellulose,

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

Persil Biological Tablets

Sodium carbonate, Sodium Carbonate Peroxide, Sodium bicarbonate, Zeolite, Aqua, Sodium Silicate, Sodium Lauryl Sulfate, Cellulose, TAED, Sodium Dodecylbenzenesulfonate, Hemicellulose, Lignin, Lauryl Glucoside, Sodium Acrylic Acid/MA Copolymer, Bentonite, Sodium chloride, Parfum, Tetrasodium Etidronate, Sodium sulfate, Sodium Polyacrylate, Dimethicone, Disodium AnilinomorpholinotriazinylamiNOS:tilbenesulfonate, Dodecylbenzene Sulfonic Acid, Trimethylsiloxysilicate, Calcium carbonate, Cellulose, PEG-75, Titanium dioxide, Dextrin, Protease, Corn Starch Modified, Sucrose, CI 12490, Sodium Polyaryl Sulphonate, Sodium Thiosulfate, Amylase, Kaolin,

Persil Colour Care Biological Powder

Subtilisin, Imidazolidinone, Hexyl Cinnamal, Sucrose, Sorbitol, Aluminum Silicate, Polyoxymethylene Melamine, CI 61585, CI 45100, Lipase, Amylase, Xanthan gum, Hydroxypropyl methyl cellulose, CI 12490, Disodium Distyrylbiphenyl Disulfonate, Sodium Thiosulfate, CI 42090, Mannanase, CI 11680, Etidronic Acid, Tetrasodium EDTA.

Persil Colour Care Biological Tablets

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

Persil Dual Action Capsules Bio

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

Persil 2 in1 with Comfort Sunshiny Days Powder

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

Persil Small & Mighty 2in1 with Comfort Sunshiny Days

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

Persil Small & Mighty Bio

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

Persil Small & Mighty Capsules Biological

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

Denatonium Benzoate, Polymeric Yellow Colourant, Mannanase.

Persil Small & Mighty Capsules Colour Care

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

Persil Small & Mighty Colour Care

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

Composition of Fairy Non Bio (liquid)

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

Composition of Model detergent T (powder)

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

Composition of Model detergent X (powder)

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

Composition of Ariel Actilift Colour&Style (powder)

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

Composition of Ariel Actilift (powder)

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

Composition of Persil Megaperls (powder)

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

Gain Liquid, Original:

Ingredients: Water, Alcohol Ethoxysulfate, Diethylene Glycol, Alcohol Ethoxylate, Ethanolamine, Linear Alkyl Benzene Sulfonate, Sodium Fatty Acids, Polyethyleneimine Ethoxylate, Citric Acid, Borax, Sodium Cumene Sulfonate, Propylene Glycol, DTPA, Disodium DiamiNOS:tilbene Disulfonate, Dipropylethyl Tetramine, Sodium Hydroxide, Sodium Formate, Calcium Formate, Dimethicone, Amylase, Protease, Liquitint™, Hydrogenated Castor Oil, Fragrance

Tide Liquid, Original:

Ingredients: Linear alkylbenzene sulfonate, propylene glycol, citric acid, sodium hydroxide, borax, ethanolamine, ethanol , alcohol sulfate, polyethyleneimine ethoxylate, sodium fatty acids, diquaternium ethoxysulfate, protease, diethylene glycol, laureth-9, alkyldimethylamine oxide, fragrance, amylase, disodium diaminostilbene disulfonate, DTPA, sodium formate, calcium formate, polyethylene glycol 4000, mannanase, Liquitint™ Blue, dimethicone.

Liquid Tide, Free and Gentle:

Water, sodium alcoholethoxy sulfate, propylene glycol, borax, ethanol, linear alkylbenzene sulfonate sodium, salt, polyethyleneimine ethoxylate, diethylene glycol, trans sulfated & ethoxylated hexamethylene diamine, alcohol ethoxylate, linear alkylbenzene sulfonate, MEA salt, sodium formate, sodium alkyl sulfate, DTPA, amine oxide, calcium formate, disodium diamiNOS:tilbene, disulfonate, amylase, protease, dimethicone, benzisothiazolinone

Tide Coldwater Liquid, Fresh Scent:

Water, alcoholethoxy sulfate, linear alkylbenzene sulfonate, diethylene glycol, propylene

glycol, ethanolamine, citric acid, Borax, alcohol sulfate, sodium hydroxide, polyethyleneimine, ethoxylate, sodium fatty acids, ethanol, protease, Laureth-9, diquaternium ethoxysulfate, lauramine oxide, sodium cumene, sulfonate, fragrance, DTPA, amylase, disodium, diamiNOS:tilbene, disulfonate, sodium formate, disodium distyrylbiphenyl disulfonate, calcium formate, polyethylene glycol 4000, mannanase, pectinase, Liquitint™ Blue, dimethicone

Tide TOTALCARE™ Liquid, Cool Cotton:

Water, alcoholethoxy sulfate, propylene glycol, sodium fatty acids, laurtrimonium chloride, ethanol, sodium hydroxide, sodium cumene sulfonate, citric acid, ethanolamine, diethylene glycol, silicone polyether, borax, fragrance, polyethyleneimine ethoxylate, protease, Laureth-9,

DTPA, polyacrylamide quaternium chloride, disodium diaminostilbene disulfonate, sodium formate, Liquitint™ Orange, dipropylethyl tetraamine, dimethicone, cellulase,

Liquid Tide Plus Bleach Alternative™, Vivid White and Bright, Original and Clean Breeze:

Water, sodium alcoholethoxy sulfate, sodium alkyl sulfate, MEA citrate, linear alkylbenzene sulfonate, MEA salt, propylene glycol, diethylene glycol, polyethyleneimine ethoxylate, ethanol, sodium fatty acids, ethanolamine, lauramine oxide, borax, Laureth-9, DTPA, sodium cumene sulfonate, sodium formate, calcium formate, linear alkylbenzene sulfonate, sodium salt, alcohol sulfate, sodium hydroxide, diquaternium ethoxysulfate, fragrance, amylase, protease, mannanase, pectinase, disodium diamiNOS:tilbene disulfonate, benzisothiazolinone, Liquitint™ Blue, dimethicone, dipropylethyl tetraamine.

Liquid Tide HE, Original Scent:

Water, Sodium alcoholethoxy sulfate, MEA citrate, Sodium Alkyl Sulfate, alcohol ethoxylate, linear alkylbenzene sulfonate, MEA salt, sodium fatty acids, polyethyleneimine ethoxylate, diethylene glycol, propylene glycol, diquaternium ethoxysulfate, borax, polyethyleneimine, ethoxylate propoxylate, ethanol, sodium cumene sulfonate, fragrance, DTPA, disodium diamiNOS:tilbene disulfonate, Mannanase, cellulase, amylase, sodium formate, calcium formate, Lauramine oxide, Liquitint™ Blue, Dimethicone / polydimethyl silicone.

Tide TOTALCARE HE Liquid, renewing Rain:

Water, alcoholethoxy sulfate, linear alkylbenzene sulfonate, alcohol ethoxylate, citric acid, Ethanolamine, sodium fatty acids, diethylene glycol, propylene glycol, sodium hydroxide, borax, polyethyleneimine ethoxylate, silicone polyether, ethanol, protease, sodium cumene sulfonate, diquaternium ethoxysulfate, Laureth-9, fragrance, amylase, DTPA, disodium diamiNOS:tilbene

disulfonate, disodium distyrylbiphenyl disulfonate, sodium formate, calcium formate, mannanase, Liquitint™ Orange, dimethicone, polyacrylamide quaternium chloride, cellulase, dipropylethyl tetraamine.

Tide liquid HE Free:

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

Tide Coldwater HE Liquid, Fresh Scent:

Water, alcoholethoxy sulfate, MEA Citrate, alcohol sulfate, Alcohol ethoxylate, Linear alkylbenzene sulfonate MEA, sodium fatty acids, polyethyleneimine ethoxylate, diethylene glycol, propylene glycol, diquaternium ethoxysulfate, borax, polyethyleneimine ethoxylate propoxylate, ethanol, sodium cumene sulfonate, fragrance, DTPA, disodium diamiNOS:tilbene disulfonate, protease, mannanase, cellulase, amylase, sodium formate, calcium formate, lauramine oxide, Liquitint™ Blue, dimethicone.

Tide for Coldwater HE Free Liquid:

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

Tide Simply Clean & Fresh:

Water, alcohol ethoxylate sulfate, linear alkylbenzene sulfonate Sodium/Mea salts, propylene glycol, diethylene glycol, sodium formate, ethanol, borax, sodium fatty acids, fragrance, lauramine oxide, DTPA, Polyethylene amine ethoxylate, calcium formate, disodium diamiNOS:tilbene disulfonate, dimethicone, tetramine, Liquitint™ Blue.

Tide Pods, Ocean Mist, Mystic Forest, Spring Meadow:

Linear alkylbenzene sulfonates, C12-16 Pareth-9, propylene glycol, alcoholethoxy sulfate,

water, polyethyleneimine ethoxylate, glycerine, fatty acid salts, PEG-136 polyvinyl acetate, ethylene Diamine disuccinic salt, monoethanolamine citrate, sodium bisulfite, diethylenetriamine pentaacetate sodium, disodium distyrylbiphenyl disulfonate, calcium formate, mannanase, exyloglucanase, sodium formate, hydrogenated castor oil, natalase, dyes, termamyl, subtilisin, benzisothiazolin, perfume.

Tide to Go:

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

Tide Stain Release Liquid:

Water, Alkyl Ethoxylate, Linear Alkylbenzenesulfonate, Hydrogen Peroxide, Diquaternium Ethoxysulfate, Ethanolamine, Disodium Distyrylbiphenyl Disulfonate, tetrabutyl Ethylidinebisphenol, F&DC Yellow 3, Fragrance.

Tide Stain Release Powder:

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

Tide Stain Release, Pre Treater Spray:

Water, Alkyl Ethoxylate, MEA Borate, Linear Alkylbenzenesulfonate, Propylene Glycol, Diquaternium Ethoxysulfate, Calcium Chlorideenzyme, Protease, Ethanolamine, Benzoisothiazolinone, Amylase, Sodium Citrate, Sodium Hydroxide, Fragrance.

Tide to Go Stain Eraser:

Water, Alkyl Amine Oxide, Dipropylene Glycol Phenyl Ether, Hydrogen Peroxide, Citric Acid, Ethylene Diamine Disuccinic Acid Sodium salt, Sodium Alkyl Sulfate, Fragrance.

Tide boost with Oxi:

Sodium bicarbonate, sodium carbonate, sodium percarbonate, alcohol ethoxylate, sodium chloride, maleic/acrylic copolymer, nonanoyloxy benzene sulfonate, sodium sulfate, colour ant, diethylenetriamine pentaacetate sodium salt, hydrated aluminosilicate (zeolite), polyethylene glycol,

sodium alkylbenzene sulfonate, sodium palmitate, starch, water, fragrance.

Tide Stain Release boost Duo Pac:

Polyvinyl Alcoholpouch film, wherein there is packed a liquid part and a powder part:

Liquid Ingredients: Dipropylene Glycol, diquaternium Ethoxysulfate, Water, Glycerin, LiquitintTM Orange, **Powder Ingredients:** sodium percarbonate, nonanoyloxy benzene sulfonate, sodium carbonate, sodium sulfate, sodium aluminosilicate, sodium polyacrylate, sodium alkylbenzenesulfonate, maleic/acrylic copolymer, water, amylase, polyethylene glycol, sodium palmitate, modified starch, protease, glycerine, DTPA, fragrance.

Tide Ultra Stain Release:

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

Ultra Tide with a Touch of Downy® Powdered Detergent, April Fresh/Clean Breeze/April Essence:

Sodium Carbonate, Sodium AlumiNOS:ilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Bentonite, Water, Sodium Percarbonate, Sodium Polyacrylate, Silicate, Alkyl Sulfate, Nonanoyloxybenzenesulfonate, DTPA, Polyethylene Glycol 4000, Silicone, Ethoxylate, fragrance, Polyethylene Oxide, Palmitic Acid, Disodium Diaminostilbene Disulfonate, Protease, Liquitint™ Red, FD&C Blue 1, Cellulase.

Ultra Tide with a Touch of Downy Clean Breeze:

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

Ultra Tide with Downy Sun Blossom:

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

Ultra Tide with Downy April Fresh/ Sweet Dreams:

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

Ultra Tide Free Powdered Detergent:

Sodium Carbonate, Sodium Aluminosilicate, Alkyl Sulfate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Water, Sodium polyacrylate, Silicate, Ethoxylate, Sodium percarbonate, Polyethylene Glycol 4000, Protease, Disodium Diaminostilbene Disulfonate, Silicone, Cellulase.

Ultra Tide Powdered Detergent, Clean Breeze/Spring Lavender/mountain Spring:

Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Alkyl Sulfate, Sodium Percarbonate, Water, Sodium Polyacrylate, Silicate, Nonanoyloxybenzenesulfonate, Ethoxylate, Polyethylene Glycol 4000, Fragrance, DTPA, Disodium DiamiNOS:tilbene Disulfonate, Palmitic Acid, Protease, Silicone, Cellulase.

Ultra Tide HE (high Efficiency) Pwdered Detergent, Clean Breeze:

Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Water, Nonanoyloxybenzenesulfonate, Alkyl Sulfate, Sodium Polyacrylate, Silicate, Sodium Percarbonate, Ethoxylate, Polyethylene Glycol 4000, Fragrance, DTPA, Palmitic Acid, Disodium DiamiNOS:tilbene Disulfonate, Protease, Silicone, Cellulase.

Ultra Tide Coldwater Powdered Detergent, Fresh Scent:

Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Sodium Percarbonate, Alkyl Sulfate, Linear Alkylbenzene Sulfonate, Water, Nonanoyloxybenzenesulfonate, Sodium

Polyacrylate, Silicate, Ethoxylate, Polyethylene Glycol 4000, DTPA, Fragrance, Natalase, Palmitic Acid, Protease, Disodium, DiamiNOS:tilbene Disulfonate, FD&C Blue 1, Silicone, Cellulase, Alkyl Ether Sulfate.

Ultra Tide with bleach Powdered Detergent, Clean Breeze:

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

Ultra Tide with Febreeze Freshness™ Powdered Detergent, Spring Renewal:

Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate, Sodium Percarbonate , Alkyl Sulfate, Water, Sodium Polyacrylate, Silicate, Nonanoyloxybenzenesulfonate, Ethoxylate, Polyethylene Glycol 4000, DTPA, Fragrance, Cellulase, Protease, Disodium DiamiNOS:tilbene Disulfonate, Silicone, FD&C Blue 1.

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

Water, Sodium alcoholethoxy sulfate, MEA citrate, linear alkylbenzene sulfonate, sodium salt, linear alkylbenzene sulfonate: MEA salt, alcohol ethoxylate, sodium fatty acids, propylene glycol, diethylene glycol, polyethyleneimine ethoxylate propoxylate, diquaternium ethoxysulfate, Ethanol, sodium cumene sulfonate, borax, fragrance, DTPA, Sodium bisulfate, disodium diaminostilbene disulfonate, Mannanase, cellulase, amylase, sodium formate, calcium formate, Lauramine oxide, Liquitint™ Blue, Dimethicone / polydimethyl silicone.

Tide Plus Febreeze Freshness Spring & Renewal:

Water, sodium alcoholethoxy sulfate, linear alkyl benzene sulfonate: sodium/MEA salts, MEA citrate, propylene glycol, polyethyleneimine ethoxylate, fragrance, ethanol, diethylene glycol, polyethyleneimine propoxyethoxylate, protease, alcohol sulfate, borax, sodium fatty acids, DTPA, disodium diaminostilbene disulfonate, MEA, mannanase, gluconase, sodium formate, dimethicone, Liquitint™ Blue, tetramine.

Liquid Tide Plus with Febreeze Freshness, Sport HE Victory Fresh:

Water, Sodium alcoholethoxy sulfate, MEA citrate, linear alkylbenzene sulfonate, sodium salt, linear alkylbenzene sulfonate: MEA salt, alcohol ethoxylate, sodium fatty acids, propylene

glycol, diethylene glycol, polyethyleneimine ethoxylate propoxylate, diquaternium ethoxysulfate, ethanol, sodium cumene sulfonate, borax, fragrance, DTPA, Sodium bisulfate, disodium diamiNOS:tilbene disulfonate, Mannanase, cellulase, amylase, sodium formate, calcium formate, Lauramine oxide, Liquitint™ Blue, Dimethicone / polydimethyl silicone.

Tide Vivid White + Bright Powder, Original:

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

HEY SPORT TEX WASH Detergent

Aqua, dodecylbenzenesulfonsäure, laureth-11, peg-75 lanolin, propylene glycol, alcohol denat., potassium soyate, potassium hydroxide, disodium cocoamphodiacetate, ethylendiamine triacetate cocosalkyl acetamide, parfum, zinc ricinoleate, sodium chloride, benzisothiazolinone, methylisothiazolinone, ci 16255, benzyl alcohol.

The products named Tide, Ariel, Gain and Fairy are commercially available products supplied by Procter & Gamble. The products named Persil are commercially available products supplied by Unilever and Henkel. The products named Hey Sport are commercially available products supplied by Hey Sport.

Ingredient	Amount (in wt %)
Anionic detersive surfactant (such as alkyl benzene sulphonate, alkyl ethoxylated sulphate and mixtures	from 8 wt % to 15 wt % thereof)
Non-ionic detersive surfactant (such as alkyl ethoxylated alcohol)	from 0.5 wt % to 4 wt %
Cationic detersive surfactant (such as quaternary ammonium compounds)	from 0 to 4 wt %
Other detersive surfactant (such as zwiterionic detersive surfactants, amphoteric surfactants and mixtures thereof)	from 0 wt % to 4 wt %
Carboxylate polymer (such as co-polymers of maleic acid and acrylic acid)	from 1 wt % to 4 wt %
Polyethylene glycol polymer (such as a polyethylene glycol polymer	from 0.5 wt % to 4

comprising poly vinyl acetate side chains)	wt %
Polyester soil release polymer (such as Repel-o-tex from and/or Texcare polymers)	0.1 to 2 wt %
Cellulosic polymer (such as carboxymethyl cellulose, methyl cellulose and combinations thereof)	from 0.5 wt % to 2 wt %
Other polymer (such as amine polymers, dye transfer inhibitor polymers, hexamethylenediamine derivative polymers, and mixtures thereof)	from 0 wt % to 4 wt %
Zeolite builder and phosphate builder (such as zeolite 4A and/or sodium tripolyphosphate)	from 0 wt % to 4 wt%
Other builder (such as sodium citrate and/or citric acid)	from 0 wt % to 3 wt %
Carbonate salt (such as sodium carbonate and/or sodium bicarbonate)	from 15 wt % to 30 wt %
Silicate salt (such as sodium silicate)	from 0 wt % to 10 wt %
Filler (such as sodium sulphate and/or bio-fillers)	from 10 wt % to 40 wt %
Source of available oxygen (such as sodium percarbonate)	from 10 wt % to 20 wt %
Bleach activator (such as tetraacetylethylene diamine (TAED) and/or nonanoyloxybenzenesulphonate (NOBS)	from 2 wt % to 8 wt %
Bleach catalyst (such as oxaziridinium-based bleach catalyst and/or transition metal bleach catalyst)	from 0 wt % to 0.1 wt %
Other bleach (such as reducing bleach and/or pre- formed peracid)	from 0 wt % to 10 wt %
Chelant (such as ethylenediamine-N'N'-disuccinic acid (EDDS) and/or hydroxyethane diphosphonic acid(HEDP)	from 0.2 wt % to1 wt %
Photobleach (such as zinc and/or aluminium sulphonated phthalocyanine)	from 0 wt % to 0.1 wt %
Hueing agent (such as direct violet 99, acid red 52, acid blue 80, direct violet 9, solvent violet 13 and any combination thereof)	from 0 wt % to 1 wt %
Brightener (such as brightener 15 and/or brightener 49)	from 0.1 wt % to 0.4 wt %
Protease (such as Savinase, Savinase Ultra, Purafect, FN3, FN4 and any	from 0.1 wt % to

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

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

Non-ionic detersive surfactant (such as alkyl ethoxylated alcohol)	from about 0.5 wt% to 4 wt%
Cationic detersive surfactant (such as quaternary ammonium compounds)	from about 0 wt% to about 4 wt%
Other detersive surfactant (such as zwiterionic detersive surfactants, amphoteric surfactants and mixtures thereof)	from about 0 wt% to 4 wt%
Carboxylate polymer (such as co-polymers of maleic acid and acrylic acid)	from about 1 wt% to about 4 wt%
Polyethylene glycol polymer (such as a polyethylene glycol polymer comprising poly vinyl acetate side chains)	from about 0 wt% to about 4 wt%
Polyester soil release polymer (such as Repel-O- Tex(R) and/or Texcare(R) polymers)	from about 0.1 wt% to about 2 wt%
Cellulosic polymer (such as carboxymethyl cellulose, methyl cellulose and combinations thereof)	from about 0.5wt% to about 2 wt%
Other polymer (such as amine polymers, dye transfer inhibitor polymers, hexamethylenediamine derivative polymers, and mixtures thereof)	from about 0 wt% to about 4 wt%
Zeolite builder and phosphate builder (such as zeolite 4A and/or sodium tripolyphosphate)	from about 0 wt% to about 4 wt%
Other builder (such as sodium citrate and/or citric acid)	from about 0 wt% to about 3 wt%
Carbonate salt (such as sodium carbonate and/or sodium bicarbonate)	from about 15 t% to about 30 wt%
Silicate salt (such as sodium silicate)	from about 0 wt% to about 10 wt%
Filler (such as sodium sulphate and/or bio-fillers)	from about 10 wt% to about 40 wt%
Source of available oxygen (such as sodium percarbonate)	from about 10wt% to about 20 wt%
Bleach activator (such as tetraacetylethylene diamine (TAED) and/or nonanoyloxybenzenesulphonate (NOBS)	from about 2 wt% to about 8 wt%
Bleach catalyst (such as oxaziridinium-based bleach catalyst and/or transition metal bleach catalyst)	from about 0 wt% to about 0.1 wt%
Other bleach (such as reducing bleach and/or pre formed peracid)	from about 0 wt%

	to about 10 wt%
Chelant (such as ethylenediamine-N'N'-disuccinic acid (EDDS) and/or hydroxyethane diphosphonic acid (HEDP)	from about 0.2wt% to about 1wt%
Photobleach (such as zinc and/or aluminium sulphonated phthalocyanine)	from about 0 wt% to about 0.1 wt%
Hueing agent (such as direct violet 99, acid red 52, acid blue 80, direct violet 9, solvent violet 13 and any combination thereof)	from about 0 wt% to about 0.5 wt%
Brightener (such as brightener 15 and/or brightener 49)	from about 0.1wt% to about 0.4 wt%
Protease (such as Savinase, Polarzyme, Purafect, FN3, FN4 and any combination thereof, typically having an enzyme activity of from about 20 mg to about 100mg active enzyme/g)	from about 0.1wt% to about 1.5 wt%
Amylase (such as Termamyl(R), Termamyl Ultra(R), Natalase(R), Optisize HT Plus(R), Powerase(R), Stainzyme(R) and any combination thereof, typically having an enzyme activity of from about 10 mg to about 50 mg active enzyme/ g)	from about 0.05 wt% to about 0.2 wt%
Cellulase (such as Carezyme(R), Celluzyme(R) and/or Celluclean(R), typically having an enzyme activity of about from 10 to 50mg active enzyme/ g)	from about 0.05 wt% to 0.5 wt%
Lipase (such as Lipex(R), Lipolex(R), Lipoclean(R) and any combination thereof, typically having an enzyme activity of from about 10 mg to about 50 mg active enzyme/ g)	from about 0.2 wt% to about 1 wt%
Other enzyme (such as xyloglucanase (e.g., Whitezyme(R)), cutinase, pectate lyase, mannanase, bleaching enzyme, typically having an enzyme activity of from about 10 mg to about 50 mg active enzyme/g)	from 0 wt% to 2 wt%
Fabric softener (such as montmorillonite clay and/or polydimethylsiloxane (PDMS))	from 0 wt% to 15 wt%
Flocculant (such as polyethylene oxide)	from 0 wt% to 1 wt%
Suds suppressor (such as silicone and/or fatty acid)	from 0 wt% to 0.1wt%
Perfume (such as perfume microcapsule, spray-on perfume, starch	from 0.1 wt% to 1

encapsulated perfume accords, perfume loaded zeolite, and any combination thereof)	wt%
Aesthetics (such as colour ed soap rings and/or colour ed speckles/noodles)	from 0 wt% to 1wt%
Miscellaneous	Balance

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

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

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

Sodium carbonate and sodium bicarbonate can be obtained from Solvay, Brussels, Belgium.

Polyacrylate, polyacrylate/maleate copolymers can be obtained from BASF, Ludwigshafen, Germany.

Repel-O-Tex(R) can be obtained from Rhodia, Paris, France.

Texcare(R) can be obtained from Clariant, Sulzbach, Germany. Sodium percarbonate and sodium carbonate can be obtained from Solvay, Houston, Tex., USA.

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

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

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

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

Direct violet 9 and 99 can be obtained from BASF DE, Ludwigshafen, Germany. Solvent violet 13 can be obtained from Ningbo Lixing Chemical Co., Ltd. Ningbo, Zhejiang, China.

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

All percentages and ratios are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated. It should be understood that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

Wash assays

Launder-O-Meter (LOM) Model Wash System

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

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

Mini Launder-O-Meter (MiniLOM) Model Wash System

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

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

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

Terg-O-timeter (TOM) wash assay

The Tergo-To-Meter (TOM) is a medium scale model wash system that can be applied to test 12 different wash conditions simultaneously. A TOM is basically a large temperature controlled water bath with up to 12 open metal beakers submerged into it. Each beaker constitutes one small top loader style washing machine and during an experiment, each of them will contain a solution of a specific detergent/enzyme system and the soiled and unsoiled fabrics its performance is tested on. Mechanical stress is achieved by a rotating stirring arm, which stirs the liquid within each beaker. Because the TOM beakers have no lid, it is possible to withdraw samples during a TOM experiment and assay for information on-line during wash.

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

Equipment: The water bath with 12 steel beakers and 1 rotating arm per beaker with capacity of 500 or 1200mL of detergent solution. Temperature ranges from 5 to 80ºC. The water bath has to be filled up with deionised water. Rotational speed can be set up to 70 to 120rpm/min.

Set temperature in the Terg-O-Tometer and start the rotation in the water bath. Wait for the temperature to adjust (tolerance is +/- 0,5°C). All beakers shall be clean and without traces of prior test material.

The wash solution with desired amount of detergent, temperature and water hardness is prepared in a bucket. The detergent is allowed to dissolve during magnet stirring for 10 min. Wash solution shall be used within 30 to 60 min after preparation.

800ml wash solution is added into a TOM beaker. The wash solution is agitated at 120rpm and optionally one or more enzymes are added to the beaker. The swatches are sprinkled into the beaker and then the ballast load. Time measurement starts when the swatches and ballast are added to the beaker. The swatches are washed for 20 minutes after which agitation is terminated. The wash load is subsequently transferred from the TOM beaker to a sieve and rinse with cold tap water. The soid swatches are separated from the ballast load. The soil swatches are transferred to

a 5L beaker with cold tap water under running water for 5 minuttes. The ballast load is kept separately for the coming inactivation. The water is gently pressed out of the swatches by hand and placed on a tray covered with a paper. Another paper is palced on top of the swatches. The swatches are allowed to dry overnight before subjecting the swatches to analysis, such as measuring the colour intensity using a Colour Eye as described herein.

Enzyme assays

Assay I: 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 temperated 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 are observed as colour less zones around the spotted enzyme solutions.

Assay II

Analysis of E-2-nonenal on textile using an electronic nose.

One way of testing for the presence of malodour on textiles is by using E-2-Nonenal as a marker for the malodour, as this compound contributes to the malodour on laundry.

Add a solution of E-2-nonenal to a 5 cm x 5 cm textile swatch and place the swatch in a 20 mL glass vial for GC analysis and cap the vial. Analyze 5 mL headspace from the capped vials in a Heracles II Electronic nose from Alpha M.O.S., France (double column gas chromatograph with 2 FIDs, column 1: MXT5 and column 2: MXT1701) after 20 minutes incubation at 40°C.

Examples

Methods

General methods of PCR, cloning, ligation nucleotides etc. are well-known to a person skilled in the art and may for example be found in in “Molecular cloning: A laboratory manual”, Sambrook et al. (1989), Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. et al. (eds.); “Current protocols in Molecular Biology”, John Wiley and Sons, (1995); Harwood, C. R., and Cutting, S. M. (eds.); “DNA Cloning: A Practical Approach, Volumes I and II”, D.N. Glover ed. (1985); “Oligonucleotide Synthesis”, M.J. Gait ed. (1984); “Nucleic Acid Hybridization”, B.D. Hames

& S.J. Higgins eds (1985); “A Practical Guide To Molecular Cloning”, B. Perbal, (1984).

Example 1 Cloning and expression of a DNase from Vibrissea flavovirens

The DNase was derived from fruiting body of a fungal strain isolated in Japan by standard microbiological isolation techniques. The strain was identified and taxonomy was assigned based on DNA sequencing of the ribosomal ITS region (Table 1).

Table 1

Organism name	Source
Vibrissea flavovirens FC-2743	fruiting body, Japan

Chromosomal DNA isolated from pure cultures of the Vibrissea flavovirens strain FC-2743 with the DNeasy Plant Mini Kit from Qiagen (Hilden, Germany) was subjected to full genome sequencing using Illumina technology. Genome sequencing, the subsequent assembly of reads and the gene discovery (i.e. annotation of gene functions) is known to the person skilled in the art and the service can be purchased commercially. The genome sequence was analyzed for putative DNases from the PFAM database family PF07510 (R.D. Finn et al. Nucleic Acids Research (2014) ,42:D222-D230) This analysis identified a gene encoding the putative DNase with the nucleotide sequence and deduced amino acid sequence given in SEQ ID: 1 and SEQ ID NO: 2, respectively.

The encoded protein is 209 amino acids. Using the SignalP program (Nielsen et al., 1997, Protein Engineering 10: 1-6), a signal peptide of 19 residues was determined. The mature protein contains 190 amino acids with a predicted molecular mass of 20 kDa and an isoelectric pH of 5.8.

The gene encoding the Vibrissea flavovirens DNase was amplified by PCR and cloned in the expression vector pDau109 (WO 2005/042735) by a IN-FUSION™ Cloning Kit (BD Biosciences, Palo Alto, CA, USA). Cloning of the Vibrissea flavovirens DNase gene into Bam HI-Xho I digested pDau109 resulted in the transcription of the Vibrissea flavovirens DNase gene under the control of a NA2-tpi double promoter. NA2-tpi is a mod-ified promoter from the gene encoding the Aspergillus niger neutral alpha-amylase in which the untranslated leader has been replaced by an untranslated leader from the gene encoding the Aspergillus nidulans triose phosphate isomerase. The Vibrissea flavovirens DNase gene containing expression plasmid was transformed into Aspergillus oryzae MT3568. Aspergillus oryzae MT3568 is an AMDS (acetamidase) disrupted derivative of JaL355 (WO 02/40694) in which pyrG auxotrophy was restored in the process of knocking out the Aspergillus oryzae acetamidase (AMDS) gene. MT3568 protoplasts are prepared according to the method of European Patent No. 0238023, pages 14-15, which are incorporated herein by reference. Transformants were purified on COVE sucrose selection plates through single conidia prior to sporulating them on PDA plates. Production of the Vibrissea flavovirens DNase

polypeptide by the transformants was analyzed from culture supernatants of 1 ml 96 deep well stationary cultivations at 30°C in YP+2% glucose medium. Expression was verified on an E-Page 8% SDS-PAGE 48 well gel (Invitrogen, Carlsbad, CA, USA) by Coomassie staining. Subsequently, a recombinant Aspergillus oryzae clone containing the integrated expression construct was grown in liquid culture. The culture broth was collected by filtration through a bottle top MF75 Supor MachV 0.2 µm PES filter (Thermo Fisher Scientific, Roskilde, Denmark) in order to remove the rest of the Aspergillus oryzae host cells.

The DNases from Penicillium reticulisporum (SEQ ID NO 4), Acremonium dichromosporum (SEQ ID NO 5), Preussia aemulans (SEQ ID NO 6), Colletotrichum circinans (SEQ ID NO 7), Clavicipitaceae (SEQ ID NO 8), Preussia aemulans (SEQ ID NO 9) or Trichurus spiralis (SEQ ID NO 10) was cloned and expressed in a similar way.

Example 2 MiniLOM liquid detergent

Isolating laundry specific bacterial strains

One strain of *Brevundimonas* sp. isolated from laundry was used in the present example. The *Brevundimonas* sp. was isolated during a study, where the bacterial diversity in laundry after washing at 15, 40 and 60°C, respectively, was investigated. The study was conducted on laundry collected from Danish households. For each wash, 20 g of laundry items (tea towel, towel, dish cloth, bib, T-shirt armpit, T-shirt collar, socks) in the range 4:3:2:2:1:1:1 was used. Washing was performed in a Laundr-O-Meter (LOM) at 15, 40 or 60°C. For washing at 15 and 40°C, Ariel Sensitive White & Colour was used, whereas WFK IEC-A* model detergent was used for washing at 60°C. Ariel Sensitive White & Colour was prepared by weighing out 5.1 g and adding tap water up to 1000 ml followed by stirring for 5 minutes. WFK IEC-A* model detergent (which is available from WFK Testgewebe GmbH) was prepared by weighing out 5 g and adding tap water up to 1300 ml followed by stirring for 15 min. Washing was performed for 1 hour at 15, 40 and 60°C, respectively, followed by 2 times rinsing with tap water for 20 min at 15°C.

Laundry was sampled immediately after washing at 15, 40 and 60°C, respectively. Twenty grams of laundry was added 0.9% (w/v) NaCl (1.06404; Merck, Damstadt, Germany) with 0.5% (w/w) tween 80 to yield a 1:10 dilution in stomacher bag. The mixture was homogenized using a Stomacher for 2 minutes at medium speed. After homogenization, ten-fold dilutions were prepared in 0.9% (w/v) NaCl. Bacteria were enumerated on Tryptone Soya Agar (TSA) (CM0129, Oxoid, Basingstoke, Hampshire, UK) incubated aerobically at 30°C for 5-7 days. To suppress growth of yeast and moulds, 0.2% sorbic acid (359769, Sigma) and 0.1% cycloheximide (18079; Sigma) were added. Bacterial colonies were selected from countable plates and purified by restreaking twice on TSA. For long time storage, purified isolates were stored at -80°C in TSB containing 20% (w/v)

glycerol (49779; Sigma).

Preparation of swatches with biofilm

Brevundimonas sp. was pre-grown on Tryptone Soya Agar (TSA) (pH 7.3) (CM0131; Oxoid Ltd, Basingstoke, UK) for 2-5 days at 30°C. From a single colony, a loop-full was transferred to 10 mL of TSB and incubated for 1 day at 30°C with shaking (240 rpm). After propagation, *Brevundimonas* sp. was pelleted by centrifugation (Sigma Laboratory Centrifuge 6K15) (3000 g at 21°C in 7 min) and resuspended in 10 mL of TSB diluted twice with water. Optical density (OD) at 600 nm was measured using a spectophometer (POLARstar Omega (BMG Labtech, Ortenberg, Germany). Fresh TSB diluted twice with water was inoculated to an OD_{600nm} of 0.03, and 1.6 mL was added into each well of a 12-well polystyrene flat-bottom microplate (3512; Corning Incorporated, Corning, NY, USA), in which a round swatch (diameter 2 cm) of sterile Polyester WFK30A was placed. After incubation (24 h at 15°C with shaking (100 rpm), swatches were rinsed twice with 0.9% (w/v) NaCl.

Wash experiment

Wash liquor of liquid model detergent A were prepared by weighing out and dissolving detergent in water with water with hardness 15°dH. Dosing of model detergent A was 3.33 g/L. Pigment soil (Pigmentschmutz, 09V, wfk, Krefeld, Germany) (0.7 g/L) was added to the wash liquor. DNase (0.5 ppm) was added to the wash liquor. As control, wash liquor without DNase was made. Wash liquor (10 ml) was added to a 50 ml test tube, in which five rinsed swatches with *Brevundimonas* sp. biofilm and five sterile polyester (WFK30A) swatches were placed. Test tubes were placed in a Stuart rotator (Mini LOM) for 1 hour at 30°C. Swatches were rinsed twice with tap water and dried on filter paper over night. Colour difference (L values) was measured using a Colour Eye (Macbeth Colour Eye 7000 reflectance spectrophotometer). The measurements were made without UV in the incident light and the L value from the CIE Lab colour space was extracted. The colour difference (L value, L*) represents the darkest black at L* = 0, and the brightest white at L* = 100. Data is represented as Delta L values meaning the L value of the swatch washed with DNase minus the L value of swatch washed without DNase.

Table 2 Deep cleaning of biofilm established on polyester by fungal DNases in miniLOM.

Host name	L-value Model detergent A	ΔL Model detergent A
No enzyme	88.09	0

Vibressea flavovirens	83.48	4.61
Penicillium reticulisporum	88.00	4.41
Acremonium dichromosporum	87.49	3.91
Preussia aemulans	87.87	4.29
Colletotrichum circinans	86.48	2.89
Clavicipitaceae	89.05	5.47
Preussia aemulans	85.69	2.10
Trichurus spiralis	87.33	3.75

Example 3 MiniLOM wash in powder detergent

Isolating laundry specific bacterial strains

One strain of *Brevundimonas* sp. isolated from laundry was used in the present example. The *Brevundimonas* sp. was isolated during a study, where the bacterial diversity in laundry after washing at 15, 40 and 60°C, respectively, was investigated. The study was conducted on laundry collected from Danish households. For each wash, 20 g of laundry items (tea towel, towel, dish cloth, bib, T-shirt armpit, T-shirt collar, socks) in the range 4:3:2:2:1:1:1 was used. Washing was performed in a Laundr-O-Meter (LOM) at 15, 40 or 60°C. For washing at 15 and 40°C, Ariel Sensitive White & Colour was used, whereas WFK IEC-A* model detergent was used for washing at 60°C. Ariel Sensitive White & Colour was prepared by weighing out 5.1 g and adding tap water up to 1000 ml followed by stirring for 5 minutes. WFK IEC-A* model detergent (which is available from WFK Testgewebe GmbH) was prepared by weighing out 5 g and adding tap water up to 1300 ml followed by stirring for 15 min. Washing was performed for 1 hour at 15, 40 and 60°C, respectively, followed by 2 times rinsing with tap water for 20 min at 15°C.

Laundry was sampled immediately after washing at 15, 40 and 60°C, respectively. Twenty grams of laundry was added 0.9% (w/v) NaCl (1.06404; Merck, Damstadt, Germany) with 0.5% (w/w) tween 80 to yield a 1:10 dilution in stomacher bag. The mixture was homogenized using a Stomacher for 2 minutes at medium speed. After homogenization, ten-fold dilutions were prepared in 0.9% (w/v) NaCl. Bacteria were enumerated on Tryptone Soya Agar (TSA) (CM0129, Oxoid, Basingstoke, Hampshire, UK) incubated aerobically at 30°C for 5-7 days. To suppress growth of yeast and moulds, 0.2% sorbic acid (359769, Sigma) and 0.1% cycloheximide (18079; Sigma) were added. Bacterial colonies were selected from countable plates and purified by restreaking twice on TSA. For long time storage, purified isolates were stored at -80°C in TSB containing 20% (w/v) glycerol (49779; Sigma).

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Wash experiment

Wash liquors of powder model detergent T without bleach and powder model detergent T with bleach were prepared by weighing out and dissolving detergents in water with water with hardness 15°dH. Dosing of detergent T without bleach and model detergent T with bleach model detergent was 5.30 g/L. The AEO Biosoft N25-7 (NI) (0.16 g/l) component of model detergent T without bleach and model detergent T with bleach was added separately. Pigment soil (Pigmentschmutz, 09V, wfk, Krefeld, Germany) (0.7 g/L) was added to the wash liquor. DNase (0.5 ppm) was added to the wash liquor. As control, wash liquor without DNase was made. Wash liquor (10 ml) was added to a 50 ml test tube, in which five rinsed swatches with *Brevundimonas* sp. biofilm and five sterile polyester (WFK30A) swatches were placed. Test tubes were placed in a Stuart rotator (Mini LOM) for 1 hour at 30°C. Swatches were rinsed twice with tap water and dried on filter paper over night. Colour difference (L values) was measured using a Colour Eye (Macbeth Colour Eye 7000 reflectance spectrophotometer). The measurements were made without UV in the incident light and the L value from the CIE Lab colour space was extracted. The colour difference (L value, L*) represents the darkest black at L* = 0, and the brightest white at L* = 100. Data is represented as Delta L values meaning the L value of the swatch washed with DNase minus the L value of swatch washed without DNase.

Table 3 Deep cleaning of biofilm by *Vibressea flavovirens* DNase in miniLOM.

Detergent	Type of textile	Soil (g/L)	DNase conc. (ppm)	L-value	$L\text{-value}_{with\ DNase}$ −

					L-value$_{without\ DNase}$
Model detergent T w/o bleach	Polyester	0.7	0	85.58	
Model detergent T w/o bleach	Polyester	0.7	0.5	82.39	3.19
Model detergent T w bleach	Polyester	0.7	0	85.62	
Model detergent T w bleach	Polyester	0.7	0.5	84.95	0.67

Example 4: Cloning, expression and fermentation of DNases

The DNases were cloned from fungal strains obtained from a variety of sources.

Pyrenochaetopsis sp. was isolated in Denmark and received from the University of Copenhagen and is the source for the mature peptide with SEQ ID NO 27. Aspergillus sydowii, Cladosporium cladosporioides, Rhinocladiella sp., Pyronema domesticum, Paradendryphiella salina, Purpureocillium lilacinum, Warcupiella spinulosa, and Arthrinium arundinis were isolated from environmental samples by standard microbiological isolation techniques.

Strain	Origin	Mature peptide SEQ ID:
Aspergillus sydowii	Denmark	30
Cladosporium cladosporioides	Brazil	33
Rhinocladiella sp.	Denmark	36
Paradendryphiella salina	Ireland	48
Purpureocillium lilacinum	Denmark	54
Warcupiella spinulosa	Japan	57
Arthrinium arundinis	Denmark	69

Pyronema domesticum was purchased from the University of Oslo and is the source for the mature peptide with SEQ ID NO 39.

Phialophora geniculate, Stenocarpella maydis, Acrophialophora fusispora, and Chaetomium luteum were purchased from the CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

Strain	CBS strain number	Origin	Mature peptide SEQ ID:
Phialophora geniculata	CBS680.94	Indonesia	45 and 72
Stenocarpella maydis	CBS187.55	USA	60
Acrophialophora fusispora	CBS380.55	India	63
Chaetomium luteum	CBS543.83	Pakistan	66

Genomic DNA was isolated from the strains and the genomic sequences were determined, assembled and annotated by standard methods or by purchasing the services commercially. The annotated genomes were searched for putative DNases with a NUC2_B domain.

The predicted peptides with SEQ ID NO: 26, 29, 32, 35, 38, 41, 44, 59, 62, 65, 68, and 71 were found to have a NUC2_B domain and the corresponding DNA sequences encoding them with SEQ ID NO: 25, 28, 31, 34, 37, 40, 43, 58, 61, 64, 67, and 70 were PCR amplified from genomic DNA isolated from Pyrenochaetopsis sp., Aspergillus sydowii, Cladosporium cladosporioides, Rhinocladiella sp., Pyronema domesticum, Aspergillus niger, Phialophora geniculate, Paradendryphiella salina, Purpureocillium lilacinum, Warcupiella spinulosa, Stenocarpella maydis, Acrophialophora fusispora, Chaetomium luteum, and Arthrinium arundinis and cloned into the *Aspergillus* expression vector pMStr57(WO 04/032648).

The sequences of the NUC2_B encoding genes cloned in the expression vector were confirmed, and the expression constructs were transformed into the *Aspergillus oryzae* strain MT3568 (WO 11/057140). Transformants were selected on acetamide during regeneration from protoplasts and subsequently re-isolated under selection (Christensen *et al.*, 1988, *Biotechnology* 6, 1419-1422 and WO 04/032648).

For production of the recombinant DNases, a single *Aspergillus* transformant was selected for each DNase and the transformants were cultured in 500ml baffled flasks containing 150 ml of DAP-4C-1 medium (WO 12/103350). The cultures were shaken on a rotary table at 150 RPM at 30°C for 4 days. The culture broth was subsequently separated from cellular material by passage through a 0.22 um filter.

Example 5: Chromatographic purification of recombinant DNases

pH of the filtered sample was adjusted to around pH 7.5 and 1.8M ammonium sulfate was added. The sample was applied to a 5 ml HiTrap™ Phenyl (HS) column on an Äkta Explorer. Prior to

loading, the column had been equilibrated in 5 column volumes (CV) of 50mM HEPES + 1.8M AMS pH 7. In order to remove unbound material, the column was washed with 5 CV of 50mM HEPES + 1.8M AMS pH 7. The target protein was eluted from the column into a 10 ml loop using 50mM HEPES + 20% isopropanol pH 7. From the loop, the sample was loaded onto a desalting column (HiPrep™ 26/10 Desalting), which had been equilibrated with 3CV of 50mM HEPES+100mM NaCl pH 7.0. The target protein was eluted with 50mM HEPES+100mM NaCl pH 7.0 and relevant fractions were selected and pooled based on the chromatogram. The flow rate was 5 ml/min.

Protein concentration in the final sample was estimated by measuring absorption at 280nm.

Example 6 Cloning, expression and fermentation of DNases

Strains

Escherichia coli Top-10 strain purchased from TIANGEN (TIANGEN Biotech Co. Ltd., Beijing, China) was used to propagate our expression vector.

Aspergillus oryzae MT3568 strain was used for heterologous expression of the gene encoding a polypeptide having homology with polypeptides with phospholipase activity. *A. oryzae* MT3568 is an *amdS* (acetamidase) disrupted gene derivative of *A. oryzae* JaL355 (WO02/40694) in which *pyrG* auxotrophy was restored by disrupting the *A. oryzae* acetamidase (*amdS*) gene with the *pyrG* gene.

Media

YPM medium was composed of 10g yeast extract, 20 g Bacto-peptone, 20 g maotose, and deionised water to 1000 ml.

LB plates were composed of 10 g of Bacto-tryptone, 5 g of yeast extract, 10 g of sodium chloride, 15g of Bacto-agar, and deionised water to 1000 ml.

LB medium was composed of 1g of Bacto-tryptone, 5 g of yeast extract, and 10 g of sodium chloride, and deionised water to 1000 ml.

COVE sucrose plates were composed of 342 g of sucrose, 20 g of agar powder, 20 ml of COVE salt solution, and deionized water to 1 liter. The medium was sterilized by autoclaving at 15 psi for 15 minutes. The medium was cooled to 60°C and 10 mM acetamide, 15 mM CsCl, Triton X-100 (50 µl/500 ml) were added.

COVE-2 plate/tube for isolation: 30 g/L sucrose, 20 ml/L COVE salt solution, 10 mM acetamide, 30 g/L noble agar (Difco, Cat#214220).

COVE salt solution was composed of 26 g of $MgSO_4 \cdot 7H_2O$, 26g of KCL, 26g of KH_2PO_4, 50 ml of COVE trace metal solution, and deionised water to 1000 ml.

COVE trace metal solution was composed of 0.04g of $Na_2B_4O_7 \cdot 10H_2O$, 0.4g of $CuSO_4 \cdot 5H_2O$, 1.2g

of $FeSO_4 \cdot 7H_2O$, 0.7g of $MnSO_4 \cdot H_2O$, 0.8g of $Na_2MoO_4 \cdot 2H_2O$, 10g of $ZnSO_4 \cdot 7H_2O$, and deionised water to 1000 ml.

Methyl green DNA test agar plates was made by suspending 42.05 g "DNase Test Agar Base w/ methyl green" (HiMedia Laboratories Pvt. Ltd., Inida) in 1000 ml distilled water and sterilized by autoclaving.

Example 7: Cloning, expression and fermentation of fungal DNases

The DNases were derived from fungal strains isolated from environmental samples by standard microbiological isolation techniques. Strains were identified and taxonomy was assigned based on DNA sequencing of the internal transcribed spacer, ITS, of the 18S rRNA gene (Table 4).

Table 4:

Donor Organism name	source country	SEQ ID NO
Aspergillus insuetus	China	51

Chromosomal DNA from the strain (Table. 4) was isolated by QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). 5 µg of chromosomal DNA were sent for full genome sequencing using Illumina technology. Genome sequencing, and e.g. annotation of gene functions is known to the person skilled in the art and the service can be purchased commercially.

The genome sequences were analyzed for putative DNases from the PFAM database families NUC2 domain. This analysis identified 3 genes encoding putative DNases which were subsequently cloned and recombinantly expressed in *Aspergillus oryzae*. The gene was amplified by PCR from above isolated fungal genomic DNA. The purified PCR product was cloned into the previously digested expression vector pCaHj505 by ligation with an IN-FUSION™ CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) according to the manufacturer's instructions. The ligation mixture was used to transform *E. coli* TOP10 chemically competent cells (described in Strains). Correct colonies containing the DNase was selected and verified by DNA sequencing (by SinoGenoMax Company Limited, Beijing, China). The DNase comprising colonies were cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using a Qiagen Spin Miniprep kit (Cat. 27106) (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Using the SignalP program v.3 (Nielsen et al., 1997, Protein Engineering 10: 1-6), the signal peptide and accordingly the mature peptide of the DNase was predicted. Protoplasts of *Aspergillus oryzae* MT3568 was prepared according to WO95/002043. 100 µl of protoplasts were repectively mixed with 2.5-10 µg of each *Aspergillus*

expression vector comprising DNases and 250 µl of 60% PEG 4000, 10mM $CaCl_2$, and 10mM Tris-HCl pH7.5 and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread onto COVE sucrose plates for selection. After incubation for 4-7 days at 37°C spores of 4 transformants were inoculated into 3 ml of YPM medium. After 3 days cultivation at 30°C, the culture broths were analyzed by SDS-PAGE using Novex® 4-20% Tris-Glycine Gel (Invitrogen Corporation, Carlsbad, CA, USA) to identify the transformants producing the largest amount of recombinant DNAses with respective estimated mature peptide size. The hydrolytic activity of the DNase produced by the *Aspergillus* transformants was investigated using methyl green DNA test agar plates. 20 µl aliquots of the culture broth from the different transformants, or buffer (negative control) were distributed into punched holes with a diameter of 3 mm and incubated for 1 hour at 37°C. The plates were subsequently examined for the presence or absence of a white zone around the holes corresponding to phospholipase activity. Based on those two selection criteria, spores of the best transformant were spread on COVE-2 plates for re-isolation in order to isolate single colonies. Then a single colony was spread on a COVE-2 tube until sporulation. Spores from the best expressed transformant were cultivated in 2400 ml of YPM medium in shake flasks during 3 days at a temperature of 30°C under 80 rpm agitation. Culture broth was harvested by filtration using a 0.2 µm filter device. The filtered fermentation broth was used for enzyme characterization.

Example 8: Purification of recombinant DNase by metal ion affinity chromatography (IMAC)

The culture broth harvested in example 7 was precipitated with ammonium sulfate (80% saturated). Precipitates were re-dissolved in 50 ml of 20 mM PBS pH 7.0, and then filtered through a 0.45 µm filter. The filtered crude protein solution was applied to a 50 ml self-packed Ni sepharose excel affinity column (GE Healthcare, Buckinghamshire, UK) equilibrated with 20 mM PBS pH 7.0 and 300 mM sodium chloride. Proteins were eluted with a linear 0 - 0.5 M imidazole gradient. Fractions were analyzed by SDS-PAGE using a Mini-PROTEAN TGX Stain-Free 4-15% Precast Gel (Bio-Rad Laboratories, CA, United States). DNase activities of fractions were assessed on BD DifcoTM DNase Test Agar with Methyl Green (Becton, Dickinson and Company, New Jersey, United States) at pH 8.0, 40°C. Fractions were pooled containing recombinant protein bands and showing positive activities. Then the pooled solution was concentrated by ultrafiltration.

Example 9:

A phylogenetic tree was constructed, of public and proprietary polypeptide sequences containing an Exo_endo_phos domain, as defined in PFAM (PF03372, Pfam version 30.0 Finn (2016). Nucleic Acids Research, Database Issue 44:D279-D285). The phylogenetic tree was constructed from a multiple alignment of mature polypeptide sequences containing at least one Exo_endo_phos

domain. The sequences were aligned using the MUSCLE algorithm version 3.8.31 (Edgar, R.C. (2004). Nucleic Acids Research, 32(5), 1792-1797), and the tree was constructed using FastTree version 2.1.8 (Price et al. (2010) PloS one, 5(3)) and visualized using iTOL (Letunic, I., & Bork, P. (2007). Bioinformatics, 23(1), 127-128). The polypeptides in Exo_endo_phos can be separated into distinct sub-clusters, where we denoted one sub-cluster defined by the motif [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), corresponding to positions 396 to 399 in SEQ ID NO: 36;

Creating phylogenetic tree for NUC2

Reference SEQ ID: 36 (Rhinocladiella sp.)

A phylogenetic tree was constructed, of polypeptide sequences containing a NUC2 domain, as defined above. The phylogenetic tree was constructed from a multiple alignment of mature polypeptide sequences containing at least one NUC2 domain. The sequences were aligned using the MUSCLE algorithm version 3.8.31 (Edgar, R.C. (2004). Nucleic Acids Research, 32(5), 1792-1797), and the tree was constructed using FastTree version 2.1.8 (Price et al. (2010) PloS one, 5(3)) and visualized using iTOL (Letunic, I., & Bork, P. (2007). Bioinformatics, 23(1), 127-128).

Creating phylogenetic tree for NUC2_B

A phylogenetic tree was constructed, of polypeptide sequences containing a NUC2 domain, as defined above. The phylogenetic tree was constructed from a multiple alignment of mature polypeptide sequences containing at least one NUC2 domain. The sequences were aligned using the MUSCLE algorithm version 3.8.31 (Edgar, R.C. (2004). Nucleic Acids Research, 32(5), 1792-1797), and the tree was constructed using FastTree version 2.1.8 (Price et al. (2010) PloS one, 5(3)) and visualized using iTOL (Letunic, I., & Bork, P. (2007). Bioinformatics, 23(1), 127-128). The polypeptides in NUC2 can be separated into at least one distinct sub-clusters, one was denoted sub-clusters NUC2_B defined with motif SDH[D/H/L]P (SEQ ID NO 74), corresponding to positions 610 to 614 of SEQ ID NO: 36. The polypeptides of this sub-cluster e.g. domain e.g. clade may further comprise motif or GGNI[R/Q] corresponding to position 395 to 399 in SEQ ID NO: 36. The polypeptides have NUC2_B catalytic domains, wherein the NUC2_B catalytic domain having a trusted domain cut-off score of at least 100.0, preferably a score of at least 135, preferably a score of at least 150, preferably a score of at least 250 when queried using a Profile Hidden Markov

Hidden Markov Model (HMM):

the experimentally verified functional NUC2_B endo-nucleases were analyzed using the HMMER software package (available at http://hmmer.org; the theory behind profile HMMs is described in R.

Durbin, S. Eddy, A. Krogh, and G. Mitchison, Biological sequence analysis: probabilistic models of proteins and nucleic acids, Cambridge University Press, 1998; Krogh et al., 1994; J. Mol. Biol. 235:1501-1531), following the user guide which is available from HMMER (Janelia Farm Research Campus, Ashburn, Va., http://hmmer.org). Hidden Markov models are used in a number of databases that aim at classifying proteins, for review see Bateman A and Haft D H (2002) Brief Bioinform 3; 236-245. The output of the HMMER hmmbuild software program is a profile Hidden Markov Model (profile HMM) that characterizes the input sequences. As stated in the user guide, profile HMMs are statistical descriptions of the consensus of a multiple sequence alignment. They use position-specific scores for amino acids (or nucleotides) and position specific scores for opening and extending an insertion or deletion. Compared to other profile based methods, HMMs have a formal probabilistic basis. Profile HMMs for a large number of protein families are publicly available in the PFAM database (Janelia Farm Research Campus, Ashburn, Va.).

The profile HMM was built as follows:

Step 1. Build a Sequence Alignment

The polypeptides shown in SEQ ID NO: 10, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 33, SEQ ID NO: 36, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 45, SEQ ID NO: 48, SEQ ID NO: 51, SEQ ID NO: 54, SEQ ID NO: 57, SEQ ID NO: 6, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 66, SEQ ID NO: 69, SEQ ID NO: 7, SEQ ID NO: 72 were aligned using the MUSCLE algorithm version 3.8.31 with default parameters (Edgar, R.C. (2004). Nucleic Acids Research, 32(5), 1792-1797), and from this multiple sequence alignment the HMM was built with the software program hmmbuild version 3.1b2 (available at http://hmmer.org). hmmbuild reads the multiple sequence alignment file created by MUSCLE, builds a new profile HMM, and saves the profile HMM to a HMMER profile file. A profile HMM is completely described in a HMMER profile file, which contains all the probabilities that are used to parameterize the HMM.

Step 2. Test the Specificity and Sensitivity of the Built Profile HMMs

The Profile HMM was evaluated using hmmsearch version 3.1b2 software program with default settings, which reads a Profile HMM file and searches a sequence file for significantly similar sequence matches. The sequence file searched contained all Uniprot sequences annotated with Pfam family Exo_endo_phos (Pfam family PF03372, database version 30.0 UniProt annotated 14852 sequences). During the search, the size of the database (Z parameter) was set to 1 billion. This size setting ensures that significant E-values against the current database will remain significant in the foreseeable future. The hmmsearch trusted domain cut-off domT was set at 150.0. A hmmer search, using hmmsearch, with the profile HMM generated from the alignment of the NUC2_B experimentally active endo-nucleases, matched 3395 sequences in UniProt above a Trusted domain cut-off of 157.0; all matching pFam domain PF03372 and all comprising NUC2_B

motif SDH[DHL]P. This result indicates that members of the NUC2_B family share significant sequence similarity. A hmmer search with a Trusted domain cut-off of 150 was used to separate NUC2_B from other proteins.

Claims

1. A composition comprising a polypeptide having DNase activity, wherein the polypeptide comprises any of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R/Q] (SEQ ID NO 75), and at least on additional component, selected from,

v. a polyol, or

vi. an enzyme preferably selected from protease and lipases, or

vii. a surfactant, preferably selected from anionic or nonionic surfactants or

viii. one or more polymer.

2. A composition according to claim 1 wherein the polypeptide having DNase activity is selected from the group consisting of:

a) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 3,

b) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 4,

c) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 5,

d) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 6,

e) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 7,

f) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 8,

g) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 9,

h) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 10,

i) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 27,

j) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 30,

k) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 33,

l) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at

least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 36,

m) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 39,

n) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 42,

o) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 45,

p) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 48,

q) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 51,

r) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 54,

s) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 57,

t) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 60,

u) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 63,

v) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 66,

x) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 69,

and

y) a polypeptide having at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to the polypeptide of SEQ ID NO: 72.

3. A composition according to claim 1 or 2, wherein the polyol is selected from glycerol, (mono, di, or tri) propylene glycol, ethylene glycol, polyethylene glycol (for example PEG 200 – PEG 800), sugar alcohols, sorbitol, mannitol, erythritol, dulcitol, inositol, xylitol and adonitol.

4. A composition according to any of claims 1 to 3, wherein the enzyme is selected from

(a) at least 0.01 ppm of one or more protease variant 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 protease shown in SEQ ID NO 1 of WO 2011/036263, or

(b) at least 0.01 ppm one or more protease variant of a protease parent, wherein the protease variant comprises one or more mutation 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, L211Q, L211D, N212D, N212S, M216S, A226V, K229L, Q230H, Q239R, N246K, N255W, N255D, N255E, L256E, L256D T268A and R269H, wherein the positions correspond to the positions of the protease shown in SEQ ID NO 1 of WO 2011/036263, wherein the protease parent is selected from the protease shown in SEQ ID NO 1 of WO 2011/036263 (Savinase®) and the Bacillus amylolichenifaciens protease (BPN') shown in SEQ ID NO 2 of WO 2011/036263 and wherein the protease variants have at least 80% sequence identity to SEQ ID NO 1 or SEQ ID NO 2 of WO 2011/036263.

(c) at least 0.01 ppm of one or more amylase variants selected from the group consisting of:

(i) variants comprising one or more substitutions in the following positions: 9, 26, 30, 33, 82, 37, 106, 118, 128, 133, 149, 150, 160, 178, 182, 186, 193, 195, 202, 203, 214, 231, 256, 257, 258, 269, 270, 272, 283, 295, 296, 298, 299, 303, 304, 305, 311, 314, 315, 318, 319, 320, 323, 339, 345, 361, 378, 383, 419, 421, 437, 441, 444, 445, 446, 447, 450, 458, 461, 471, 482, 484, wherein the positions corresponds to positions of SEQ ID NO 2 of WO2000/060060;

(ii) variants exhibiting at least 90 percent identity with SEQ ID NO 2 of WO96/023873, with deletions in the 183 and 184 positions; or

(iii) variants exhibiting at least 95 percent identity with SEQ ID NO 3 of WO2008/112459, comprising mutations in one or more of the following positions M202, M208, S255, R172 and/or M261, or

(d) at least 0.01 ppm one or more lipase.

5. A composition according to any of claims 1 to 4, wherein the surfactant is selected from soap, LAS, AEOS and/or SLES.

6. A composition according to any of the proceeding claims wherein the polymer is selected from (carboxymethyl)cellulose (CMC), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) and poly(ethylene oxide) (PEG).

7. A composition according to any of the proceeding claims wherein the composition is a detergent composition, such as a laundry or dish wash composition.

8. A detergent composition according to claim 7 comprising a polypeptide having DNase activity selected from the group consisting of SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 or DNases having at least 80% sequence identity hereto and a detergent adjunct ingredient.

9. Use of a polypeptide having DNase activity, wherein the polypeptide comprises any of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R|Q] (SEQ ID NO 75) for preventing, reducing or removing a biofilm from an item, wherein the item is a textile.

10. Use according to claim 9 for preventing, reducing or removing stickiness of the item.

11. Use according to any of claims 9 to 10 for pretreating stains on the item.

12. Use according to any of claims 9 to 11 for preventing, reducing or removing redeposition of soil during a wash cycle.

13. Use according to any of claims 9 to 12 for preventing, reducing or removing adherence of soil to the item.

14. Use according to any of the preceding claims for maintaining or improving the whiteness of the item.

15. Use according to any of the preceding claims, wherein a malodour is reduced or removed from the item.

16. A polypeptide having DNase activity, wherein the polypeptide comprises one or more of the motifs [G/Y/W/F/A/H]NI[R/Q/D/E/V] (SEQ ID NO 73), SDH[D/H/L]P (SEQ ID NO 74) or GGNI[R|Q] (SEQ ID NO 75) and wherein the polypeptide is selected from the polypeptides:

a) a polypeptide having at least 72% sequence identity to the polypeptide of SEQ ID NO: 6,
b) a polypeptide having at least 90% sequence identity to the polypeptide of SEQ ID NO: 7,
c) a polypeptide having at least 84,5% sequence identity to the polypeptide of SEQ ID NO: 10,
d) a polypeptide having at least 82% sequence identity to the polypeptide of SEQ ID NO: 27,
e) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 30,
f) a polypeptide having at least 60% sequence identity to the polypeptide of SEQ ID NO: 33,
g) a polypeptide having at least 65% sequence identity to the polypeptide of SEQ ID NO: 36,
h) a polypeptide having at least 91% sequence identity to the polypeptide of SEQ ID NO: 39,
i) a polypeptide having at least 99% sequence identity to the polypeptide of SEQ ID NO: 42,
j) a polypeptide having at least 68% sequence identity to the polypeptide of SEQ ID NO: 45,
k) a polypeptide having at least 94% sequence identity to the polypeptide of SEQ ID NO: 48,
l) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 51,
m) a polypeptide having at least 73% sequence identity to the polypeptide of SEQ ID NO: 57,
n) a polypeptide having at least 90% sequence identity to the polypeptide of SEQ ID NO: 60,
o) a polypeptide having at least 84% sequence identity to the polypeptide of SEQ ID NO: 63,
p) a polypeptide having at least 80% sequence identity to the polypeptide of SEQ ID NO: 66,
q) a polypeptide having at least 72% sequence identity to the polypeptide of SEQ ID NO: 69, and
r) a polypeptide having at least 68% sequence identity to the polypeptide of SEQ ID NO: 72.

17. The polypeptide of claim 16, comprising or consisting of a polypeptide selected from the group consisting of SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72.

18. The polypeptide of any of claims 16 to 17, which is a variant of the polypeptide of SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69 or SEQ ID NO 72 comprising a substitution, deletion, and/or insertion at one or more positions.

19. A laundering method for laundering an item comprising the steps of:

a) Exposing an item to a wash liquor comprising a polypeptide of claims 16-18 or a detergent composition according to any of claim 8;

b) Completing at least one wash cycle; and

c) Optionally rinsing the item,

wherein the item is a textile.

20. Method according to claim 19, wherein the pH of the wash liquor is in the range 5.5 to 11, such as in the range of 7 to 9, in the range of 7 to 8 or in the range of 7 to 8.5.

21. Method according to claim 19 or 20, wherein the temperature of the wash liquor is in the range of 5ºC to 95ºC, or in the range of 10ºC to 80ºC, in the range of 10ºC to 70ºC, in the range of 10ºC to 60ºC, in the range of 10ºC to 50ºC, in the range of 15ºC to 40ºC or in the range of 20ºC to 30ºC.

22. a method of producing a polypeptide, wherein the polypeptide is selected from the group consisting of polypeptides in shown in SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 33, SEQ ID NO 36, SEQ ID NO 39, SEQ ID NO 42, SEQ ID NO 45, SEQ ID NO 48, SEQ ID NO 51, SEQ ID NO 54, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 72 and polypeptide having at least 80% sequence identity hereto, wherein the polypeptide has DNase activity

(a) cultivating the recombinant host cell under conditions conducive for production of the polypeptide; and

(b) recovering the polypeptide.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/074102

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/22 C11D3/386 C11D3/48
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 617 824 A1 (INDP ADMINISTRATIVE INST NIMS [JP]) 24 July 2013 (2013-07-24) sequence 3	1-22
X	DATABASE UniProt [Online] 13 October 2009 (2009-10-13), "SubName: Full=Bacterial-type extracellular deoxyribonuclease {ECO:0000313|EMBL:EEU45937.1};", XP002765068, retrieved from EBI accession no. UNIPROT:C7YPZ7 Database accession no. C7YPZ7 sequence	1-22

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 2016	17/02/2017

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

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE UniProt [Online] 1 October 2014 (2014-10-01), "SubName: Full=Uncharacterized protein {ECO:0000313¦EMBL:KEQ94839.1};", XP002765069, retrieved from EBI accession no. UNIPROT:A0A074YFK3 Database accession no. A0A074YFK3 sequence -----	1-22
X	DATABASE UniProt [Online] 18 September 2013 (2013-09-18), "SubName: Full=DNase I-like protein {ECO:0000313¦EMBL:EPE36386.1};", XP002765070, retrieved from EBI accession no. UNIPROT:S3DWR8 Database accession no. S3DWR8 sequence -----	1-22
A	DATABASE UniProt [Online] 18 September 2013 (2013-09-18), "SubName: Full=Uncharacterized protein {ECO:0000313¦EMBL:EPE32502.1};", XP002765071, retrieved from EBI accession no. UNIPROT:S3D1S1 Database accession no. S3D1S1 sequence -----	1-22
A	MARTIN ET AL: "Do non-long terminal repeat retrotransponsons have nuclease activity?", TRENDS IN BIOCHEMICAL SCIENCES, ELSEVIER, AMSTERDAM, NL, vol. 21, no. 8, 1 August 1996 (1996-08-01), pages 283-285, XP022263465, ISSN: 0968-0004 figure 1 -----	1-22
A	WO 2011/098579 A1 (UNIV NEWCASTLE [GB]; BURGESS JAMES GRANT [GB]; HALL MICHAEL JOHN [GB];) 18 August 2011 (2011-08-18) cited in the application sequence 5 abstract -----	1-22
A	WO 2014/087011 A1 (NOVOZYMES AS [DK]) 12 June 2014 (2014-06-12) cited in the application the whole document -----	1-22

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/074102

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

1-22(partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-22(partially)

Polypeptide having DNAse activity according to SEQ ID NO:3. Furthermore compositions, uses, detergents, methods relating thereto.

2-24. claims: 1-22(partially)

as invention 1, but relating to SEQ ID NOs -10, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72 respectively.

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25. claims: 1-22(partially)

As invention 1, but relating to polypeptides not covered by the above inventions. This invention is expected to comprise further inventions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/074102

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