

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

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

26 March 2020 (26.03.2020)



(10) International Publication Number

WO 2020/057510 A1

(51) International Patent Classification:

C11D 3/386 (2006.01)

(21) International Application Number:

PCT/CN2019/106223

(22) International Filing Date:

17 September 2019 (17.09.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PCT/CN2018/106187

18 September 2018 (18.09.2018) CN

(71) Applicant: **NOVOZYMES A/S** [DK/DK]; Krogshoejvej 36, DK-2880 Bagsvaerd (DK).

(72) Inventor; and

(71) Applicant (*for BW only*): **SUN, Tianqi** [CN/CN]; 14 Xinx Road, Shangdi Zone, Haidian District, Beijing 100085 (CN).

(72) Inventors: **SEGURA, Dorotea** Raventos; Krogshoejvej 36, 2880 Bagsvaerd (DK). **NISSEN, Dorota**; Krogshoejvej 36, 2880 Bagsvaerd (DK). **BARRIENTOS, Fabian**; Krogshoejvej 36, 2880 Bagsvaerd (DK). **SCHNORR, Kirk Matthew**; Krogshoejvej 36, 2880 Bagsvaerd (DK). **MORANT, Marc Dominique**; Krogshoejvej 36, 2880 Bagsvaerd (DK). **GJERMANSEN, Morten**; Krogshoejvej 36, 2880 Bagsvaerd (DK). **STRINGER, Mary Ann**; Krogshoejvej 36, 2880 Bagsvaerd (DK).

(74) Agent: **LIU, SHEN & ASSOCIATES**; 10th Floor, Building 1, 10 Caihefang Road, Haidian District, Beijing 100080 (CN).

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

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

(54) Title: T2 RNASES FOR CLEANING

(57) Abstract: Disclosed are new T2 RNase polypeptides and cleaning compositions containing the polypeptides. Also disclosed are methods for using the polypeptides and cleaning compositions. Also disclosed are polynucleotides encoding the polypeptides, and vectors and cells containing the polynucleotides.

WO 2020/057510 A1

T2 RNASES FOR CLEANING

Reference to a Sequence Listing

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

Field of the Invention

This application describes polypeptides with RNase activity, compositions containing the
polypeptides, and polynucleotides encoding the polypeptides. Also disclosed are nucleic acid
10 constructs and vectors encoding the polypeptides, and host cells expressing the constructs and vectors,
for producing the polypeptides. Methods of using the polypeptides are disclosed.

Background

Compositions for cleaning that contain enzymes are known in the art. Generally, the enzymes
15 in the cleaning compositions may remove stains, improve whiteness, and eliminate malodor. The
enzymes may degrade or remove molecules like proteins, polysaccharides or fats that may be present
in soils and stains. In some instances, these molecules and others may be present in organic stains
such as body soils, sweat, sebum, dead cells or biofilms, which are formations of microorganisms
within a matrix, the matrix generally composed of an extracellular polymeric substance (EPS), that
20 forms on surfaces. Biofilms are natural habitats for certain microorganisms and, as the microorganisms
grow in the biofilms, they secrete molecules like polysaccharides, proteins, lipids, nucleic acids (e.g.,
DNA and RNA), etc.

New cleaning compositions, including those containing enzymes, and those capable of
removing stains, improving whiteness and eliminating malodor continue to be developed.

Summary

We have shown that compositions containing RNases, specifically T2 RNases, have cleaning
activity on items, including fabrics. Although other enzymes have been shown to be efficacious in
removing soils and stains, use of RNases has not been described.

30 In one aspect, the disclosed invention relates to isolated polypeptides with RNase activity,
selected from amino acid sequences having at least 60% sequence identity, or at least 60% sequence
identity but less than 100% sequence identity, to one of the groups of SEQ ID NOs. 2 and/or 3, 5 and/or
6, 8 and/or 9, 11 and/or 12, 14 and/or 15, 17 and/or 18, 20 and/or 21, 23 and/or 24, 26 and/or 27, 29

and/or 30, 32 and/or 33, 35 and/or 36, 38 and/or 39, 41 and/or 42, 44 and/or 45, 47 and/or 48, 50 and/or 51, 53 and/or 54, 56 and/or 57, 59 and/or 60, 62 and/or 63, 65 and/or 66, 68 and/or 69, 71 and/or 72, 74 and/or 75, 77 and/or 78, 80 and/or 81, 83 and/or 84, 86 and/or 87, 89 and/or 90, 92 and/or 93, 95 and/or 96, 98 and/or 99, 101 and/or 102, or 109 and/or 110.

5 In one aspect, the invention is related to the isolated polypeptides set forth above that are variants. The variants may have one or more amino acid substitutions, deletions, insertions, or combinations thereof. The variants may extensions of amino acids at the N-terminal, C-terminal, or both N- and C-terminal ends of the polypeptides. The extensions may be His- or HQ-tags. In some examples, the variants may have amino acid substitutions and/or deletions outside of PF00445 domains of the polypeptides.

10 In one aspect, the invention is related to compositions that contain any of the polypeptides described above and herein. The compositions generally are useful for cleaning. One aspect of the invention relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component.

15 The compositions may contain other enzymes, in addition to the polypeptides having RNase activity, including proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases, deoxyribonucleases and/or mannanases. In some cases, one or more of those additional enzyme(s) may be specifically excluded from the compositions. One aspect relates to the use of the T2 RNases of the invention. One aspect relates to the use of a cleaning composition, preferably a laundry composition, comprising; at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, for cleaning an item by:

- 25 i. preventing, reducing or removing stickiness of the item;
ii. preventing, reducing or removing biofilm or biofilm components from the item;
iii. reducing or removing stains comprises RNA from the item;
iv. preventing, reducing or removing redeposition of soil during cleaning of the item;
v. preventing, reducing or removing adherence of soil to the item;
30 vi. maintaining or improving whiteness of the item; or
vii. preventing, reducing or removing malodor from the item,
wherein the item is a textile, a hard surface or a dish ware.

In one aspect, the invention is related to methods of using the polypeptides or compositions described above and herein. The methods are generally methods for cleaning an item by exposing the

item to the polypeptides or compositions. The item may be a textile. One aspect of the invention relates to a method for laundering an item, comprising:

- (a) exposing the item to a cleaning composition, preferably a laundry composition, comprising; at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component;
- (b) completing at least one wash cycle; and
- (c) optionally, rinsing the item, wherein the item is a textile, a hard surface or a dish ware.

In other aspects, the invention is related to polynucleotides encoding the polypeptides described above and herein, nucleic acid constructs or expression vectors comprising the polynucleotides, and recombinant host cells comprising the nucleic acid constructs or expression vectors.

Overview of Sequences

SEQ ID NO: 1 is a DNA sequence from *Trichoderma reesei* that encodes a full-length polypeptide.

SEQ ID NO: 2 is a full-length polypeptide encoded by SEQ ID NO: 1.

SEQ ID NO: 3 is a mature polypeptide derived from SEQ ID NO: 2.

SEQ ID NO: 4 is a DNA sequence from *Trichoderma harzianum* that encodes a full-length polypeptide.

SEQ ID NO: 5 is a full-length polypeptide encoded by SEQ ID NO: 4.

SEQ ID NO: 6 is a mature polypeptide derived from SEQ ID NO: 5.

SEQ ID NO: 7 is a DNA sequence from *Aspergillus oryzae* that encodes a full-length polypeptide.

SEQ ID NO: 8 is a full-length polypeptide encoded by SEQ ID NO: 7.

SEQ ID NO: 9 is a mature polypeptide derived from SEQ ID NO: 8.

SEQ ID NO: 10 is a DNA sequence from *Trichoderma harzianum* that encodes a full-length polypeptide.

SEQ ID NO: 11 is a full-length polypeptide encoded by SEQ ID NO: 10.

SEQ ID NO: 12 is a mature polypeptide derived from SEQ ID NO: 11.

SEQ ID NO: 13 is a DNA sequence from *Trichoderma harzianum* that encodes a full-length polypeptide.

SEQ ID NO: 14 is a full-length polypeptide encoded by SEQ ID NO: 13.

SEQ ID NO: 15 is a mature polypeptide derived from SEQ ID NO: 14.

SEQ ID NO: 16 is a DNA sequence from *Trichoderma harzianum* that encodes a full-length polypeptide.

SEQ ID NO: 17 is a full-length polypeptide encoded by SEQ ID NO: 16.

SEQ ID NO: 18 is a mature polypeptide derived from SEQ ID NO: 17.

SEQ ID NO: 19 is a DNA sequence from *Aeromonas molluscorum* that encodes a full-length polypeptide.

SEQ ID NO: 20 is a full-length polypeptide encoded by SEQ ID NO: 19.

- SEQ ID NO: 21 is a mature polypeptide derived from SEQ ID NO: 20.
- SEQ ID NO: 22 is a DNA sequence from *Paracoccus carotinifaciens* that encodes a full-length polypeptide.
- SEQ ID NO: 23 is a full-length polypeptide encoded by SEQ ID NO: 22.
- 5 SEQ ID NO: 24 is a mature polypeptide derived from SEQ ID NO: 23.
- SEQ ID NO: 25 is a DNA sequence from *Mycobacterium* sp-62710 that encodes a full-length polypeptide.
- SEQ ID NO: 26 is a full-length polypeptide encoded by SEQ ID NO: 25.
- SEQ ID NO: 27 is a mature polypeptide derived from SEQ ID NO: 26.
- 10 SEQ ID NO: 28 is a DNA sequence from *Shinella granuli* that encodes a full-length polypeptide.
- SEQ ID NO: 29 is a full-length polypeptide encoded by SEQ ID NO: 28.
- SEQ ID NO: 30 is a mature polypeptide derived from SEQ ID NO: 29.
- SEQ ID NO: 31 is a DNA sequence from *Agrobacterium fabrum* that encodes a full-length polypeptide.
- SEQ ID NO: 32 is a full-length polypeptide encoded by SEQ ID NO: 31.
- 15 SEQ ID NO: 33 is a mature polypeptide derived from SEQ ID NO: 32.
- SEQ ID NO: 34 is a DNA sequence from *Trichoderma parapiluliferum* that encodes a full-length polypeptide.
- SEQ ID NO: 35 is a full-length polypeptide encoded by SEQ ID NO: 34.
- SEQ ID NO: 36 is a mature polypeptide derived from SEQ ID NO: 35.
- 20 SEQ ID NO: 37 is a DNA sequence from *Lecanicillium* sp-WMM742 that encodes a full-length polypeptide.
- SEQ ID NO: 38 is a full-length polypeptide encoded by SEQ ID NO: 37.
- SEQ ID NO: 39 is a mature polypeptide derived from SEQ ID NO: 38.
- SEQ ID NO: 40 is a DNA sequence from *Ascochyta fabae* that encodes a full-length polypeptide.
- 25 SEQ ID NO: 41 is a full-length polypeptide encoded by SEQ ID NO: 41.
- SEQ ID NO: 42 is a mature polypeptide derived from SEQ ID NO: 42.
- SEQ ID NO: 43 is a DNA sequence from *Simplicillium lamellicola* that encodes a full-length polypeptide.
- SEQ ID NO: 44 is a full-length polypeptide encoded by SEQ ID NO: 43.
- SEQ ID NO: 45 is a mature polypeptide derived from SEQ ID NO: 44.
- 30 SEQ ID NO: 46 is a DNA sequence from *Penicillium polonicum* that encodes a full-length polypeptide.
- SEQ ID NO: 47 is a full-length polypeptide encoded by SEQ ID NO: 46.
- SEQ ID NO: 48 is a mature polypeptide derived from SEQ ID NO: 47.
- SEQ ID NO: 49 is a DNA sequence from *Simplicillium lamellicola* that encodes a full-length polypeptide.
- SEQ ID NO: 50 is a full-length polypeptide encoded by SEQ ID NO: 49.

- SEQ ID NO: 51 is a mature polypeptide derived from SEQ ID NO: 50.
- SEQ ID NO: 52 is a DNA sequence from *Setosphaeria rostrate* that encodes a full-length polypeptide.
- SEQ ID NO: 53 is a full-length polypeptide encoded by SEQ ID NO: 52.
- SEQ ID NO: 54 is a mature polypeptide derived from SEQ ID NO: 53.
- 5 SEQ ID NO: 55 is a DNA sequence from *Trichoderma parapiluliferum* that encodes a full-length polypeptide.
- SEQ ID NO: 56 is a full-length polypeptide encoded by SEQ ID NO: 55.
- SEQ ID NO: 57 is a mature polypeptide derived from SEQ ID NO: 56.
- SEQ ID NO: 58 is a DNA sequence from *Trichoderma atroviride* that encodes a full-length polypeptide.
- 10 SEQ ID NO: 59 is a full-length polypeptide encoded by SEQ ID NO: 58.
- SEQ ID NO: 60 is a mature polypeptide derived from SEQ ID NO: 59.
- SEQ ID NO: 61 is a DNA sequence from *Acremonium* sp-XZ2020 that encodes a full-length polypeptide.
- SEQ ID NO: 62 is a full-length polypeptide encoded by SEQ ID NO: 61.
- 15 SEQ ID NO: 63 is a mature polypeptide derived from SEQ ID NO: 62.
- SEQ ID NO: 64 is a DNA sequence from *Clathrosphaerina* sp that encodes a full-length polypeptide.
- SEQ ID NO: 65 is a full-length polypeptide encoded by SEQ ID NO: 64.
- SEQ ID NO: 66 is a mature polypeptide derived from SEQ ID NO: 65.
- SEQ ID NO: 67 is a DNA sequence from *Thermoascus aurantiacus* that encodes a full-length
- 20 polypeptide.
- SEQ ID NO: 68 is a full-length polypeptide encoded by SEQ ID NO: 67.
- SEQ ID NO: 69 is a mature polypeptide derived from SEQ ID NO: 68.
- SEQ ID NO: 70 is a DNA sequence from *Cadophora malorum* that encodes a full-length polypeptide.
- SEQ ID NO: 71 is a full-length polypeptide encoded by SEQ ID NO: 70.
- 25 SEQ ID NO: 72 is a mature polypeptide derived from SEQ ID NO: 71.
- SEQ ID NO: 73 is a DNA sequence from *Cladosporium* sp that encodes a full-length polypeptide.
- SEQ ID NO: 74 is a full-length polypeptide encoded by SEQ ID NO: 73.
- SEQ ID NO: 75 is a mature polypeptide derived from SEQ ID NO: 74.
- SEQ ID NO: 76 is a DNA sequence from *Zoogloea ramigera* that encodes a full-length polypeptide.
- 30 SEQ ID NO: 77 is a full-length polypeptide encoded by SEQ ID NO: 76.
- SEQ ID NO: 78 is a mature polypeptide derived from SEQ ID NO: 77.
- SEQ ID NO: 79 is a DNA sequence from an unknown microorganism that encodes a full-length polypeptide.
- SEQ ID NO: 80 is a full-length polypeptide encoded by SEQ ID NO: 79.

SEQ ID NO: 81 is a mature polypeptide derived from SEQ ID NO: 80.

SEQ ID NO: 82 is a DNA sequence from *Buttiauxella brennerae* that encodes a full-length polypeptide.

SEQ ID NO: 83 is a full-length polypeptide encoded by SEQ ID NO: 82.

SEQ ID NO: 84 is a mature polypeptide derived from SEQ ID NO: 83.

5 SEQ ID NO: 85 is a DNA sequence from *Caulobacter vibrioides* that encodes a full-length polypeptide.

SEQ ID NO: 86 is a full-length polypeptide encoded by SEQ ID NO: 85.

SEQ ID NO: 87 is a mature polypeptide derived from SEQ ID NO: 86.

SEQ ID NO: 88 is a DNA sequence from *Cadophora malorum* that encodes a full-length polypeptide that is a truncated version of the polypeptide encoded by SEQ ID NO: 70. The truncation was made to

10 remove a C-terminal domain of the encoded protein.

SEQ ID NO: 89 is the polypeptide encoded by SEQ ID NO: 88.

SEQ ID NO: 90 is a mature polypeptide derived from SEQ ID NO: 89.

SEQ ID NO: 91 is a DNA sequence from *Trichoderma atroviride* that encodes a full-length polypeptide that is a truncated version of the polypeptide encoded by SEQ ID NO: 58. The truncation was made to

15 remove a C-terminal domain of the encoded protein.

SEQ ID NO: 92 is the polypeptide encoded by SEQ ID NO: 91.

SEQ ID NO: 93 is a mature polypeptide derived from SEQ ID NO: 92.

SEQ ID NO: 94 is a DNA sequence from *Trichoderma parapoluliferum* that encodes a full-length polypeptide that is a truncated version of the polypeptide encoded by SEQ ID NO: 55. The truncation

20 was made to remove a C-terminal domain of the encoded protein.

SEQ ID NO: 95 is the polypeptide encoded by SEQ ID NO: 94.

SEQ ID NO: 96 is a mature polypeptide derived from SEQ ID NO: 95.

SEQ ID NO: 97 is a DNA sequence from *Acremonium sp.-XZ2020* that encodes a full-length polypeptide that is a truncated version of the polypeptide encoded by SEQ ID NO: 61. The truncation

25 was made to remove a C-terminal domain of the encoded protein.

SEQ ID NO: 98 is the polypeptide encoded by SEQ ID NO: 97.

SEQ ID NO: 99 is a mature polypeptide derived from SEQ ID NO: 98.

SEQ ID NO: 100 is a DNA sequence from *Clathrospira sp.* that encodes a full-length polypeptide that is a truncated version of the polypeptide encoded by SEQ ID NO: 64. The truncation was made to

30 remove a C-terminal domain of the encoded protein.

SEQ ID NO: 101 is the polypeptide encoded by SEQ ID NO: 100.

SEQ ID NO: 102 is a mature polypeptide derived from SEQ ID NO: 101.

SEQ ID NO: 103 is an amino acid motif found in many T2 RNases polypeptides from fungi.

SEQ ID NO: 104 is a T2 RNase from the fungus, *Aspergillus niger*.

SEQ ID NO: 105 is a first amino acid motif found in many T2 RNases polypeptides from bacteria.

SEQ ID NO: 106 is a second amino acid motif found in many T2 RNases polypeptides from bacteria.

SEQ ID NO: 107 is a T2 RNase from the bacterium, *Escherichia coli*.

5 SEQ ID NO: 108 is a DNA sequence from *Trichoderma harzianum* that encodes a full-length polypeptide that is a truncated version of the polypeptide encoded by SEQ ID NO: 4. The truncation was made to remove a C-terminal domain of the encoded protein.

SEQ ID NO: 109 is the polypeptide encoded by SEQ ID NO: 108.

SEQ ID NO: 110 is a mature polypeptide derived from SEQ ID NO: 109.

10 Definitions

The term "allelic variant" means any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation may arise naturally through mutation and may result in polymorphism within populations. These genetic changes, if within a polypeptide-coding sequence, may 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.

The term "biofilm" means a film produced by any group of microorganisms in which cells stick to each other or stick to a surface, such as a textile, dishware, hard surface or another kind of surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS is a polymeric conglomeration, generally composed of extracellular macromolecules, for example, DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces. The microbial cells growing in a biofilm may be 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 may have different properties from planktonic bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment for the microorganisms is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community.

30 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.

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.

5 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
10 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.

15 The term “deep cleaning” means, in this context disruption, reduction or removal of organic components such as polysaccharides, proteins, RNA, DNA, soil or other components present in organic matter such as biofilm. In some examples, reducing or removing biofilms from textiles/fabrics is deep cleaning.

20 The term “detergent adjunct ingredient” refers to ingredients different from the RNases 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 aids, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric agents, clay soil removal/anti-
25 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.

30 The term “cleaning component” includes any component (which is not water) useful in laundry and dish wash, including hand dish wash compositions and includes but are not limited to surfactants, builders, flocculating aids, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, builders and co-builders, fabric hueing agents, anti-foaming agents, dispersants, processing aids,

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

The term "detergent composition" or "cleaning composition" refers to compositions that find use in the removal of undesired compounds from items to be cleaned, such as textiles. The terms "detergent compositions" and "cleaning compositions" are used interchangeably in the present application. The detergent and cleaning composition may be used to, e.g., clean textiles for both household cleaning and industrial cleaning. The terms encompass any materials/compounds selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, gel, powder, granulate, paste, or spray compositions) and include, but are not limited to, detergent compositions such as liquid and/or solid laundry detergents and fine fabric detergents; fabric fresheners; fabric softeners; and textile and laundry pre-spotters/pretreatment. In addition to containing the enzyme of the invention, the detergent formulation may contain one or more additional enzymes (such as proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases and mannanases, or any mixture thereof), and/or detergent adjunct ingredients such as surfactants, builders, chelators or chelating agents, bleach systems or bleach components, polymers, fabric conditioners, foam boosters, suds suppressors, dyes, perfume, tannish inhibitors, optical brighteners, bactericides, fungicides, soil suspending agents, anti-corrosion agents, enzyme inhibitors or stabilizers, enzyme activators, transferase(s), hydrolytic enzymes, oxido reductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers.

The term "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.

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.

A "His-tag" refers to a polyhistidine tag typically comprising at least 6 histidine residues, that may be added to the N- or C-terminal. His-tags are known in the art for use in e.g. protein purification but may also be used for improving solubility at low pH values. Similarly, an "HQ-tag", i.e. a histidine-glutamine tag, may also be used for the purpose of purification as is known in the art.

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.

The term "immature polypeptide" means a polypeptide that is not in its final form following translation. For example, an immature polypeptide may undergo post-translation modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc., before it is considered to be a mature polypeptide. Herein, an immature polypeptide may be referred to as a full-length polypeptide.

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.

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 (i.e., manually).

The term "laundry" generally refers to household (e.g., clothes, towels, sheets, and the like) or industrial fabric items that are soiled (e.g., have less than optimal whiteness; produce a malodor).

By the term "malodor" is meant an odor which is not desired on clean items. The cleaned item should smell fresh and clean, without malodors adhered to the item. One example of malodor is compounds with an unpleasant smell which may be produced by microorganisms and trapped within a biofilm or stick to the "glue" of a biofilm. Other examples of unpleasant smells are sweat, or body odor adhered to an item which has been in contact with a human or animal. Other examples of malodor are odors from spices which stick to items, for example curry or other exotic spices with a strong smell.

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.

It is known in the art that a host cell may produce a mixture of two or 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
5 expressing the same polynucleotide.

The term "mature polypeptide coding sequence" means a polynucleotide that encodes a mature polypeptide having RNase activity.

The term "microorganism" generally means small organisms that are visible through a microscope. Microorganisms often exist as single cells or as colonies of cells. Some microorganisms
10 may be multicellular. Microorganisms include prokaryotic (e.g., bacteria and archaea) and eukaryotic (e.g., some fungi, algae, protozoa) organisms. Herein, viruses may be considered microorganisms.

The term "naturally occurring" generally means existing in nature, without human intervention. More specifically, "naturally occurring" encompasses things that are not patentable in the United States
15 under Section 101 of Title 35 of the United States Code.

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.

20 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.

The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

25 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
30 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).

The term "RNase" is an abbreviation of the term ribonuclease, which means a nuclease having

RNase activity that catalyzes degradation of RNA into smaller components. Ribonucleases can be divided into endoribonucleases and exoribonucleases. For purposes of the present invention, RNase activity is determined according to procedures described in the Examples. Generally, the RNases disclosed herein are polypeptides and are enzymes. 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 RNase activity of any of the mature polypeptides shown in SEQ ID NOs: 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 99, 102 or 110. Assays that measure RNase activity generally measure degradation of RNA into smaller components.

Generally, the RNases that are the subject of this application are T2 RNases. T2 RNases are generally defined by the presence of an amino acid domain described as Pfam PF00445 (PF00445, Pfam version 31.0 Fin, (2016) *Nucleic Acids Research*, Database Issue 44:D279-D285; Pfam: Family: Ribonuclease_T2 (PF00445) [WWW Document], n.d. URL <http://Pfam.xfam.org/family/PF00445>). Other domains may be present in the T2 RNases

The term "textile" means cloth or fabric.

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

The term "whiteness" refers to the property or quality of being white. Generally, whiteness of a textile may correlate with its cleanliness. Deep cleaning of a soiled item may increase the whiteness of the item.

Nomenclature

For purposes of this disclosure, the nomenclature [E/Q] or simply [EQ] 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] or [VGAI] 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.

Compositions for Cleaning

Generally, the T2 RNase-containing compositions disclosed herein, and methods of using the

compositions, are useful for cleaning. In some instances, the compositions may be useful for deep cleaning of items that contain organic stains, such as body soils (e.g., sebum, sweat and dead cells/cell debris) and/or biofilms. In some instances, the compositions are useful for stain/soil removal, improving whiteness, prevention/reduction/removal of malodor, and/or for prevention or reduction of soil redeposition. There are no descriptions in the prior art that RNases have cleaning activity or activity against such stains. However:

WO 2004/041988 (US 2005/0079594) discloses methods for removing biofilms using solutions containing combinations of enzymes, one which may be RNases;

WO 2006/031554 discloses methods for preventing, removing, reducing or disrupting a biofilm using alpha-amylases, which may be combined with other enzymes, including RNases;

WO 2008/153805 discloses detergents that may contain enzymes. Among many enzymes, RNases are disclosed.

In all the above disclosures, RNases are disclosed as part of laundry lists on enzymes that might be included in compositions. No effect of, or results from, use of RNases are described. No specific RNases are disclosed in those disclosures, for use in the methods or compositions that are disclosed therein.

RNases

The RNases that are disclosed herein are T2 RNases. The T2 RNases that have been characterized are transferase-type RNases. Families of transferase-type RNases include RNase A, RNase T1 and RNase T2. The RNases that are the subject of this patent application generally are T2 RNases. T2 RNases are classified as such based on their similarity to the RNase T2 from *Aspergillus oryzae* (e.g., see Ozeki, K. et al. Cloning and nucleotide sequence of the genomic ribonuclease T2 gene (*rntB*) from *Aspergillus oryzae*. Curr Genet. 1991;19:367–373).

Herein, molecules were classified as T2 RNases if they include a T2 domain, as defined by Pfam PF00445 (Pfam version 31.0 Fin, (2016) *Nucleic Acids Research*, Database Issue 44:D279-D285; Pfam: Family: Ribonuclease_T2 (PF00445) [WWW Document], n.d. URL <http://Pfam.xfam.org/family/PF00445>).

The Pfam protein family database is a resource of the European Bioinformatics Institute (EMBL-EBI) (<https://Pfam.xfam.org/>). The Pfam PF00445 domain is an amino acid domain. For Pfam domains/families, including PF00445, a group of protein sequences called seeds were chosen to define the family. For PF00445, 228 sequences were used. These were aligned to make a seed alignment (<https://Pfam.xfam.org/family/PF00445/alignment/seed/html>). The seed alignment was used to construct a profile hidden Markov model (HMM) using HMMER3 software (<http://hmmer.org/>)

(Accelerated profile HMM searches. S. R. Eddy. PLOS Comp. Biol., 7:e1002195, 2011). The HMM profile is here: <https://Pfam.xfam.org/family/PF00445/hmm>; and can be visualized here: <https://Pfam.xfam.org/family/PF00445>. This profile HMM was used to search other protein sequences, with all matches scoring greater than or equal to a curated threshold (called the gathering threshold) considered as members of the PF00445 family. These members were subsequently aligned to the profile HMM to generate a full alignment.

The Pfam domain is as explained above characteristic for the T2 RNases. This group of RNases are distinct from other RNases e.g. T1 RNases, Luhtala, N. and Parker, R. Trends Biochem Sci. 2010 May; 35(5): 253–259. The T2 RNase of the invention is preferably from E.C. class EC 3.1.27.1.

The T2 RNases preferably belongs to the E.C class (EC 3.1.27.1).

Generally, all known organisms encode T2 RNases. This application encompasses compositions/methods of using compositions that contain T2 RNases from any source. In some examples, the RNases encompassed here are T2 RNases from microorganisms. In some examples, the RNases encompassed here are T2 RNases from fungi. In some examples, the RNases encompassed here include T2 RNases that contain a HGLWPD (SEQ ID NO: 103) amino acid motif, which is discussed herein. In some examples, the RNases encompassed here include T2 RNases from bacteria. In some examples, the RNases encompassed here include T2 RNases that include a [VL]HGLWP[QNEG] (SEQ ID NO: 105) amino acid motif. In some examples, the RNases encompassed here include T2 RNases that include a L[SFT]WX[PT]XXC amino acid motif (SEQ ID NO: 106), where X may be any naturally-occurring amino acid.

In one aspect, the polypeptides disclosed herein are immature T2 RNase polypeptides. Specifically, disclosed herein are:

SEQ ID NO: 2 is a full-length T2 RNase polypeptide from *Trichoderma reesei*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-203 of SEQ ID NO: 2. SEQ ID NO: 3 is amino acids 1-238 of SEQ ID NO: 2.

SEQ ID NO: 5 is a full-length T2 RNase polypeptide from *Trichoderma harzianum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 31-202 of SEQ ID NO: 5. SEQ ID NO: 6 is amino acids 1-387 of SEQ ID NO: 5.

SEQ ID NO: 8 is a full-length T2 RNase polypeptide from *Aspergillus oryzae*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 25-198 of SEQ ID NO: 8. SEQ ID NO: 9 is amino acids 1-253 of SEQ ID NO: 8.

SEQ ID NO: 11 is a full-length T2 RNase polypeptide from *Trichoderma harzianum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 42-260 of SEQ ID NO: 11. SEQ ID NO: 12 is amino acids 1-305 of SEQ ID NO: 11.

5 SEQ ID NO: 14 is a full-length T2 RNase polypeptide from *Trichoderma harzianum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-204 of SEQ ID NO: 14. SEQ ID NO: 15 is amino acids 1-247 of SEQ ID NO: 14.

10 SEQ ID NO: 17 is a full-length T2 RNase polypeptide from *Trichoderma harzianum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 29-201 of SEQ ID NO: 17. SEQ ID NO: 18 is amino acids 1-384 of SEQ ID NO: 17.

15 SEQ ID NO: 20 is a full-length T2 RNase polypeptide from *Aeromonas molluscorum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 3-166 of SEQ ID NO: 20. SEQ ID NO: 21 is amino acids 1-189 of SEQ ID NO: 20.

20 SEQ ID NO: 23 is a full-length T2 RNase polypeptide from *Paracoccus carotinifaciens*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 7-173 of SEQ ID NO: 23. SEQ ID NO: 24 is amino acids 1-192 of SEQ ID NO: 23.

SEQ ID NO: 26 is a full-length T2 RNase polypeptide from *Mycobacterium sp-62710*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 25-136 of SEQ ID NO: 26. SEQ ID NO: 27 is amino acids 1-229 of SEQ ID NO: 26.

25 SEQ ID NO: 29 is a full-length T2 RNase polypeptide from *Shinella granuli*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-197 of SEQ ID NO: 29. SEQ ID NO: 30 is amino acids 1-216 of SEQ ID NO: 29.

30 SEQ ID NO: 32 is a full-length T2 RNase polypeptide from *Agrobacterium fabrum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 45-212 of SEQ ID NO: 32. SEQ ID NO: 33 is amino acids 1-231 of SEQ ID NO: 32.

SEQ ID NO: 35 is a full-length T2 RNase polypeptide from *Trichoderma parapiluliferum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-203 of SEQ ID NO: 35. SEQ ID NO: 36 is amino acids 1-245 of

SEQ ID NO: 35.

SEQ ID NO: 38 is a full-length T2 RNase polypeptide from *Lecanicillium sp-WMM742*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 29-202 of SEQ ID NO: 38. SEQ ID NO: 39 is amino acids 1-239 of
5 SEQ ID NO: 38.

SEQ ID NO: 41 is a full-length T2 RNase polypeptide from *Ascochyta fabae*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 32-203 of SEQ ID NO: 41. SEQ ID NO: 42 is amino acids 1-248 of SEQ ID NO: 41.

SEQ ID NO: 44 is a full-length T2 RNase polypeptide from *Simplicillium lamellicola*. The region
10 of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 41-260 of SEQ ID NO: 44. SEQ ID NO: 45 is amino acids 1-310 of SEQ ID NO: 44.

SEQ ID NO: 47 is a full-length T2 RNase polypeptide from *Penicillium polonicum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes
15 approximate amino acids 30-203 of SEQ ID NO: 47. SEQ ID NO: 48 is amino acids 1-233 of SEQ ID NO: 47.

SEQ ID NO: 50 is a full-length T2 RNase polypeptide from *Simplicillium lamellicola*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 33-207 of SEQ ID NO: 50. SEQ ID NO: 51 is amino acids 1-243 of SEQ ID
20 NO: 50.

SEQ ID NO: 53 is a full-length T2 RNase polypeptide from *Setosphaeria rostrate*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 40-258 of SEQ ID NO: 53. SEQ ID NO: 54 is amino acids 1-307 of SEQ ID
NO: 53.

SEQ ID NO: 56 is a full-length T2 RNase polypeptide from *Trichoderma parapiluliferum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-202 of SEQ ID NO: 56. SEQ ID NO: 57 is amino acids 1-389 of
25 SEQ ID NO: 56.

SEQ ID NO: 59 is a full-length T2 RNase polypeptide from *Trichoderma atroviride*. The region
30 of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 31-202 of SEQ ID NO: 59. SEQ ID NO: 60 is amino acids 1-388 of SEQ ID NO: 59.

SEQ ID NO: 62 is a full-length T2 RNase polypeptide from *Acremonium sp-XZ2020*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes

approximate amino acids 34-205 of SEQ ID NO: 62. SEQ ID NO: 63 is amino acids 1-385 of SEQ ID NO: 62.

SEQ ID NO: 65 is a full-length T2 RNase polypeptide from *Clathrosphaerina sp.* The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-201 of SEQ ID NO: 65. SEQ ID NO: 66 is amino acids 1-372 of SEQ ID NO: 65.

SEQ ID NO: 68 is a full-length T2 RNase polypeptide from *Thermoascus aurantiacus*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 31-206 of SEQ ID NO: 68. SEQ ID NO: 69 is amino acids 1-238 of SEQ ID NO: 68.

SEQ ID NO: 71 is a full-length T2 RNase polypeptide from *Cadophora malorum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 138-308 of SEQ ID NO: 71. SEQ ID NO: 72 is amino acids 1-476 of SEQ ID NO: 71.

SEQ ID NO: 74 is a full-length T2 RNase polypeptide from *Cladosporium sp.* The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 42-259 of SEQ ID NO: 74. SEQ ID NO: 75 is amino acids 1-307 of SEQ ID NO: 74.

SEQ ID NO: 77 is a full-length T2 RNase polypeptide from *Zoogloea ramigera*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 32-199 of SEQ ID NO: 77. SEQ ID NO: 78 is amino acids 1-218 of SEQ ID NO: 77.

SEQ ID NO: 80 is a full-length T2 RNase polypeptide from an unknown microorganism. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 7-173 of SEQ ID NO: 80. SEQ ID NO: 81 is amino acids 1-191 of SEQ ID NO: 80.

SEQ ID NO: 83 is a full-length T2 RNase polypeptide from *Buttiauxella brennerae*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 12-213 of SEQ ID NO: 83. SEQ ID NO: 84 is amino acids 1-245 of SEQ ID NO: 83.

SEQ ID NO: 86 is a full-length T2 RNase polypeptide from *Caulobacter vibrioides*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 34-195 of SEQ ID NO: 86. SEQ ID NO: 87 is amino acids 1-214 of SEQ ID NO: 86.

SEQ ID NO: 89 is a full-length truncated version of a T2 RNase polypeptide from *Cadophora*

malorum. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 138-308 of SEQ ID NO: 89. SEQ ID NO: 90 is amino acids 1-354 of SEQ ID NO: 89.

SEQ ID NO: 92 is a full-length truncated version of a T2 RNase polypeptide from *Trichoderma atroviride*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 31-202 of SEQ ID NO: 92. SEQ ID NO: 93 is amino acids 1-236 of SEQ ID NO: 92.

SEQ ID NO: 95 is a full-length truncated version of a T2 RNase polypeptide from *Trichoderma parapiluliferum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-202 of SEQ ID NO: 95. SEQ ID NO: 96 is amino acids 1-246 of SEQ ID NO: 95.

SEQ ID NO: 98 is a full-length truncated version of a T2 RNase polypeptide from *Acremonium* sp. XZ2020. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 34-205 of SEQ ID NO: 98. SEQ ID NO: 99 is amino acids 1-239 of SEQ ID NO: 98.

SEQ ID NO: 101 is a full-length truncated version of a T2 RNase polypeptide from *Clathrosphaerina* sp. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 30-201 of SEQ ID NO: 101. SEQ ID NO: 102 is amino acids 1-236 of SEQ ID NO: 101.

SEQ ID NO: 109 is a full-length truncated version of a T2 RNase polypeptide from *Trichoderma harzianum*. The region of this polypeptide which contains the domain identifying the polypeptide as a T2 RNase includes approximate amino acids 31-202 of SEQ ID NO: 109. SEQ ID NO: 110 is amino acids 1-240 of SEQ ID NO: 109.

In one aspect, the polypeptides disclosed herein are mature T2 RNase polypeptides. Specifically disclosed herein are:

In one aspect, the mature polypeptide is amino acids 1 to 238 of SEQ ID NO: 2. Amino acids -19 to -1 of SEQ ID NO: 2 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 3.

In one aspect, the mature polypeptide is amino acids 1 to 387 of SEQ ID NO: 5. Amino acids -19 to -1 of SEQ ID NO: 5 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 6.

In one aspect, the mature polypeptide is amino acids 1 to 253 of SEQ ID NO: 8. Amino acids -23 to -1 of SEQ ID NO: 8 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 9.

In one aspect, the mature polypeptide is amino acids 1 to 305 of SEQ ID NO: 11. Amino acids -17 to -1 of SEQ ID NO: 11 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 12.

In one aspect, the mature polypeptide is amino acids 1 to 247 of SEQ ID NO: 14. Amino acids - 21 to -1 of SEQ ID NO: 14 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 15.

In one aspect, the mature polypeptide is amino acids 1 to 384 of SEQ ID NO: 17. Amino acids - 19 to -1 of SEQ ID NO: 17 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 18.

In one aspect, the mature polypeptide is amino acids 1 to 189 of SEQ ID NO: 20. Amino acids - 24 to -1 of SEQ ID NO: 20 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 21.

In one aspect, the mature polypeptide is amino acids 1 to 192 of SEQ ID NO: 23. Amino acids - 19 to -1 of SEQ ID NO: 23 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 24.

In one aspect, the mature polypeptide is amino acids 1 to 229 of SEQ ID NO: 26. Amino acids - 24 to -1 of SEQ ID NO: 26 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 27.

In one aspect, the mature polypeptide is amino acids 1 to 216 of SEQ ID NO: 29. Amino acids - 26 to -1 of SEQ ID NO: 29 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 30.

In one aspect, the mature polypeptide is amino acids 1 to 231 of SEQ ID NO: 32. Amino acids - 21 to -1 of SEQ ID NO: 32 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 33.

In one aspect, the mature polypeptide is amino acids 1 to 245 of SEQ ID NO: 35. Amino acids - 19 to -1 of SEQ ID NO: 35 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 36.

In one aspect, the mature polypeptide is amino acids 1 to 239 of SEQ ID NO: 38. Amino acids - 17 to -1 of SEQ ID NO: 38 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 39.

In one aspect, the mature polypeptide is amino acids 1 to 248 of SEQ ID NO: 41. Amino acids - 16 to -1 of SEQ ID NO: 41 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 42.

In one aspect, the mature polypeptide is amino acids 1 to 310 of SEQ ID NO: 44. Amino acids - 19 to -1 of SEQ ID NO: 44 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 45.

In one aspect, the mature polypeptide is amino acids 1 to 233 of SEQ ID NO: 47. Amino acids -

23 to -1 of SEQ ID NO: 47 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 48.

In one aspect, the mature polypeptide is amino acids 1 to 243 of SEQ ID NO: 50. Amino acids - 19 to -1 of SEQ ID NO: 50 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 51.

In one aspect, the mature polypeptide is amino acids 1 to 307 of SEQ ID NO: 53. Amino acids - 21 to -1 of SEQ ID NO: 53 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 54.

In one aspect, the mature polypeptide is amino acids 1 to 389 of SEQ ID NO: 56. Amino acids - 18 to -1 of SEQ ID NO: 56 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 57.

In one aspect, the mature polypeptide is amino acids 1 to 388 of SEQ ID NO: 59. Amino acids - 18 to -1 of SEQ ID NO: 59 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 60.

In one aspect, the mature polypeptide is amino acids 1 to 385 of SEQ ID NO: 62. Amino acids - 20 to -1 of SEQ ID NO: 62 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 63.

In one aspect, the mature polypeptide is amino acids 1 to 372 of SEQ ID NO: 65. Amino acids - 16 to -1 of SEQ ID NO: 65 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 66.

In one aspect, the mature polypeptide is amino acids 1 to 238 of SEQ ID NO: 68. Amino acids - 27 to -1 of SEQ ID NO: 68 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 69.

In one aspect, the mature polypeptide is amino acids 1 to 476 of SEQ ID NO: 71. Amino acids - 19 to -1 of SEQ ID NO: 72 is the signal peptide.

In one aspect, the mature polypeptide is amino acids 1 to 307 of SEQ ID NO: 74. Amino acids - 17 to -1 of SEQ ID NO: 74 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 75.

In one aspect, the mature polypeptide is amino acids 1 to 218 of SEQ ID NO: 77. Amino acids - 24 to -1 of SEQ ID NO: 77 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 78.

In one aspect, the mature polypeptide is amino acids 1 to 191 of SEQ ID NO: 80. Amino acids - 19 to -1 of SEQ ID NO: 80 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 81.

In one aspect, the mature polypeptide is amino acids 1 to 245 of SEQ ID NO: 83. Amino acids - 22 to -1 of SEQ ID NO: 83 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 84

In one aspect, the mature polypeptide is amino acids 1 to 214 of SEQ ID NO: 86. Amino acids - 19 to -1 of SEQ ID NO: 86 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 87.

In one aspect, the mature polypeptide is amino acids 1 to 354 of SEQ ID NO: 89. Amino acids - 19 to -1 of SEQ ID NO: 89 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 90.

In one aspect, the mature polypeptide is amino acids 1 to 236 of SEQ ID NO: 92. Amino acids - 18 to -1 of SEQ ID NO: 92 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 93.

In one aspect, the mature polypeptide is amino acids 1 to 246 of SEQ ID NO: 95. Amino acids - 18 to -1 of SEQ ID NO: 95 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 96.

In one aspect, the mature polypeptide is amino acids 1 to 239 of SEQ ID NO: 98. Amino acids - 20 to -1 of SEQ ID NO: 98 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 99.

In one aspect, the mature polypeptide is amino acids 1 to 236 of SEQ ID NO: 101. Amino acids -16 to -1 of SEQ ID NO: 101 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 102.

In one aspect, the mature polypeptide is amino acids 1 to 240 of SEQ ID NO: 109. Amino acids -19 to -1 of SEQ ID NO: 109 is the signal peptide. The mature polypeptide is also shown in SEQ ID NO: 110.

In some embodiments, the invention may relate to the amino acid sequences of SEQ ID NO: 2 or SEQ ID NO: 3 (mature polypeptide), SEQ ID NO: 5 or SEQ ID NO: 6 (mature polypeptide), SEQ ID NO: 8 or SEQ ID NO: 9 (mature polypeptide), SEQ ID NO: 11 or SEQ ID NO: 12 (mature polypeptide), SEQ ID NO: 14 or SEQ ID NO: 15 (mature polypeptide), SEQ ID NO: 17 or SEQ ID NO: 18 (mature polypeptide), SEQ ID NO: 20 or SEQ ID NO: 21 (mature polypeptide), SEQ ID NO: 23 or SEQ ID NO: 24 (mature polypeptide), SEQ ID NO: 26 or SEQ ID NO: 27 (mature polypeptide), SEQ ID NO: 29 or SEQ ID NO: 30 (mature polypeptide), SEQ ID NO: 32 or SEQ ID NO: 33 (mature polypeptide), SEQ ID NO: 35 or SEQ ID NO: 36 (mature polypeptide), SEQ ID NO: 38 or SEQ ID NO: 39 (mature polypeptide), SEQ ID NO: 41 or SEQ ID NO: 42 (mature polypeptide), SEQ ID NO: 44 or SEQ ID NO: 45 (mature polypeptide), SEQ ID NO: 47 or SEQ ID NO: 48 (mature polypeptide), SEQ ID NO: 50 or

SEQ ID NO: 51 (mature polypeptide), SEQ ID NO: 53 or SEQ ID NO: 54 (mature polypeptide), SEQ ID NO: 56 or SEQ ID NO: 57 (mature polypeptide), SEQ ID NO: 59 or SEQ ID NO: 60 (mature polypeptide), SEQ ID NO: 62 or SEQ ID NO: 63 (mature polypeptide), SEQ ID NO: 65 or SEQ ID NO: 66 (mature polypeptide), SEQ ID NO: 68 or SEQ ID NO: 69 (mature polypeptide), SEQ ID NO: 71 or SEQ ID NO: 72 (mature polypeptide), SEQ ID NO: 74 or SEQ ID NO: 75 (mature polypeptide), SEQ ID NO: 77 or SEQ ID NO: 78 (mature polypeptide), SEQ ID NO: 80 or SEQ ID NO: 81 (mature polypeptide), SEQ ID NO: 83 or SEQ ID NO: 84 (mature polypeptide), SEQ ID NO: 86 or SEQ ID NO: 87 (mature polypeptide), SEQ ID NO: 89 or SEQ ID NO: 90 (mature polypeptide), SEQ ID NO: 92 or SEQ ID NO: 93 (mature polypeptide), SEQ ID NO: 95 or SEQ ID NO: 96 (mature polypeptide), SEQ ID NO: 98 or SEQ ID NO: 99 (mature polypeptide), SEQ ID NO: 101 or SEQ ID NO: 102 (mature polypeptide), or SEQ ID NO: 109 or SEQ ID NO: 110 (mature polypeptide).

In some embodiments, the invention may be peptides comprising or consisting of an amino acid sequence having sequence identity to any of the above mature polypeptides of at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.

In some embodiments, the invention may be polypeptides comprising or consisting of an amino acid sequence with identity to any of the above mature polypeptides of less than 100%, but at least as much sequence identity as set forth in one of the instances above (i.e., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%).

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 3.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 6.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 9.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 12.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 15.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 18.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 21.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 24.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID

NO: 27.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 30.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 33.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 36.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 39.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 42.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 45.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 48.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%,
5 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 51.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%,
10 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 54.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%,
15 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 57.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%,
20 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 60.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%,
25 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 63.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%,
30 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 66.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%,

71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 69.

5 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 72.

10 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 75.

15 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 78.

20 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 81.

25 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 84.

30 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 87.

In some embodiments, the invention may be a polypeptide with RNase activity comprising an

amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 90.

5 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 93.

10 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 96.

15 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 99.

20 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 102.

25 In some embodiments, the invention may be a polypeptide with RNase activity comprising an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 110.

30 In some embodiments, the invention relates to an isolated polypeptide with RNase activity, selected from the group consisting of:

an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity

to one of the groups of SEQ ID NOs. below, or

an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, but less than 100%

5 sequence identity to one of the groups of SEQ ID NOs. below:

- (a) SEQ ID NO: 2 or SEQ ID NO: 3;
- (b) SEQ ID NO: 5 or SEQ ID NO: 6;
- (c) SEQ ID NO: 8 or SEQ ID NO: 9;
- (d) SEQ ID NO: 11 or SEQ ID NO: 12;
- 10 (e) SEQ ID NO: 14 or SEQ ID NO: 15;
- (f) SEQ ID NO: 17 or SEQ ID NO: 18;
- (g) SEQ ID NO: 20 or SEQ ID NO: 21;
- (h) SEQ ID NO: 23 or SEQ ID NO: 24;
- (i) SEQ ID NO: 26 or SEQ ID NO: 27;
- 15 (j) SEQ ID NO: 29 or SEQ ID NO: 30;
- (k) SEQ ID NO: 32 or SEQ ID NO: 33;
- (l) SEQ ID NO: 35 or SEQ ID NO: 36;
- (m) SEQ ID NO: 38 or SEQ ID NO: 39;
- (n) SEQ ID NO: 41 or SEQ ID NO: 42;
- 20 (o) SEQ ID NO: 44 or SEQ ID NO: 45;
- (p) SEQ ID NO: 47 or SEQ ID NO: 48;
- (q) SEQ ID NO: 50 or SEQ ID NO: 51;
- (r) SEQ ID NO: 53 or SEQ ID NO: 54;
- (s) SEQ ID NO: 56 or SEQ ID NO: 57;
- 25 (t) SEQ ID NO: 59 or SEQ ID NO: 60;
- (u) SEQ ID NO: 62 or SEQ ID NO: 63;
- (v) SEQ ID NO: 65 or SEQ ID NO: 66;
- (w) SEQ ID NO: 68 or SEQ ID NO: 69;
- (x) SEQ ID NO: 71 or SEQ ID NO: 72;
- 30 (y) SEQ ID NO: 74 or SEQ ID NO: 75;
- (z) SEQ ID NO: 77 or SEQ ID NO: 78;
- (aa) SEQ ID NO: 80 or SEQ ID NO: 81;
- (bb) SEQ ID NO: 83 or SEQ ID NO: 84;
- (cc) SEQ ID NO: 86 or SEQ ID NO: 87;

- (dd) SEQ ID NO: 89 or SEQ ID NO: 90;
- (ee) SEQ ID NO: 92 or SEQ ID NO: 93;
- (ff) SEQ ID NO: 95 or SEQ ID NO: 96;
- (gg) SEQ ID NO: 98 or SEQ ID NO: 99;
- 5 (hh) SEQ ID NO: 101 or SEQ ID NO: 102; and
- (ii) SEQ ID NO: 109 or SEQ ID NO: 110.

In some embodiments, the isolated polypeptide with RNase activity is selected from the group consisting of: (a) SEQ ID NO: 3; (b) SEQ ID NO: 6; (c) SEQ ID NO: 9; (d) SEQ ID NO: 12; (e) SEQ ID NO: 15; (f) SEQ ID NO: 18; (g) SEQ ID NO: 21; (h) SEQ ID NO: 24; (i) SEQ ID NO: 27; (j) SEQ ID NO: 30; (k) SEQ ID NO: 33; (l) SEQ ID NO: 36; (m) SEQ ID NO: 39; (n) SEQ ID NO: 42; (o) SEQ ID NO: 45; (p) SEQ ID NO: 48; (q) SEQ ID NO: 51; (r) SEQ ID NO: 54; (s) SEQ ID NO: 57; (t) SEQ ID NO: 60; (u) SEQ ID NO: 63; (v) SEQ ID NO: 66; (w) SEQ ID NO: 69; (x) SEQ ID NO: 72; (y) SEQ ID NO: 75; (z) SEQ ID NO: 78; (aa) SEQ ID NO: 81; (bb) SEQ ID NO: 84; (cc) SEQ ID NO: 87; (dd) SEQ ID NO: 90; 15 (ee) SEQ ID NO: 93; (ff) SEQ ID NO: 96; (gg) SEQ ID NO: 99; (hh) SEQ ID NO: 102; and (ii) SEQ ID NO: 110.

In some preferred embodiment of the invention the polypeptide of the invention having RNase activity, comprises a C-terminal domain, i.e. a specific domain near the C-terminal, which is in the polypeptide with SEQ ID NO: 71 and in the mature polypeptide with SEQ ID NO: 72 approximately amino acids 364 to 473, in the polypeptide with SEQ ID NO: 59 and in the mature polypeptide with SEQ ID NO: 60 approximately amino acids 267 to 388, in the polypeptide with SEQ ID NO: 56 and in the mature polypeptide with SEQ ID NO: 57 approximately amino acids 268 to 389, in the polypeptide with SEQ ID NO: 62 and in the mature polypeptide with SEQ ID NO: 63 approximately amino acids 263 to 385, in the polypeptide with SEQ ID NO: 65 and in the mature polypeptide with SEQ ID NO: 66 approximately amino acid 259 to 371, and in the polypeptide with SEQ ID NO: 5 and in the mature polypeptide with SEQ ID NO: 6 approximately amino acids 268 to 387. 20

The exact start and end amino acid may shift a couple of amino acids, the location of the domain is estimated by prediction and may change slightly over time. However, the domains can be visually identified by aligning the sequences of the T2 RNases and the person skilled in the art would from the alignment and the approximated annotations be able to identify the domain. As shown in example 5 the polypeptides comprising this domain have shown higher activity in detergent. 30

Thus, a preferred embodiment of the invention relates to a T2 RNase comprising a C-domain. Another preferred embodiment relates to a polypeptide having RNase activity, preferably a T2 RNase, having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acids sequence shown in SEQ ID NO 6. Another preferred embodiment relates to a polypeptide having RNase activity, preferably a T2 RNase, having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acids sequence shown in SEQ ID NO 57. Another preferred embodiment relates to a polypeptide having RNase activity, preferably a T2 RNase, having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acids sequence shown in SEQ ID NO 60. Another preferred embodiment relates to a polypeptide having RNase activity, preferably a T2 RNase, having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acids sequence shown in SEQ ID NO 63. Another preferred embodiment relates to a polypeptide having RNase activity, preferably a T2 RNase, having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acids sequence shown in SEQ ID NO 66. Another preferred embodiment relates to a polypeptide having RNase activity, preferably a T2 RNase, having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acids sequence shown in SEQ ID NO 72.

In some embodiments, the invention may be polypeptides that comprise or consist of allelic variants of any of the mature amino acid sequences set forth above, or fragments thereof that have RNase activity.

In some embodiments, the polypeptide may comprise or consist of any of the mature amino acid sequences set forth above; comprise or consist of one of those amino acid sequences and an N-terminal and/or C-terminal His-tag and/or HQ-tag; comprise or consist of one of those amino acid sequences and an N-terminal and/or C-terminal extension of between 1 and 10 amino acids; or a

fragment thereof having RNase activity, and having at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of the length of one of those amino acid sequences.

In some embodiments, the invention may relate to variants of any of the mature polypeptides set forth above, comprising a substitution, deletion, and/or insertion at one or more (e.g., several) positions. In some embodiments, the number of amino acid substitutions, deletions and/or insertions is up to 10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

The changes in amino acid sequences of the inventive T2 RNase polypeptides, as compared to the amino acid sequences set forth in the Sequence Listing that is a part of this patent application (e.g., when the inventive polypeptide is not 100% identical to one of amino acid sequences set forth in the Sequence Listing), may be described as set forth below.

The amino acid changes may be of a minor nature, like 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, like 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).

Essential amino acids in a polypeptide may be defined as amino acids that cannot be substituted or deleted without loss of RNase activity. These amino acids can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis. In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant molecules are tested for RNase activity to identify amino acid residues that are critical to the activity of the molecule. Generally, these amino acids can be identified by substituting or deleting them, and then testing the substituted/deletion molecule for RNase activity. These methods are well known in the art. Amino acids that, when deleted or substituted, result in loss of some, but not all RNase activity may exist in the polypeptides.

The active site of the enzyme or other biological interaction can be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative

contact site amino acids. The identity of essential amino acids can also be inferred from an alignment with a related polypeptide.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure. Other methods that can be used include error-prone PCR, phage display, and region-directed mutagenesis.

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

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

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

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

Sources of Polypeptides Having RNase Activity

A polypeptide having T2 RNase activity may be obtained from any organism. In some examples, the T2 RNases are obtained from microorganisms. The microorganisms may be from any genus. In some examples, the T2 RNases were obtained from fungi or bacteria. Herein, the disclosed polynucleotides encoding T2 RNase polypeptides were obtained from the microorganisms set forth below (fungi or bacteria). In other words, these microorganisms may be considered natural hosts for the RNases. The RNases may be obtained from other microorganisms, however. In some examples, the RNases may be obtained from organisms into which a polynucleotide encoding the RNase has been inserted (e.g., a recombinant organism). Other organisms may be a source for T2 RNases.

In one aspect, the polynucleotides and/or polypeptides disclosed herein may be obtained from

bacteria:

In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Aeromonas*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Aeromonas molluscorum*.

5 In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Agrobacterium*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Agrobacterium fabrum*.

10 In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Buttiauxella*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Buttiauxella brennerae*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Caulobacter*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Caulobacter vibrioides*.

15 In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Mycobacterium*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Mycobacterium sp-62710*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Paracoccus*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Paracoccus carotinifaciens*.

20 In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Shinella*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Shinella granulii*.

25 In one aspect, the polynucleotides and/or polypeptides may be obtained from bacteria of the genus, *Zoogloea*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Zoogloea ramigera*.

In one aspect, the polynucleotides and/or polypeptides disclosed herein may be obtained from fungi:

30 In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Acremonium*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Acremonium sp-XZ2020*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Ascochyta*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Ascochyta fabae*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus,

Aspergillus. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Aspergillus oryzae*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Cadophora*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide
5 obtained from *Cadophora malorum*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Cladosporium*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Clathrosphaeria*.

10 In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Lecanicillium*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Lecanicillium sp-WMM742*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Penicillium*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide
15 obtained from *Penicillium polonicum*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Setosphaeria*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Setosphaeria rostrate*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Simplicillium*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide
20 obtained from *Simplicillium lamellicola*.

In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Thermoascus*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Thermoascus aurantiacus*.

25 In one aspect, the polynucleotides and/or polypeptides may be obtained from fungi of the genus, *Trichoderma*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Trichoderma harzianum*. One embodiment of this aspect is, for example, a polynucleotide and/or polypeptide obtained from *Trichoderma reesei*.

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

Strains of these species are readily accessible to the public in a number of culture collections, such as the American Type Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen und

Zellkulturen GmbH (DSMZ), Centraalbureau Voor Schimmelcultures (CBS), and Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL).

The polypeptide may be identified and obtained from other sources including microorganisms isolated from nature (*e.g.*, soil, composts, water, etc.) or DNA samples obtained directly from natural materials (*e.g.*, soil, composts, water, etc.) using the above-mentioned probes. Techniques for isolating microorganisms and DNA directly from natural habitats are well known in the art. A polynucleotide encoding the polypeptide may then be obtained by similarly screening a genomic DNA or cDNA library of another microorganism or mixed DNA sample. Once a polynucleotide encoding a polypeptide has been detected with the probe(s), the polynucleotide can be isolated or cloned by utilizing techniques that are known to those of ordinary skill in the art.

Compositions Containing RNases

The invention relates to compositions comprising an RNase of the present invention in combination with one or more additional components. The choice of additional components is within the skill of the artisan and includes conventional ingredients, including the exemplary non-limiting components set forth below. The compositions of the invention are preferably cleaning compositions, such as laundry or dish wash compositions.

One embodiment of the invention relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component. The Pfam domain is as explained above characteristic for the T2 RNases. This group of RNases are distinct from other RNases *e.g.* T1 RNases, Luhtala, N. and Parker, R. Trends Biochem Sci. 2010 May; 35(5): 253–259. The T2 RNase of the invention is preferably from E.C. class EC 3.1.27.1.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase preferably is from E.C. class EC 3.1.27.1.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase comprises at least one of the amino acid sequences [VL]HGLWP[QNEG] (SEQ ID NO: 105) or L[SFT]WX[PT]XXC (SEQ ID NO: 106), where X is any naturally-occurring amino acid.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably

comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase comprises the amino acid sequence HGLWPD (SEQ ID NO: 103).

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase is of microbial origin.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase is or bacterial or fungal origin.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase comprises a C-terminal domain.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising a T2 RNase and at least one cleaning component, wherein the T2 RNase comprises a C-terminal domain.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase comprises a C-terminal domain and wherein the T2 RNase is a polypeptide having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO 6, SEQ ID NO 57, SEQ ID NO 60, SEQ ID NO 63, SEQ ID NO 66 or SEQ ID NO 72.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase is a polypeptide having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99, SEQ ID NO: 102 or SEQ ID NO: 110. One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component, wherein the T2 RNase is a polypeptide having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99, SEQ ID NO: 102 or SEQ ID NO: 110, wherein the composition comprises a fabric care component selected from the group consisting of cationic softening-compounds, silicone softening-compounds, paraffins, waxes, dispersible polyolefins and mixtures thereof.

One embodiment of the invention relates to a composition comprising:

a) at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm, of at least one polypeptide having RNase activity, wherein the RNase is selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99, SEQ ID NO: 102 and SEQ ID NO: 110, and polypeptides having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity thereto; and

b) at least one detergent adjunct ingredient.

One embodiment of the invention relates to a cleaning composition comprising:

a) at least 0.001 ppm, including at least 0.01 ppm or at least 0.1 ppm, of at least one polypeptide having RNase activity, wherein the RNase is selected from the group consisting of: SEQ ID

NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99, SEQ ID NO: 102, and SEQ ID NO: 110, and polypeptides having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity hereto;

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

The choice of cleaning components may include, for textile care, the consideration of the type of textile to be cleaned, the type and/or degree of soiling, the temperature at which cleaning is to take place, and the formulation of the detergent product. Although components mentioned below are categorized by general header according to a particular functionality, this is not to be construed as a limitation, as a component may comprise additional functionalities as will be appreciated by the skilled artisan.

In addition to an RNase, in some embodiments, the inventive compositions may contain a detergent/cleaning composition component and or a detergent adjunct ingredient. Examples of some of these are described below.

Surfactants

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

When included therein, the detergent will usually contain from about 1% to about 40% by weight of an anionic surfactant, such as from about 5% to about 30%, including from about 5% to about 15%, or from about 15% to about 20%, or from about 20% to about 25% of an anionic surfactant. Preferably the composition comprises from about 0.5 wt% to about 80 wt% of an anionic surfactant. Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, linear

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

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

When included therein the detergent will usually contain from about 0.2% to about 40% by weight of a nonionic surfactant, for example from about 0.5% to about 30%, in particular from about 1% to about 20%, from about 3% to about 10%, such as from about 3% to about 5%, from about 8% to about 12%, or from about 10% to about 12%. Preferably the composition comprises from about 0.1 wt% to about 40 wt% of a non-ionic surfactant. Non-limiting examples of nonionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxyated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxyated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (Pfam), polyhydroxyalkyl fatty acid amides, or N-acyl N-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamides, FAGA), as well as products available under the trade names SPAN® and TWEEN®, and combinations thereof.

When included therein the detergent will usually contain from about 0.1% to about 10% by weight of a semipolar surfactant. Non-limiting examples of semipolar surfactants include amine oxides (AO) such as alkyltrimethylamineoxide, N-(coco alkyl)-N,N-dimethylamine oxide and N-(tallow-alkyl)-

N,N-bis(2-hydroxyethyl)amine oxide, , and combinations thereof.

When included therein the detergent will usually contain from about 0.1% to about 10% by weight of a zwitterionic surfactant. Non-limiting examples of zwitterionic surfactants include betaines such as alkyldimethylbetaines, sulfobetaines, and combinations thereof.

5

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain and/or a C-domain, and at least one cleaning component, preferably wherein the T2 RNase is a polypeptide having at least 60%, 61%, 62%, 63%, 64%, 65%, 10 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, 15 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99, SEQ ID NO: 102 or SEQ ID NO: 110, preferably wherein the composition comprises from about 0.5 wt% to about 80 wt % of an anionic surfactant, preferably wherein the anionic surfactant is selected from the 20 group consisting of 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), secondary alkanesulfonates (SAS), 25 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.

30 One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain and/or a C-domain, and at least one cleaning component, preferably wherein the T2 RNase is a polypeptide having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99, SEQ ID NO: 102 or SEQ ID NO: 110, preferably wherein the composition comprises from about 0.1 wt% to about 40 wt% of a non-ionic surfactant, preferably wherein the nonionic surfactant is selected from the group consisting of 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) and combinations thereof.

Builders and Co-Builders

The cleaning or detergent composition may contain about 0-65% by weight, such as about 5% to about 50% of a detergent builder or co-builder, or a mixture thereof. In a dish wash detergent, the level of builder is typically 40-65%, particularly 50-65%. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in cleaning detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (e.g., SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as 2,2'-iminodiethan-1-ol), triethanolamine (TEA, also known as 2,2',2''-nitrilotriethan-1-ol), and (carboxymethyl)inulin (CMI), and combinations thereof.

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

acid. Additional specific examples include 2,2',2"-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-N,N'-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-N,N-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis (methylenephosphonic acid) (DTMPA or DTPMPA), N-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-N-monoacetic acid (ASMA), aspartic acid-N,N-diacetic acid (ASDA), aspartic acid-N-monopropionic acid (ASMP), iminodisuccinic acid (IDA), N-(2-sulfomethyl)-aspartic acid (SMAS), N-(2-sulfoethyl)-aspartic acid (SEAS), N-(2-sulfomethyl)-glutamic acid (SMGL), N-(2-sulfoethyl)-glutamic acid (SEGL), N-methyliminodiacetic acid (MIDA), α -alanine-N,N-diacetic acid (α -ALDA), serine-N,N-diacetic acid (SEDA), isoserine-N,N-diacetic acid (ISDA), phenylalanine-N,N-diacetic acid (PHDA), anthranilic acid-N,N-diacetic acid (ANDA), sulfanilic acid-N,N-diacetic acid (SLDA), taurine-N,N-diacetic acid (TUDA) and sulfomethyl-N,N-diacetic acid (SMDA), N-(2-hydroxyethyl)ethylenediamine-N,N',N"-triacetic acid (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain and/or a C-domain, and at least one cleaning component, preferably wherein the T2 RNase is a polypeptide having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99, SEQ ID NO: 102 or SEQ ID NO: 110, preferably wherein the composition comprises from about 0.5 wt% to about 65 wt% by weight of a builder and/or a co-builder, preferably wherein the builder is selected from the group consisting of zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (e.g., SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as 2,2'-iminodiethan-1-ol), triethanolamine (TEA, also known as 2,2',2"-nitrilotriethan-1-ol), (carboxymethyl)inulin (CMI), 2,2',2"-nitrilotriacetic acid (NTA),

ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-N,N'-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-N,N-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis (methylenephosphonic acid) (DTMPA or DTPMPA), N-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-N-monoacetic acid (ASMA), aspartic acid-N,N-diacetic acid (ASDA), aspartic acid-N-monopropionic acid (ASMP), iminodisuccinic acid (IDA), N-(2-sulfomethyl)-aspartic acid (SMAS), N-(2-sulfoethyl)-aspartic acid (SEAS), N-(2-sulfomethyl)-glutamic acid (SMGL), N-(2-sulfoethyl)-glutamic acid (SEGL), N-methyliminodiacetic acid (MIDA), α -alanine-N,N-diacetic acid (α -ALDA), serine-N,N-diacetic acid (SEDA), isoserine-N,N-diacetic acid (ISDA), phenylalanine-N,N-diacetic acid (PHDA), anthranilic acid-N,N-diacetic acid (ANDA), sulfanilic acid-N,N-diacetic acid (SLDA), taurine-N,N-diacetic acid (TUDA) and sulfomethyl-N,N-diacetic acid (SMDA), N-(2-hydroxyethyl)ethylenediamine-N,N',N''-tri-acetic acid (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof.

Bleaching Systems

The cleaning or 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, peroxy-carboxylic acids such as peroxybenzoic acid and its ring-substituted derivatives, peroxy- α -naphthoic acid, peroxyphthalic acid, peroxy-lauric acid, peroxy-stearic acid, ϵ -phthalimidoperoxy-caproic acid [phthalimidoperoxyhexanoic acid (PAP)], and o-carboxybenzamidoperoxy-caproic acid; aliphatic and aromatic diperoxydicarboxylic

acids such as diperoxydodecanedioic acid, diperoxyazelaic acid, diperoxysebacic acid, diperoxybrassylic acid, 2-decyldiperoxybutanedioic acid, and diperoxyphthalic, -isophthalic and -terephthalic acids; perimidic acids; peroxymonosulfuric acid; peroxydisulfuric acid; peroxyphosphoric acid; peroxysilicic acid; and mixtures of said compounds. It is understood that the peracids mentioned
 5 may in some cases be best added as suitable salts, such as alkali metal salts (e.g., Oxone®) or alkaline earth-metal salts.

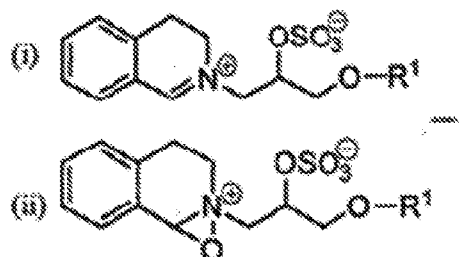
b) Suitable bleach activators include those belonging to the class of esters, amides, imides, nitriles or anhydrides and, where applicable, salts thereof. Suitable examples are tetraacetylenediamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene-1-sulfonate
 10 (ISONOBS), sodium 4-(dodecanoyloxy)benzene-1-sulfonate (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.
 15 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

20 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-
 25 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
 30 bleach booster may be used having one of the following formulae:



or (iii) and mixtures thereof; wherein each R^1 is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R^1 is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R^1 is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylononyl, 2-hexyldecyl, dodecyl, tetradecyl, hexadecyl, octadecyl, isononyl, isodecyl, isotridecyl and isopentadecyl.

Other exemplary bleaching systems are described in the art. Suitable photobleaches may for example be sulfonated zinc or aluminium phthalocyanines.

Metal care agents

Metal care agents may prevent or reduce the tarnishing, corrosion or oxidation of metals, including aluminium, stainless steel and non-ferrous metals, such as silver and copper. Suitable examples include one or more of the following:

(a) benzotriazoles, including benzotriazole or bis-benzotriazole and substituted derivatives thereof. Benzotriazole derivatives are those compounds in which the available substitution sites on the aromatic ring are partially or completely substituted. Suitable substituents include linear or branch-chain $\text{C}_1\text{-C}_{20}$ -alkyl groups (e.g., $\text{C}_1\text{-C}_{20}$ -alkyl groups) and hydroxyl, thio, phenyl or halogen such as fluorine, chlorine, bromine and iodine.

(b) metal salts and complexes chosen from the group consisting of zinc, manganese, titanium, zirconium, hafnium, vanadium, cobalt, gallium and cerium salts and/or complexes, the metals being in one of the oxidation states II, III, IV, V or VI. In one aspect, suitable metal salts and/or metal complexes may be chosen from the group consisting of Mn(II) sulphate, Mn(II) citrate, Mn(II) stearate, Mn(II) acetylacetonate, K^+TiF_6 (e.g., K_2TiF_6), K^+ZrF_6 (e.g., K_2ZrF_6), CoSO_4 , $\text{Co(NO}_3)_2$ and $\text{Ce(NO}_3)_3$, zinc salts, for example zinc sulphate, hydrozincite or zinc acetate.;

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

Further suitable organic and inorganic redox-active substances that act as silver/copper corrosion inhibitors are disclosed in the art. Preferably the composition of the invention comprises from

0.1 to 5% by weight of the composition of a metal care agent, preferably the metal care agent is a zinc salt.

Hydrotropes

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

Polymers

15 The cleaning or detergent may contain 0-10% by weight, such as 0.5-5%, 2-5%, 0.5-2% or 0.2-1% of a polymer. Any polymer known in the art for use in detergents may be utilized. The polymer may function as a co-builder as mentioned above, or may provide antiredeposition, fiber protection, soil release, dye transfer inhibition, grease cleaning and/or anti-foaming properties. Some polymers may have more than one of the above-mentioned properties and/or more than one of the below-mentioned motifs. Exemplary polymers include (carboxymethyl)cellulose (CMC), poly(vinyl alcohol) (PVA),
20 poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) or poly(ethylene oxide) (PEG), ethoxylated poly(ethyleneimine), carboxymethyl inulin (CMI), and polycarboxylates such as PAA, PAA/PMA, poly-aspartic acid, and lauryl methacrylate/acrylic acid copolymers, hydrophobically modified CMC (HM-CMC) and silicones, copolymers of terephthalic acid and oligomeric glycols, copolymers of poly(ethylene terephthalate) and poly(oxyethylene terephthalate) (PET-POET), PVP, poly(vinylimidazole)
25 (PVI), poly(vinylpyridine-N-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Suitable examples include PVP-K15, PVP-K30, ChromaBond S-400, ChromaBond S- 403E and Chromabond S-100 from Ashland Aqualon, and Sokalan® HP 165, Sokalan® HP 50 (Dispersing agent), Sokalan® HP 53 (Dispersing agent), Sokalan® HP 59 (Dispersing agent), Sokalan® HP 56 (dye transfer inhibitor), Sokalan® HP 66 K (dye transfer inhibitor) from BASF. Further exemplary polymers
30 include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated. Particularly preferred polymer is ethoxylated homopolymer Sokalan® HP 20 from BASF, which helps to prevent redeposition of soil in the wash liquor.

Fabric Hueing Agents

The cleaning or detergent compositions of the present invention may also include fabric hueing agents such as dyes or pigments, which when formulated in detergent compositions can deposit onto a fabric when said fabric is contacted with a wash liquor comprising said detergent compositions and thus altering the tint of said fabric through absorption/reflection of visible light. Fluorescent whitening agents emit at least some visible light. In contrast, fabric hueing agents alter the tint of a surface as they absorb at least a portion of the visible light spectrum. Suitable fabric hueing agents include dyes and dye-clay conjugates and may also include pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct Blue, Direct Red, Direct Violet, Acid Blue, Acid Red, Acid Violet, Basic Blue, Basic Violet and Basic Red, or mixtures thereof. 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. Other suitable hueing agents are described in the art.

Enzymes

The detergent additive as well as the cleaning or detergent composition may comprise one or more additional enzymes such as at least one lipase, cutinase, amylase, carbohydrase, cellulase, deoxyribonuclease, pectinase, mannanase, arabinase, galactanase, xylanase, oxidase, e.g., 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.

Examples of additional enzymes are described below.

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*.

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits.

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™, Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

Mannanases

Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. The mannanase may be an alkaline mannanase of Family 5 or 26. It may be a wild-type from *Bacillus* or *Humicola*, particularly *B. agaradhaerens*, *B. licheniformis*, *B. halodurans*, *B. clausii*, or *H. insolens*. A commercially available mannanase is Mannaway (Novozymes A/S).

Peroxidases/Oxidases

A peroxidase may be 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 derived therefrom, exhibiting peroxidase activity.

Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g., from *C. cinereus*, and variants thereof. Commercially available peroxidases include Guardzyme™ (Novozymes A/S).

A suitable peroxidase 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. Preferably, the haloperoxidase is a vanadium haloperoxidase, i.e., a vanadate-containing haloperoxidase. 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.*

A suitable oxidase includes in particular, any laccase enzyme comprised by the enzyme classification EC 1.10.3.2, or any fragment derived 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 derived 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. versicolor*, *Rhizoctonia*, e.g., *R. solani*, *Coprinopsis*, e.g., *C. cinerea*, *C. comatus*, *C. friesii*, and *C. plicatilis*, *Psathyrella*, e.g., *P. condelleana*, *Panaeolus*, e.g., *P. papilionaceus*, *Myceliophthora*, e.g., *M. thermophila*, *Schytalidium*, e.g., *S. thermophilum*, *Polyporus*, e.g., *P. pinsitus*, *Phlebia*, e.g., *P. radiata*, or *Coriolus*, e.g., *C. hirsutus*. Suitable examples from bacteria include a laccase derivable from a strain of *Bacillus*. A laccase derived from *Coprinopsis* or *Myceliophthora* is preferred; in particular, a laccase derived from *Coprinopsis* *cinerea*; or from *Myceliophthora* *thermophila*.

Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutant enzymes are included. Examples include lipase from *Thermomyces*, e.g. from *T. lanuginosus* (previously named *Humicola lanuginosa*), cutinase from *Humicola*, e.g. *H. insolens*, lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g., *P. alcaligenes* or *P. pseudoalcaligenes*, *P. cepacia*, *P. sp.* strain SD705, *P. wisconsinensis*, GDSL-type *Streptomyces* lipases, cutinase from *Magnaporthe grisea*, cutinase from *Pseudomonas mendocina*, lipase from *Thermobifida fusca*, *Geobacillus stearothermophilus* lipase, lipase from *Bacillus subtilis*, and lipase from *Streptomyces griseus* and *S. pristinaespiralis*.

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

49

Amylases

Suitable amylases include alpha-amylases and/or a glucoamylases and may be of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus*, e.g., a special strain of *Bacillus licheniformis*,
 5 described in more detail in GB 1,296,839.

Suitable amylases include amylases having SEQ ID NO: 2 in WO 1995/010603 or variants having 90% sequence identity to SEQ ID NO: 3 thereof. Preferred variants are described in WO 1994/002597, WO 1994/018314, WO 1997/043424 and SEQ ID NO: 4 of WO 1999/019467, such as
 10 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 2002/010355 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.

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

M197T;

25 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 1999/019467 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:
 30 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 1996/023873 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 2008/153815, SEQ ID NO: 10 in WO 2001/066712 or variants thereof having 90% sequence identity to SEQ ID NO: 2 of WO 2008/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 2001/066712. Preferred variants of SEQ ID NO: 10 in WO 2001/066712 are those having a substitution, a deletion or an insertion in one or more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264.

Further suitable amylases are amylases having SEQ ID NO: 2 of WO 2009/061380 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 or more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320, Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one or more of the following positions: Q87E,R, Q98R, S125A, N128C, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 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.

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

G477K and/or deletion in position R178 and/or S179 or of T180 and/or G181. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

E187P+I203Y+G476K

E187P+I203Y+R458N+T459S+D460T+G476K

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

Further suitable amylases are amylases having SEQ ID NO: 1 of WO 2010/104675 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: N21, D97,
10 V128 K177, R179, S180, I181, G182, M200, L204, E242, G477 and G478. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: N21D, D97N, V128I K177L, M200L, L204YF, E242QA, G477K and G478K and/or deletion in position R179 and/or S180 or of I181 and/or G182. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

15 N21D+D97N+V128I

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

Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO 2001/066712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those
20 having a substitution, a deletion or an insertion in one of more of the following positions of SEQ ID NO: 12 in WO 2001/066712: 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
25 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 WO 2011/098531, WO 2013/001078 and WO 2013/001087.

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

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

The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 sub-divisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

Examples of subtilases are those derived from *Bacillus* such as *Bacillus lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii*, and subtilisin *lentus*, subtilisin *Novo*, subtilisin *Carlsberg*, *Bacillus licheniformis*, subtilisin *BPN'*, subtilisin 309, subtilisin 147 and subtilisin 168 and protease PD138 described in (WO 1993/018140). Other useful proteases may be those described in WO 1992/175177, WO 2001/016285, WO 2002/026024 and WO 2002/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease, and the chymotrypsin proteases derived from *Cellulomonas*.

A further preferred protease is the alkaline protease from *Bacillus lentus* DSM 5483, and variants thereof.

Examples of metalloproteases are the neutral metalloprotease, such as those derived from *Bacillus amyloliquefaciens*.

Examples of useful proteases are the variants described in: WO 1992/019729, WO 1996/034946, WO 98/20115, WO 98/20116, WO 99/011768, WO 01/44452, WO 03/006602, WO 04/03186, WO 04/041979, WO 07/006305, WO 11/036263, WO 11/036264, 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 one or more of the following 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 amyloliquefaciens* protease (BPN') shown in SEQ ID NO 2 of WO2016/001449. The protease variants preferably have at least 80% sequence identity to SEQ ID NO 1 or SEQ ID NO 2 of WO 2016/001449.

A protease variant comprising a substitution at one or more positions corresponding to positions 171, 173, 175, 179, or 180 of SEQ ID NO: 1 of WO2004/067737, wherein said protease variant has a sequence identity of at least 75% but less than 100% to SEQ ID NO: 1 of 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 Ox®, Purafect OxP®, Puramax®, FN2®, FN3®, FN4®, Excellase®, Excellenz P1000TM, Excellenz P1250TM, Eraser®, Preferenz P100TM, Purafect Prime®, Preferenz P110TM, Effectenz P1000TM, Purafect®TM, Effectenz P1050TM, Purafect Ox®TM, Effectenz P2000TM, Purafast®, Properase®, Opticlean® and Optimase® (Danisco/DuPont), Axapem™ (Gist-Brocades N.V.), BLAP (sequence shown in Figure 29 of US5352604) and variants hereof (Henkel AG) and KAP (*Bacillus alkalophilus subtilisin*) from Kao.

One embodiment relates to a cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain and/or a C-domain, and at least one cleaning component, preferably wherein the T2 RNase is a polypeptide having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 21, SEQ ID NO: 24, 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, SEQ ID NO: 75, SEQ ID NO: 78, SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 90, SEQ ID NO: 93, SEQ ID NO: 96, SEQ ID NO: 99,

SEQ ID NO: 102 or SEQ ID NO: 110, preferably wherein the composition comprises an enzyme selected from the group consisting of proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases, deoxyribonucleases and mannanases.

5

Dispersants

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

10

Dye Transfer Inhibiting Agents

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

20

Fluorescent Whitening Agent

The cleaning or detergent compositions of the present invention will preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agent or optical brighteners. Where present the brightener is preferably at a level of about 0.01% to about 0.5%. Any fluorescent whitening agent suitable for use in a laundry detergent composition may be used in the composition of the present invention. The most commonly used fluorescent whitening agents are those belonging to the classes of diaminostilbene-sulfonic acid derivatives, diarylpyrazoline derivatives and bisphenyl-distyryl derivatives. Examples of the diaminostilbene-sulfonic acid derivative type of fluorescent whitening agents include the sodium salts of: 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2-anilino-4-(N-methyl-N-2-hydroxy-ethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(4-phenyl-1,2,3-triazol-2-yl)stilbene-2,2'-disulfonate and sodium 5-(2H-naphtho[1,2-

30

d][1,2,3]triazol-2-yl)-2-[(E)-2-phenylvinyl]benzenesulfonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt of 4,4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenyl-styryl)-disulfonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins. Suitable fluorescent brightener levels include lower levels of from about 0.01, from 0.05, from about 0.1 or even from about 0.2 wt % to upper levels of 0.5 or even 0.75 wt%.

Soil Release Polymers

The cleaning or detergent compositions of the present invention may also include one or more soil release polymers which aid the removal of soils from fabrics such as cotton and polyester based fabrics, in particular the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalate based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, or polyester polyamides. Another type of soil release polymers are amphiphilic alkoxylated grease cleaning polymers comprising a core structure and a plurality of alkoxylate groups attached to that core structure. The core structure may comprise a polyalkylenimine structure or a polyalkanolamine structure. Furthermore, random graft co-polymers are suitable soil release polymers. Suitable graft co-polymers are described in more detail in the prior art. Suitable polyethylene glycol polymers include random graft co-polymers comprising: (i) hydrophilic backbone comprising polyethylene glycol; and (ii) side chain(s) selected from the group consisting of: C4-C25 alkyl group, polypropylene, polybutylene, vinyl ester of a saturated C1-C6 mono-carboxylic acid, C1-C 6 alkyl ester of acrylic or methacrylic acid, and mixtures thereof. Suitable polyethylene glycol polymers have a polyethylene glycol backbone with random grafted polyvinyl acetate side chains. The average molecular weight of the polyethylene glycol backbone can be in the range of from 2,000 Da to 20,000 Da, or from 4,000 Da to 8,000 Da. The molecular weight ratio of the polyethylene glycol backbone to the polyvinyl acetate side chains can be in the range of from 1: 1 to 1:5, or from 1: 1.2 to 1:2. The average number of graft sites per ethylene oxide units can be less than 1, or less than 0.8, the average number of graft sites per ethylene oxide units can be in the range of from 0.5 to 0.9, or the average number of graft sites per ethylene oxide units can be in the range of from 0.1 to 0.5, or from 0.2 to 0.4. A suitable polyethylene glycol polymer is Sokalan® HP22. Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose derivatives. 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.

5

Anti-Redeposition Agents

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

10

Rheology Modifiers

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

20

Other Components

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

25

Polynucleotides

The present invention also relates to polynucleotides encoding a polypeptide of the present invention, as described herein. In one embodiment, the polynucleotide encoding the polypeptide of the present invention has been isolated.

30

In one embodiment, the present invention relates to a polynucleotide encoding a polypeptide having RNase activity, wherein the polynucleotide has a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58,

61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100 or 108 of at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 94%, 96%, 97%, 98%, 99% or 100%. In some embodiments, the polynucleotide has a sequence identity of at least any of the above values but is less than 100% identical to any of the SEQ ID NOs. listed above. The polynucleotides encode polypeptides that have RNase activity. The polynucleotide may have been isolated.

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

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.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 771 of SEQ ID NO: 1, and nucleotides 1 to 57 of SEQ ID NO: 1 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 1218 of SEQ ID NO: 4, and nucleotides 1 to 57 of SEQ ID NO: 4 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 70 to 828 of SEQ ID NO: 7, and nucleotides 1 to 69 of SEQ ID NO: 7 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 52 to 966 of SEQ ID NO: 10, and nucleotides 1 to 51 of SEQ ID NO: 10 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 64 to 804 of SEQ ID NO: 13, and nucleotides 1 to 63 of SEQ ID NO: 13 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 1209 of SEQ ID NO: 16, and nucleotides 1 to 57 of SEQ ID NO: 16 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 73 to 639 of SEQ ID NO: 19, and nucleotides 1 to 72 of SEQ ID NO: 19 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 633 of SEQ ID NO: 22, and nucleotides 1 to 57 of SEQ ID NO: 22 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 73 to 759 of SEQ ID NO: 25, and nucleotides 1 to 72 of SEQ ID NO: 25 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 79 to 726 of SEQ ID NO:

28, and nucleotides 1 to 78 of SEQ ID NO: 28 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 64 to 756 of SEQ ID NO: 31, and nucleotides 1 to 63 of SEQ ID NO: 31 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 792 of SEQ ID NO: 34, and nucleotides 1 to 57 of SEQ ID NO: 34 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 52 to 768 of SEQ ID NO: 37, and nucleotides 1 to 51 of SEQ ID NO: 37 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 49 to 792 of SEQ ID NO: 40, and nucleotides 1 to 48 of SEQ ID NO: 40 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 987 of SEQ ID NO: 43, and nucleotides 1 to 57 of SEQ ID NO: 43 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 70 to 768 of SEQ ID NO: 46, and nucleotides 1 to 69 of SEQ ID NO: 46 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 786 of SEQ ID NO: 49, and nucleotides 1 to 57 of SEQ ID NO: 49 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 64 to 984 of SEQ ID NO: 52, and nucleotides 1 to 63 of SEQ ID NO: 52 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 55 to 1221 of SEQ ID NO: 55, and nucleotides 1 to 54 of SEQ ID NO: 55 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 55 to 1218 of SEQ ID NO: 58, and nucleotides 1 to 54 of SEQ ID NO: 59 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 61 to 1215 of SEQ ID NO: 61, and nucleotides 1 to 60 of SEQ ID NO: 61 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 49 to 1164 of SEQ ID NO: 64, and nucleotides 1 to 48 of SEQ ID NO: 64 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 82 to 795 of SEQ ID NO: 67, and nucleotides 1 to 81 of SEQ ID NO: 67 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 1485 of SEQ ID NO: 70, and nucleotides 1 to 57 of SEQ ID NO: 70 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 52 to 972 of SEQ ID NO: 73, and nucleotides 1 to 51 of SEQ ID NO: 73 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 73 to 726 of SEQ ID NO: 76, and nucleotides 1 to 72 of SEQ ID NO: 76 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 630 of SEQ ID NO:

79, and nucleotides 1 to 57 of SEQ ID NO: 79 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 67 to 801 of SEQ ID NO: 82, and nucleotides 1 to 66 of SEQ ID NO: 82 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 669 of SEQ ID NO: 85, and nucleotides 1 to 57 of SEQ ID NO: 85 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 1119 of SEQ ID NO: 88, and nucleotides 1 to 57 of SEQ ID NO: 88 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 55 to 762 of SEQ ID NO: 91, and nucleotides 1 to 54 of SEQ ID NO: 91 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 55 to 792 of SEQ ID NO: 94, and nucleotides 1 to 54 of SEQ ID NO: 94 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 61 to 777 of SEQ ID NO: 97, and nucleotides 1 to 60 of SEQ ID NO: 97 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 49 to 756 of SEQ ID NO: 100, and nucleotides 1 to 48 of SEQ ID NO: 100 encode a signal peptide.

In one aspect, the mature polypeptide coding sequence is nucleotides 58 to 777 of SEQ ID NO: 108, and nucleotides 1 to 57 of SEQ ID NO: 108 encode a signal peptide.

The above sequences may or may not be genome sequences. For at least some of the sequences obtained from fungi, introns encoded in genomic DNA were removed to yield the above-referenced sequences that encode the mature polypeptides.

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 variant, 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, *E. coli* *lac* operon, *E. coli* *trc* promoter, *Streptomyces coelicolor* agarase gene (*dagA*), and prokaryotic beta-lactamase gene, as well as the *tac* promoter.

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, *Fusarium venenatum* amyloglucosidase, *Fusarium venenatum* Daria, *Fusarium venenatum* Quinn, *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 variant, truncated, and hybrid promoters thereof.

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.

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.

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.

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 known in the art.

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.

5 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.

10 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*.

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.

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, *Rhizomucor miehei* aspartic proteinase, and *Saccharomyces cerevisiae* alpha-factor.

30 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 adenylyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus* cell are *Aspergillus nidulans* or *Aspergillus oryzae* *amdS* and *pyrG* genes and a *Streptomyces hygroscopicus* *bar* gene. Preferred for use in a *Trichoderma* cell are *adeA*, *adeB*, *amdS*, *hph*, and *pyrG* genes.

The selectable marker may be a dual selectable marker system. In one aspect, the dual selectable marker is an *hph-tk* dual selectable marker system.

The vector preferably contains one or more elements that permit 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 pAMB1 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. Isolation of the AMA1 gene and construction of plasmids or vectors comprising the gene can be accomplished according to methods known in the art.

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.

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 altitudinis*, *Bacillus amyloliquefaciens*, *B. amyloliquefaciens* subsp. *plantarum*, *Bacillus brevis*,
 5 *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*,
Bacillus licheniformis, *Bacillus megaterium*, *Bacillus methylotrophicus*, *Bacillus pumilus*, *Bacillus safensis*, *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.
 10 The bacterial host cell may also be any *Streptomyces* cell including, but not limited to, *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, and *Streptomyces lividans* cells.

Introduction of DNA into a *Bacillus* cell may be effected by protoplast transformation, competent cell transformation or conjugation. The introduction of DNA into an *E. coli* cell may be effected by
 15 protoplast transformation or electroporation. The introduction of DNA into a *Streptomyces* cell may be effected by protoplast transformation, electroporation, conjugation, or transduction. The introduction of DNA into a *Pseudomonas* cell may be effected by electroporation or conjugation. The introduction of DNA into a *Streptococcus* cell may be effected by natural competence, protoplast transformation, electroporation, or conjugation. However, any method known in the art for introducing DNA into a host
 20 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. The fungal host cell may be a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (Endomycetales),
 25 basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (Blastomycetes).

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*
 30 cell.

The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision Eumycota and Oomycota. 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*,
 5 *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*,
 10 *Ceriporiopsis aneirina*, *Ceriporiopsis caregiea*, *Ceriporiopsis gilvescens*, *Ceriporiopsis pannocinta*,
Ceriporiopsis rivulosa, *Ceriporiopsis subrufa*, *Ceriporiopsis subvermisporea*, *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*
 15 *crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium*
heterosporum, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*,
Fusarium sambucinum, *Fusarium sarcochroum*, *Fusarium solani*, *Fusarium sporotrichioides*, *Fusarium*
sulphureum, *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum*, *Humicola insolens*,
Humicola lanuginosa, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium*
 20 *purpurogenum*, *Phanerochaete chrysosporium*, *Phlebia radiata*, *Pleurotus eryngii*, *Thielavia terrestris*,
Trametes villosa, *Trametes versicolor*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma*
longibrachiatum, *Trichoderma reesei*, or *Trichoderma viride* cell.

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
 25 transformation of *Aspergillus* and *Trichoderma* host cells are known in the art. Suitable methods for transforming *Fusarium* species are known in the art. Yeast may also be transformed using the procedures known in the art.

Formulations

30 The compositions of the invention may be in any convenient form, e.g., a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

Pouches can be configured as single or multicompartments. It can be of any form, shape and material which is suitable for hold the composition, e.g. without allowing the release of the composition

to release of the composition from the pouch prior to water contact. The pouch is made from water soluble film which encloses an inner volume. Said inner volume can be divided into compartments of the pouch. Preferred films are polymeric materials preferably polymers which are formed into a film or sheet. Preferred polymers, copolymers or derivatives thereof are selected polyacrylates, and water soluble acrylate copolymers, methyl cellulose, carboxy methyl cellulose, sodium dextrin, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, malto dextrin, poly methacrylates, most preferably polyvinyl alcohol copolymers and, hydroxypropyl methyl cellulose (HPMC). Preferably the level of polymer in the film for example PVA is at least about 60%. Preferred average molecular weight will typically be about 20,000 to about 150,000. Films can also be of blended compositions comprising hydrolytically degradable and water soluble polymer blends such as polylactide and polyvinyl alcohol (known under the Trade reference M8630 as sold by MonoSol LLC, Indiana, USA) plus plasticisers like glycerol, ethylene glycerol, propylene glycol, sorbitol and mixtures thereof. The pouches can comprise a solid laundry cleaning composition or part components and/or a liquid cleaning composition or part components separated by the water soluble film. The compartment for liquid components can be different in composition than compartments containing solids.

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

A liquid or gel detergent, which is not unit dosed, may be aqueous, typically containing at least 20% by weight and up to 95% water, such as up to about 70% water, up to about 65% water, up to about 55% water, up to about 45% water, up to about 35% water. Other types of liquids, including without limitation, alkanols, amines, diols, ethers and polyols may be included in an aqueous liquid or gel. An aqueous liquid or gel detergent may contain from 0-30% organic solvent. A liquid or gel detergent may be non-aqueous.

Liquid formulations may contain the RNase and may contain other small molecular material from the production process (e.g., fermentation of microorganisms expressing the RNase), for example, like salts, peptides, metabolites and the like.

Optionally, stabilizers (e.g., polyols, salts and the like) and other ingredients (e.g., inhibitors, antioxidants, anti-reductants, preservatives, alcohols, pH controllants, viscosity controllers and the like), may be contained in the formulations. The liquid formulations can be clear, hazy, or may contain sedimentation.

Polyols (or polyhydric alcohol) contained in liquid formulations generally are alcohols with two or more hydroxyl groups. The polyols typically have less than 10 carbons, such as 9, 8, 7, 6, 5, 4, or 3

carbons. The molecular weight is typically less than 500 g/mol, such as 400 g/mol or 300 g/mol. Examples of suitable polyols include, but are not limited to, glycerol, propylene glycol, ethylene glycol, sorbitol, mannitol, erythritol, dulcitol, inositol, xylitol, adonitol and the like. In some embodiments, the amount of polyol(s) in the liquid enzyme formulation is less than about 50% (w/w), 40% (w/w), 30%
5 (w/w), 20% (w/w), or 10% (w/w). In some embodiments, the liquid enzyme formulation does not contain polyol. Inhibitors includes added reversible inhibitors of the enzyme in question or of other enzymes intended for the same application or be an inhibitor of an unwanted side activity in the product or in the application.

In some examples, salts, especially those used as stabilizers, may be for the purpose of
10 reducing water activity. Salts can be organic or inorganic and are typically dissolved in the liquid formulation. Examples of cations in the salts may include Na⁺, Ca⁺⁺, K⁺, Mg⁺⁺, and the like. Examples of anions in the salts may include chloride, formate, acetate, sulfate, and the like. The formulation can also be without added salt.

Antioxidants or anti-reductants may include, for example, methionine, scavengers, sulfites, and
15 the like. Preservatives may include any food grade or technical grade preservative. Examples include sorbate, benzoate, isothiazolinones, BAC, phenoxyethanol, and the like.

Granular Detergent Formulations

The RNase-containing compositions may be formulated as granules. The granule is generally
20 composed of a core, and optionally one or more coatings (outer layers) surrounding the core.

The core of a granule may include additional materials such as fillers, fiber materials (cellulose or synthetic fibres), stabilizing agents, solubilizing 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
25 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
30 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:

a) Spray dried products, wherein a liquid enzyme-containing solution is atomized in a spray drying tower to form small droplets which during their way down the drying tower dry to form an

enzyme-containing particulate material. Very small particles can be produced this way;

b) Layered products, wherein the enzyme is coated as a layer around a pre-formed inert core particle, wherein an enzyme-containing solution is atomized, typically in a fluid bed apparatus wherein the pre-formed core particles are fluidized, and the enzyme-containing solution adheres to the core particles and dries up to leave a layer of dry enzyme on the surface of the core particle. Particles of a desired size can be obtained this way if a useful core particle of the desired size can be found;

c) Absorbed core particles, wherein rather than coating the enzyme as a layer around the core, the enzyme is absorbed onto and/or into the surface of the core;

d) Extrusion or pelletized products, wherein an enzyme-containing paste is pressed to pellets or under pressure is extruded through a small opening and cut into particles which are subsequently dried. Such particles usually have a considerable size because of the material in which the extrusion opening is made (usually a plate with bore holes) sets a limit on the allowable pressure drop over the extrusion opening. Also, very high extrusion pressures when using a small opening increase heat generation in the enzyme paste, which is harmful to the enzyme;

e) Prilled products, wherein an enzyme-containing powder is suspended in molten wax and the suspension is sprayed, e.g., through a rotating disk atomiser, into a cooling chamber where the droplets quickly solidify. The product obtained is one wherein the enzyme is uniformly distributed throughout an inert material instead of being concentrated on its surface;

f) Mixer granulation products, wherein a liquid is added to a dry powder composition of, e.g., conventional granulating components, the enzyme being introduced either via the liquid or the powder or both. The liquid and the powder are mixed and as the moisture of the liquid is absorbed in the dry powder, the components of the dry powder will start to adhere, and agglomerate and particles will build up, forming granulates comprising the enzyme. In a particular product of this process, wherein various high-shear mixers can be used as granulators, granulates consisting of enzyme as enzyme, fillers and binders etc. are mixed with cellulose fibers to reinforce the particles to give the so-called T-granulate. Reinforced particles, being more robust, release less enzymatic dust;

g) Size reduction, wherein the cores are produced by milling or crushing of larger particles, pellets, tablets, briquettes etc. containing the enzyme. The wanted core particle fraction is obtained by sieving the milled or crushed product. Over and undersized particles can be recycled;

h) Fluid bed granulation, involves suspending particulates in an air stream and spraying a liquid onto the fluidized particles via nozzles. Particles hit by spray droplets get wetted and become tacky. The tacky particles collide with other particles and adhere to them and form a granule; or

i) The cores may be subjected to drying, such as in a fluid bed drier. Other known methods for drying granules in the feed or detergent industry can be used by the skilled person. The drying

preferably takes place at a product temperature of from 25 to 90°C. For some enzymes it is important the cores comprising the enzyme contain a low amount of water before coating. If water sensitive enzymes are coated before excessive water is removed, it will be trapped within the core and it may affect the activity of the enzyme negatively. After drying, the cores preferably contain 0.1-10 % w/w water.

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).

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 may 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.

5 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). Specific examples of suitable salts are NaCl (CH₂₀°C=76%), Na₂CO₃ (CH₂₀°C=92%), NaNO₃ (CH₂₀°C=73%), Na₂HPO₄ (CH₂₀°C=95%), Na₃PO₄ (CH₂₅°C=92%), NH₄Cl (CH₂₀°C = 79.5%), (NH₄)₂HPO₄ (CH₂₀°C = 93.0%), NH₄H₂PO₄ (CH₂₀°C = 93.1%), (NH₄)₂SO₄ (CH₂₀°C=81.1%), KCl
10 (CH₂₀°C=85%), K₂HPO₄(CH₂₀°C=92%), KH₂PO₄ (CH₂₀°C=96.5%), KNO₃ (CH₂₀°C=93.5%), Na₂SO₄ (CH₂₀°C=93%), K₂SO₄ (CH₂₀°C=98%), KHSO₄ (CH₂₀°C=86%), MgSO₄ (CH₂₀°C=90%), ZnSO₄ (CH₂₀°C=90%) and sodium citrate (CH₂₅°C=86%). Other examples include NaH₂PO₄, (NH₄)H₂PO₄, CuSO₄, Mg(NO₃)₂ and magnesium acetate.

15 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. Specific examples include anhydrous sodium sulfate (Na₂SO₄), anhydrous magnesium sulfate (MgSO₄), magnesium sulfate heptahydrate (MgSO₄·7H₂O), zinc sulfate heptahydrate (ZnSO₄·7H₂O), sodium phosphate dibasic heptahydrate (Na₂HPO₄·7H₂O), magnesium nitrate hexahydrate (Mg(NO₃)₂·6H₂O), sodium citrate dihydrate and magnesium acetate tetrahydrate. Preferably the salt is applied as a solution of the salt, e.g., using a fluid bed.

20 Non-dusting granulates may be produced and may 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
25 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, generally prior to forming granules. Protected enzymes may be prepared.

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

- 30 (a) a core comprising a T2 RNase according to the invention, and
(b) optionally a coating consisting of one or more layer(s) surrounding the core.

The RNase-containing compositions may also be formulated as co-granules that combine 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 are known in the art.

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

The multi-enzyme co-granule may comprise an RNase of the invention and (a) one or more enzymes selected from lipases, hemicellulases, proteases, amylases, cellulases, cellobiose dehydrogenases, deoxyribonuclease, xylanases, phospho lipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, tannases, pentosanases, lichenases glucanases, arabinosidases, hyaluronidase, chondroitinase, amylases, and mixtures thereof.

Fermentation Broth or Cells 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 some embodiments, 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.

Methods of Using the RNases and Compositions

The polypeptides of the invention having RNase, and the compositions containing the RNase activity, may be used for cleaning of items, such as textiles. One embodiment of the invention relates to the use of an RNase of the invention for prevention or reduction of anti-redeposition and/or for improvement of whiteness of a textile subjected to multiple washes. The RNases of the invention therefore reduce the greyness of textiles when applied in the compositions of the invention to a cleaning process such as laundry. One embodiment of the invention relates to the use of an RNase and/or composition for prevention, reduction or removal of malodor.

In some examples, the RNases and/or compositions containing the RNases, may be used sequentially with other components of a detergent composition and/or detergent adjunct ingredients.

For example, in the case of cleaning to remove a biofilm, perhaps a first enzyme applied to a biofilm makes available substrates for a second enzyme. In some examples, therefore, RNase may not be the first enzyme applied to a biofilm especially, for example, if RNA in the biofilm is relatively less accessible than other components of the biofilm (e.g., if the RNA is contained within the EPS of the biofilm, and it would be more accessible to RNase if the EPS itself were reduced or removed). Therefore, cleaning of biofilms, at least with RNases, could comprise multiple sequential steps, where application of RNase is one of the steps.

In some examples, the RNases and/or compositions containing the RNases, may or may not be used in combination with other components of a cleaning or detergent composition and/or cleaning components or detergent adjunct ingredients. For example, RNase may not be used together with a protease. Instead, a protease may be used before or after the RNase.

In addition, the RNases, compositions containing the RNase, and methods described herein may be used for antimicrobial purposes in human or animal health.

One embodiment relates to the use of a cleaning composition comprising a T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component for cleaning an item by:

- i. preventing, reducing or removing stickiness of the item;
 - ii. preventing, reducing or removing biofilm or biofilm components from the item;
 - iii. reducing or removing stains comprises RNA from the item;
 - iv. preventing, reducing or removing redeposition of soil during cleaning of the item;
 - v. preventing, reducing or removing adherence of soil to the item;
 - vi. maintaining or improving whiteness of the item; or
 - vii. preventing, reducing or removing malodor from the item,
- wherein the item is a textile, a hard surface or a dish ware.

One embodiment relates to the use of a cleaning composition comprising a T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component for cleaning an item by:

- i. preventing, reducing or removing stickiness of the item;
- ii. preventing, reducing or removing biofilm or biofilm components from the item;
- iii. reducing or removing stains comprises RNA from the item;
- iv. preventing, reducing or removing redeposition of soil during cleaning of the item;
- v. preventing, reducing or removing adherence of soil to the item;
- vi. maintaining or improving whiteness of the item; or
- vii. preventing, reducing or removing malodor from the item,

wherein the item is a textile, a hard surface or a dish ware and wherein the T2 RNase is from E.C. class EC 3.1.27.1.

One embodiment relates to the use of a cleaning composition comprising a T2 RNase, preferably comprising the Pfam PF00445 domain, preferably comprising a C-domain, and at least one cleaning component for cleaning an item by:

- i. preventing, reducing or removing stickiness of the item;
 - 5 ii. preventing, reducing or removing biofilm or biofilm components from the item;
 - iii. reducing or removing stains comprises RNA from the item;
 - iv. preventing, reducing or removing redeposition of soil during cleaning of the item;
 - v. preventing, reducing or removing adherence of soil to the item;
 - vi. maintaining or improving whiteness of the item; or
 - 10 vii. preventing, reducing or removing malodor from the item,
- wherein the item is a textile, a hard surface or a dish ware and wherein the T2 RNase is from E.C. class EC 3.1.27.1.

One embodiment relates to the use of a cleaning composition comprising a T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component for cleaning an item by:

- i. preventing, reducing or removing stickiness of the item;
 - ii. preventing, reducing or removing biofilm or biofilm components from the item;
 - iii. reducing or removing stains comprises RNA from the item;
 - iv. preventing, reducing or removing redeposition of soil during cleaning of the item;
 - 20 v. preventing, reducing or removing adherence of soil to the item;
 - vi. maintaining or improving whiteness of the item; or
 - vii. preventing, reducing or removing malodor from the item,
- wherein the item is a textile, a hard surface or a dish ware and wherein the T2 RNase comprises at least one of the amino acid sequences HGLWPD (SEQ ID NO: 103), [VL]HGLWP[QNEG] (SEQ ID NO: 25 105) or L[SFT]WX[PT]XXC (SEQ ID NO: 106), where X is any naturally-occurring amino acid.

One embodiment relates to a method for laundering an item, comprising:

- (a) exposing the item to the composition comprising a T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component;
- 30 (b) completing at least one wash cycle; and
- (c) optionally, rinsing the textile.

One embodiment relates to a method for laundering an item, comprising:

- (a) exposing the item to the composition comprising a T2 RNase, preferably comprising the

Pfam PF00445 domain, and at least one cleaning component;

(b) completing at least one wash cycle; and

(c) optionally, rinsing the textile.

5 One embodiment relates to a method for laundering an item, comprising:

(a) exposing the item to the composition comprising a T2 RNase, preferably comprising the Pfam PF00445 domain, preferably comprising a C-domain, and at least one cleaning component;

(b) completing at least one wash cycle; and

(c) optionally, rinsing the textile, wherein the T2 RNase is from E.C. class EC 3.1.27.1.

10

One embodiment relates to a method for laundering an item, comprising:

(a) exposing the item to the composition comprising a T2 RNase, preferably comprising the Pfam PF00445 domain, preferably comprising a C-domain, and at least one cleaning component;

(b) completing at least one wash cycle; and

15

(c) optionally, rinsing the textile, wherein the T2 RNase comprises at least one of the amino acid sequences HGLWPD (SEQ ID NO: 103), [VL]HGLWP[QNEG] (SEQ ID NO: 105) or L[SFT]WX[PT]XXC (SEQ ID NO: 106), where X is any naturally-occurring amino acid.

Embodiments

20

Embodiments of the invention are set forth in the following numbered paragraphs:

1. An isolated polypeptide with RNase activity, selected from the group consisting of:

an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 25 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of the groups of SEQ ID NOs. below, or

an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, but less than 100%

30

sequence identity to one of the groups of SEQ ID NOs. below:

(a) SEQ ID NO: 2 or SEQ ID NO: 3;

(b) SEQ ID NO: 5 or SEQ ID NO: 6;

(c) SEQ ID NO: 8 or SEQ ID NO: 9;

(d) SEQ ID NO: 11 or SEQ ID NO: 12;

- (e) SEQ ID NO: 14 or SEQ ID NO: 15;
(f) SEQ ID NO: 17 or SEQ ID NO: 18;
(g) SEQ ID NO: 20 or SEQ ID NO: 21;
(h) SEQ ID NO: 23 or SEQ ID NO: 24;
5 (i) SEQ ID NO: 26 or SEQ ID NO: 27;
(j) SEQ ID NO: 29 or SEQ ID NO: 30;
(k) SEQ ID NO: 32 or SEQ ID NO: 33;
(l) SEQ ID NO: 35 or SEQ ID NO: 36;
(m) SEQ ID NO: 38 or SEQ ID NO: 39;
10 (n) SEQ ID NO: 41 or SEQ ID NO: 42;
(o) SEQ ID NO: 44 or SEQ ID NO: 45;
(p) SEQ ID NO: 47 or SEQ ID NO: 48;
(q) SEQ ID NO: 50 or SEQ ID NO: 51;
(r) SEQ ID NO: 53 or SEQ ID NO: 54;
15 (s) SEQ ID NO: 56 or SEQ ID NO: 57;
(t) SEQ ID NO: 59 or SEQ ID NO: 60;
(u) SEQ ID NO: 62 or SEQ ID NO: 63;
(v) SEQ ID NO: 65 or SEQ ID NO: 66;
(w) SEQ ID NO: 68 or SEQ ID NO: 69;
20 (x) SEQ ID NO: 71 or SEQ ID NO: 72;
(y) SEQ ID NO: 74 or SEQ ID NO: 75;
(z) SEQ ID NO: 77 or SEQ ID NO: 78;
(aa) SEQ ID NO: 80 or SEQ ID NO: 81;
(bb) SEQ ID NO: 83 or SEQ ID NO: 84;
25 (cc) SEQ ID NO: 86 or SEQ ID NO: 87;
(dd) SEQ ID NO: 89 or SEQ ID NO: 90;
(ee) SEQ ID NO: 92 or SEQ ID NO: 93;
(ff) SEQ ID NO: 95 or SEQ ID NO: 96;
(gg) SEQ ID NO: 98 or SEQ ID NO: 99;
30 (hh) SEQ ID NO: 101 or SEQ ID NO: 102; and
(ii) SEQ ID NO: 109 or SEQ ID NO: 110.

2. The isolated polypeptide of paragraph 1, wherein the groups of SEQ ID NOs. from which the polypeptide is selected consists of:

(a) SEQ ID NO: 3;
(b) SEQ ID NO: 6;
(c) SEQ ID NO: 9;
(d) SEQ ID NO: 12;
5 (e) SEQ ID NO: 15;
(f) SEQ ID NO: 18;
(g) SEQ ID NO: 21;
(h) SEQ ID NO: 24;
(i) SEQ ID NO: 27;
10 (j) SEQ ID NO: 30;
(k) SEQ ID NO: 33;
(l) SEQ ID NO: 36;
(m) SEQ ID NO: 39;
(n) SEQ ID NO: 42;
15 (o) SEQ ID NO: 45;
(p) SEQ ID NO: 48;
(q) SEQ ID NO: 51;
(r) SEQ ID NO: 54;
(s) SEQ ID NO: 57;
20 (t) SEQ ID NO: 60;
(u) SEQ ID NO: 63;
(v) SEQ ID NO: 66;
(w) SEQ ID NO: 69;
(x) SEQ ID NO: 72;
25 (y) SEQ ID NO: 75;
(z) SEQ ID NO: 78;
(aa) SEQ ID NO: 81;
(bb) SEQ ID NO: 84;
(cc) SEQ ID NO: 87;
30 (dd) SEQ ID NO: 90;
(ee) SEQ ID NO: 93;
(ff) SEQ ID NO: 96;
(gg) SEQ ID NO: 99;
(hh) SEQ ID NO: 102; and

(ii) SEQ ID NO: 110.

3. The isolated polypeptide of paragraphs 1 or 2, wherein the isolated polypeptide is obtained from a microorganism.

4. The isolated polypeptide of any one of paragraphs 1-3, wherein the isolated polypeptide is obtained from a bacterium.

5. The isolated polypeptide of any one of paragraphs 1-3, wherein the isolated polypeptide is obtained from a fungus.

6. The isolated polypeptide of any of paragraphs 1-4, wherein the isolated polypeptide additionally comprises at least one of the amino acid sequences [VL]HGLWP[QNEG] (SEQ ID NO: 105) or L[SFT]WX[PT]XXC (SEQ ID NO: 106), where X is any naturally-occurring amino acid.

7. The isolated polypeptide of any of paragraphs 1-4 or 6, wherein the isolated polypeptide includes at least one of SEQ ID NOs: 21, 24, 27, 30, 33, 78, 84 and 87.

8. The isolated polypeptide of paragraphs 1, 3 or 5, wherein the isolated polypeptide additionally comprises the amino acid sequence HGLWPD (SEQ ID NO: 103).

9. The isolated polypeptide of paragraphs 1, 3, 5 or 8, wherein the isolated polypeptide includes at least one of SEQ ID NOs: 3, 6, 9, 12, 15, 18, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 81 and 110.

10. The isolated polypeptide of any one of paragraphs 1-9, wherein the isolated polypeptide is a variant that has one or more amino acid substitutions, or one or more amino acid deletions, or one or more amino acid insertions, or any combination thereof, in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 positions.

11. The isolated polypeptide of any one of paragraphs 1-10, wherein the isolated polypeptide has an N-terminal, a C-terminal, or an N-terminal and a C-terminal amino acid extension of between 1 and 10 amino acids.

12. The isolated polypeptide of paragraph 11, wherein the amino acid extension is a His-tag or HQ-tag.

13. The isolated polypeptide of any one of paragraphs 1-12, wherein the isolated polypeptide is a variant that has one or more amino acid substitutions, one or more amino acid deletions, one or more amino acid insertions, or any combination thereof, in a region of the polypeptide that is not within a PF00445 domain of the polypeptide.

14. The isolated polypeptide of any one of paragraphs 1-13, wherein the isolated polypeptide is a variant that has one or more amino acids deleted from the C-terminal end of the polypeptide, with the proviso that the PF00445 domain is intact.

15. The isolated polypeptide of paragraph 14, including:

- (a) SEQ ID NO: 89 or SEQ ID NO: 90;
- (b) SEQ ID NO: 92 or SEQ ID NO: 93;
- (c) SEQ ID NO: 95 or SEQ ID NO: 96;
- (d) SEQ ID NO: 98 or SEQ ID NO: 99;
- (e) SEQ ID NO: 101 or SEQ ID NO: 102; or
- (f) SEQ ID NO: 109 or SEQ ID NO: 110.

16. A composition, comprising the isolated polypeptide of any of paragraphs 1-15, and at least one cleaning composition component and/or detergent adjunct ingredient.

17. The composition of paragraph 16, additionally comprising an enzyme selected from the group consisting of proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases and mannanases.

18. A method for cleaning an item, comprising contacting the item with the composition of paragraph 16 or 17.

19. A method for laundering an item, comprising:

- (a) exposing the item to the composition of paragraph 16 or 17;
- (b) completing at least one wash cycle; and
- (c) optionally, rinsing the textile.

20. A method for cleaning an item, comprising contacting an item with an isolated polypeptide having RNase activity, the polypeptide having a Pfam PF00445 amino acid domain.

21. The method of paragraph 20, wherein the item is a textile.

22. Use of the isolated polypeptide of any one of paragraph 1-15 for cleaning an item.

23. Use of the composition of paragraph 16 or 17 for cleaning an item.

24. A polynucleotide encoding one of the isolated polypeptides of any one of paragraphs 1-15.

25. The polynucleotide of paragraph 24, wherein the nucleotide sequence of the polynucleotide is not a naturally occurring sequence.

26. An isolated polynucleotide encoding a polypeptide with RNase activity, wherein the sequence of the polynucleotide is at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to one of SEQ ID NOs: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100 or 108.

27. The isolated polynucleotide of paragraph 26, wherein the sequence of the polynucleotide is not 100% identical to any of the SEQ ID NOs. set forth in claim 26.

28. A nucleic acid construct or expression vector, comprising the isolated polynucleotide of any one of paragraphs 24-27.

29. A recombinant host cell, comprising the nucleic acid construct or expression vector of paragraph 28.

30. A detergent composition, comprising a polypeptide that includes a Pfam PF00445 amino acid domain, and a detergent adjunct ingredient.

31. The detergent composition of paragraph 30, wherein the polypeptide is a variant of a naturally-occurring polypeptide.

32. The detergent composition of paragraph 30 or 31, additionally comprising an enzyme selected from the group consisting of proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases, mannases and DNases i.e. deoxyribonucleases.

Particularly preferred embodiments

1. An isolated polypeptide with RNase activity, selected from the group consisting of:

an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of the groups of SEQ ID NOs. below, or

an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, but less than 100% sequence identity to one of the groups of SEQ ID NOs. below:

(a) SEQ ID NO: 2 or SEQ ID NO: 3;

(b) SEQ ID NO: 5 or SEQ ID NO: 6;

(c) SEQ ID NO: 8 or SEQ ID NO: 9;

(d) SEQ ID NO: 11 or SEQ ID NO: 12;

(e) SEQ ID NO: 14 or SEQ ID NO: 15;

(f) SEQ ID NO: 17 or SEQ ID NO: 18;

(g) SEQ ID NO: 20 or SEQ ID NO: 21;

(h) SEQ ID NO: 23 or SEQ ID NO: 24;

(i) SEQ ID NO: 26 or SEQ ID NO: 27;

(j) SEQ ID NO: 29 or SEQ ID NO: 30;

(k) SEQ ID NO: 32 or SEQ ID NO: 33;

(l) SEQ ID NO: 35 or SEQ ID NO: 36;

(m) SEQ ID NO: 38 or SEQ ID NO: 39;

(n) SEQ ID NO: 41 or SEQ ID NO: 42;

(o) SEQ ID NO: 44 or SEQ ID NO: 45;

(p) SEQ ID NO: 47 or SEQ ID NO: 48;

(q) SEQ ID NO: 50 or SEQ ID NO: 51;

(r) SEQ ID NO: 53 or SEQ ID NO: 54;
(s) SEQ ID NO: 56 or SEQ ID NO: 57;
(t) SEQ ID NO: 59 or SEQ ID NO: 60;
(u) SEQ ID NO: 62 or SEQ ID NO: 63;
5 (v) SEQ ID NO: 65 or SEQ ID NO: 66;
(w) SEQ ID NO: 68 or SEQ ID NO: 69;
(x) SEQ ID NO: 71 or SEQ ID NO: 72;
(y) SEQ ID NO: 74 or SEQ ID NO: 75;
(z) SEQ ID NO: 77 or SEQ ID NO: 78;
10 (aa) SEQ ID NO: 80 or SEQ ID NO: 81;
(bb) SEQ ID NO: 83 or SEQ ID NO: 84;
(cc) SEQ ID NO: 86 or SEQ ID NO: 87;
(dd) SEQ ID NO: 89 or SEQ ID NO: 90;
(ee) SEQ ID NO: 92 or SEQ ID NO: 93;
15 (ff) SEQ ID NO: 95 or SEQ ID NO: 96;
(gg) SEQ ID NO: 98 or SEQ ID NO: 99;
(hh) SEQ ID NO: 101 or SEQ ID NO: 102; and
(ii) SEQ ID NO: 109 or SEQ ID NO: 110.

20 2. The isolated polypeptide of paragraph 1, wherein the groups of SEQ ID NOs. from which the polypeptide is selected consists of:

(a) SEQ ID NO: 3;
(b) SEQ ID NO: 6;
(c) SEQ ID NO: 9;
25 (d) SEQ ID NO: 12;
(e) SEQ ID NO: 15;
(f) SEQ ID NO: 18;
(g) SEQ ID NO: 21;
(h) SEQ ID NO: 24;
30 (i) SEQ ID NO: 27;
(j) SEQ ID NO: 30;
(k) SEQ ID NO: 33;
(l) SEQ ID NO: 36;
(m) SEQ ID NO: 39;

(n) SEQ ID NO: 42;
 (o) SEQ ID NO: 45;
 (p) SEQ ID NO: 48;
 (q) SEQ ID NO: 51;
 5 (r) SEQ ID NO: 54;
 (s) SEQ ID NO: 57;
 (t) SEQ ID NO: 60;
 (u) SEQ ID NO: 63;
 (v) SEQ ID NO: 66;
 10 (w) SEQ ID NO: 69;
 (x) SEQ ID NO: 72;
 (y) SEQ ID NO: 75;
 (z) SEQ ID NO: 78;
 (aa) SEQ ID NO: 81;
 15 (bb) SEQ ID NO: 84;
 (cc) SEQ ID NO: 87;
 (dd) SEQ ID NO: 90;
 (ee) SEQ ID NO: 93;
 (ff) SEQ ID NO: 96;
 20 (gg) SEQ ID NO: 99;
 (hh) SEQ ID NO: 102; and
 (ii) SEQ ID NO: 110.

3. The isolated polypeptide of paragraph 1 or 2, wherein the isolated polypeptide additionally comprises
 25 at least one of the amino acid sequences [VL]HGLWP[QNEG] (SEQ ID NO: 105) or L[SFT]WX[PT]XXC
 (SEQ ID NO: 106), where X is any naturally-occurring amino acid.

4. The isolated polypeptide of any one of paragraphs 1-3, wherein the isolated polypeptide additionally
 comprises the amino acid sequence HGLWPD (SEQ ID NO: 103).

5. The isolated polypeptide of any one of paragraphs 1-4, wherein the isolated polypeptide has an N-
 terminal, a C-terminal, or an N-terminal and a C-terminal amino acid extension of between 1 and 10
 amino acids.

6. The isolated polypeptide of any one of paragraphs 1-5, wherein the isolated polypeptide is a variant that has one or more amino acid substitutions, one or more amino acid deletions, one or more amino acid insertions, or any combination thereof, in a region of the polypeptide that is not within a PF00445 domain of the polypeptide.

5

7. A composition, comprising the isolated polypeptide of any of paragraphs 1-6, and at least one cleaning composition component and/or detergent adjunct ingredient.

10

8. The composition of paragraph 7, additionally comprising an enzyme selected from the group consisting of proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases, deoxyribonucleases and mannanases.

15

9. A method for cleaning an item, comprising contacting the item with the composition of paragraph 7 or 8.

10. A method for laundering an item, comprising:

- (a) exposing the item to the composition of paragraph 7 or 8;
- (b) completing at least one wash cycle; and
- (c) optionally, rinsing the textile.

20

11. Use of the isolated polypeptide of any one of paragraphs 1-6 for cleaning an item.

12. Use of the composition of paragraph 7 or 8 for cleaning an item.

25

13. A polynucleotide encoding one of the isolated polypeptides of any one of paragraphs 1-6.

14. A nucleic acid construct or expression vector, comprising the isolated polynucleotide of paragraph 13.

30

15. A recombinant host cell, comprising the nucleic acid construct or expression vector of paragraph 14.

Examples

Example 1. Identification, cloning and expression of genes encoding T2 RNases

In general, chromosomal DNA was obtained from the organisms listed below, and the whole genomes were sequenced and assembled using standard methods. The assembled genomes were annotated using gene prediction software, and then were searched for T2 RNase sequences. In most cases, the peptides predicted from the whole genome sequences were searched for similarity to the PF00445 amino acid domain as described by Pfam (PF00445, Pfam version 31.0 Fin, (2016) *Nucleic Acids Research*, Database Issue 44:D279-D285; Pfam: Family: Ribonuclease_T2 (PF00445) [WWW Document], n.d. URL <http://Pfam.xfam.org/family/PF00445>). In some cases, specific T2 RNase sequences were used to search against the predicted peptide set to identify similar sequences (e.g., using sequences described in, Ozeki, K., et al., 1991. Cloning and nucleotide sequence of the genomic ribonuclease T2 gene from *Aspergillus oryzae*. *Curr Genet* 19, 367–373). PCR primers flanking the T2 RNase genes were then designed and used to amplify T2 RNase genes. The amplified regions were cloned and sequenced. Expression vectors containing the sequences were constructed and used to express the T2 RNase proteins. Generally, an *Aspergillus oryzae* expression host was used for the T2 RNases derived from fungi, and a *Bacillus subtilis* expression host was used for T2 RNases from bacteria in these studies. The proteins were expressed (using His- tags, in some instances) and then purified.

Table 1. Organisms used in these studies

Sample	Genus & species of organism	Type of organism	Source	Sequence identifier of mature peptide
1	<i>Aeromonas molluscorum</i>	Bacteria	U.S.	SEQ ID NO: 21
2	<i>Paracoccus carotinifaciens</i>	Bacteria	Denmark	SEQ ID NO: 24
3	<i>Mycobacterium</i> sp-62710	Bacteria	Senegal	SEQ ID NO: 27
4	<i>Shinella granuli</i>	Bacteria	Denmark	SEQ ID NO: 30
5	<i>Agrobacterium fabrum</i>	Bacteria	Denmark	SEQ ID NO: 33
6	<i>Zoogloea ramigera</i>	Bacteria	Denmark	SEQ ID NO: 78
7	<i>Buttiauxella brennerae</i>	Bacteria	Denmark	SEQ ID NO: 84
8	<i>Caulobacter vibrioides</i>	Bacteria	Denmark	SEQ ID NO: 87

9	<i>Trichoderma reesei</i> QM6A	Fungi	Solomon Islands, 1944	SEQ ID NO: 3
10	<i>Trichoderma harzianum</i> , deposited as CBS223.93	Fungi	Acquired Switzerland 1968	SEQ ID NO: 6 and 110
11	<i>Aspergillus oryzae</i> IFO4177	Fungi	Japan	SEQ ID NO: 9
12	<i>Trichoderma harzianum</i> , deposited as CBS223.93	Fungi	Acquired Swiss Ferment A/G, 1968	SEQ ID NO: 12
13	<i>Trichoderma harzianum</i> , deposited as CBS223.93	Fungi	Acquired Swiss Ferment A/G, 1968	SEQ ID NO: 15
14	<i>Trichoderma harzianum</i> , deposited as CBS223.93	Fungi	Acquired Swiss Ferment A/G, 1968	SEQ ID NO: 18
15	<i>Trichoderma parapiluliferum</i>	Fungi	China	SEQ ID NO: 36
16	<i>Lecanicillium</i> sp. WMM742	Fungi	China	SEQ ID NO: 39
17	<i>Ascochyta fabae</i>	Fungi	China	SEQ ID NO: 42
18	<i>Simplicillium lamellicola</i>	Fungi	China	SEQ ID NO: 45
19	<i>Penicillium polonicum</i>	Fungi	China	SEQ ID NO: 48
20	<i>Simplicillium lamellicola</i>	Fungi	China	SEQ ID NO: 51
21	<i>Setosphaeria rostrata</i>	Fungi	China	SEQ ID NO: 54
22	<i>Trichoderma parapiluliferum</i>	Fungi	China	SEQ ID NOs: 57 and 96
23	<i>Trichoderma atroviride</i>	Fungi	China	SEQ ID NOs: 60 and 93
24	<i>Acremonium</i> sp. XZ2020	Fungi	China	SEQ ID NOs: 63 and 99
25	<i>Clathrosphaerina</i> sp.	Fungi	China	SEQ ID NOs: 66 and 102
26	<i>Thermoascus</i>	Fungi	China	SEQ ID NO: 69

	<i>aurantiacus</i>			
27	<i>Cadophora malorum</i>	Fungi	China	SEQ ID NOs: 72 and 90
28	<i>Cladosporium sp.</i>	Fungi	China	SEQ ID NO: 75
29	Glass-biofilm-enrichment E, pH 7	Metagenomes	Denmark	SEQ ID NO: 81

Example 2. Construction of clades and phylogenetic trees

The RNases disclosed herein include the polypeptides having RNase activity and the T2 family domain (Pfam PF00445). In this Example, these polypeptide sequences were analyzed to identify possible groupings within the T2 RNases.

A phylogenetic tree was constructed with polypeptide sequences containing a T2 domain, as defined in Pfam (PF00445, Pfam version 31.0 Fin, (2016) *Nucleic Acids Research*, Database Issue 44:D279-D285; Pfam: Family: Ribonuclease_T2 (PF00445) [WWW Document], n.d. URL <http://Pfam.xfam.org/family/PF00445>). The phylogenetic tree was constructed from a multiple alignment of mature polypeptide sequences containing at least one T2 domain. The sequences were aligned using the MUSCLE algorithm version 3.8.31 (Edgar, 2004. *Nucleic Acids Research* 32(5): 1792-1797), and the trees were constructed using FastTree version 2.1.8 (Price et al., 2010, *PLoS one* 5(3)) and visualized using iTOL (Letunic & Bork, 2007. *Bioinformatics* 23(1): 127-128).

We found that the polypeptides in the T2 RNase family (Luhtala, N., Parker, R., 2010. T2 Family Ribonucleases: Ancient enzymes with diverse roles. *Trends Biochem Sci* 35, 253–259) could be separated into multiple distinct sub-clusters or clades. These are denoted below. Distinct motifs for each clade are discussed below.

Generation of the fungal HGLWPD clade

The HGLWPD clade comprises T2 ribonuclease polypeptides of fungal origin, having RNase activity and comprising the motif HGLWPD (SEQ ID NO: 103) which is fully conserved in the clade.

The structure of the reference enzyme RNase Actibind from *Aspergillus niger* (SEQ ID: 104) consists of a hydrophobic core including a central four-stranded twisted antiparallel beta-sheet, and the motif HGLWPD corresponds to part of the second N-terminal beta strand (residues 51-56 of SEQ ID: 104) of the central beta sheet. Amino acid changes that destroy hydrogen bonding between the beta strands in the beta-sheet decrease the overall enzyme stability. The His (H) residue of the HGLWPD motif is part of the active site and is needed for catalysis.

While not every T2 RNase from fungi contains the HGLWPD clade-identifying sequence, most molecules we examined do contain the sequence. Therefore, presence of this motif is useful for identifying a group of T2 RNases obtained from fungi.

5 Generation of the bacterial VHGLWPQ and LSWXPXXC clades

The VHGLWPQ clade comprises T2 ribonuclease polypeptides of bacterial origin, having RNase activity and comprising the motif [VL]HGLWP[QNEG] (SEQ ID NO: 105). Another motif found in the T2 RNases from bacteria is L[SFT]WX[PT]XXC (SEQ ID NO: 106), where X may be any naturally-occurring amino acid. In our analysis, we found that most T2 RNases from bacteria contain both of the above motifs. However, some of the bacterial T2 RNases contained one of the motifs and not the other. The majority of bacterial T2 RNases that we analyzed did contain at least one of these motifs.

The structure of the reference enzyme RNase 1 from *E. coli* (SEQ ID NO: 107) consists of a hydrophobic core including a central four-stranded twisted antiparallel beta-sheet, and the motif [VL]HGLWP[QNEG] (SEQ ID NO: 105) corresponds to part of the second N-terminal beta strand (residues 54-60 of SEQ ID NO: 107) of the central beta sheet. Amino acid changes that destroy hydrogen bonding between the beta strands in the beta-sheet decrease overall enzyme stability. The His (H) residue of the [VL]HGLWP[QNEG] motif is part of the active site and is needed for catalysis.

The motif L[SFT]WX[PT]XXC (SEQ ID NO: 106) corresponds to part of the first N-terminal beta strand and a following alpha helical region (residues 18-25 of SEQ ID NO: 107). Amino acid changes that destroy hydrogen bonding between the beta strands in the beta-sheet decrease the overall enzyme stability. The conserved cystine (C) of the motif (residue 25 of SEQ ID: 107) forms of a cysteine bridge with residue 39 of SEQ ID NO: 107. Cysteine bridges are known to confer increased stability of enzymes. Mutations that destroy cysteine bridges lead to lower enzyme stability (Liu, T., Wang, Y., Luo, X., Li, J., Reed, S.A., Xiao, H., Young, T.S., Schultz, P.G., 2016. Enhancing protein stability with extended disulfide bonds. PNAS 113, 5910–5915).

Again, while not every bacterial T2 RNase that we analyzed contained one of the two motifs described here, most molecules did contain at least one of the motifs. Therefore, presence of at least one of the motifs is useful for identifying a group of T2 RNases from bacteria.

30 **Example 3. RNase activity of polypeptides**

The cloned and expressed polypeptides were examined for RNase activity. RNase activity of the polypeptides was assessed in water and in a model detergent.

RNase activity was determined by fluorescence using a fluorescence-quenched oligonucleotide probe (relative fluorescence units, RFU). This probe emits a signal after nuclease degradation

(RNaseAlert™ kit, Integrated DNA Technologies, Inc., Coralville, Iowa, USA). Briefly, RNase was diluted in water (hardness 15°dH) to obtain a concentration of 0.1 ppm, or in model detergent B wash liquor (EU, 3.3 g/L), as described below, in water (hardness 15°dH) to obtain a concentration of 1 ppm. Five µl of the RNaseAlert™ substrate was added to 95 µl of the RNase sample.

Model detergent B wash liquor (used in the RNase activity assays) was prepared by dissolving 3.33 g/l of model detergent B in water. Model detergent B (diluted as above) contained 7.2% LAS, 6.6% AEO Biosoft N25-7 (NI), 4.2% AEOS (SLES), 6% MPG (mono propylene glycol), 3% ethanol, 3% TEA (triethanolamine), 2.75% cocoa soap, 2.75% soya soap, 2% glycerol, 1.2% sodium hydroxide, 2% sodium citrate, 1% sodium formate, 0.2% DTMPA and 0.2% PCA (Propenoic acid). All percentages are w/w (weight/weight).

A kinetic curve was measured for 10 min at 22°C using a SpectraMax® microplate reader, Molecular Devices (excitation 490nm, emission at 520nm). No activity was detected in controls that contained the RNaseAlert™ fluorescence-quenched oligonucleotide substrate with no enzyme. Table 2 below shows RNase activity measured in water or in model detergent B.

Table 2. RNase activity of polypeptides						
Sample	RNase		Genus & species of organism	Type of organism	Activity in water (RFU at 10 min)	Activity in detergent (RFU at 10 min)
1	SEQ NO: 21	ID	<i>Aeromonas molluscorum</i>	Bacterium	921	19
2	SEQ NO: 24	ID	<i>Paracoccus carotinifaciens</i>	Bacterium	457	83
3	SEQ NO: 30	ID	<i>Shinella granuli</i>	Bacterium	648	27
4	SEQ NO: 33	ID	<i>Agrobacterium fabrum</i>	Bacterium	516	14
5	SEQ NO: 78	ID	<i>Zoogloea ramigera</i>	Bacterium	446	18
6	SEQ NO: 84	ID	<i>Buttiauxella brennerae</i>	Bacterium	811	19
7	SEQ	ID	<i>Trichoderma reesei</i>	Fungus	52	646

	NO: 3				
8	SEQ ID	<i>Trichoderma</i>	Fungus	20	751
	NO: 6	<i>harzianum</i>			
9	SEQ ID	<i>Aspergillus oryzae</i>	Fungus	17	542
	NO: 9				
10	SEQ ID	<i>Trichoderma</i>	Fungus	13	369
	NO: 12	<i>harzianum</i>			
11	SEQ ID	<i>Trichoderma</i>	Fungus	22	425
	NO: 15	<i>harzianum</i>			
12	SEQ ID	<i>Trichoderma</i>	Fungus	18	875
	NO: 18	<i>harzianum</i>			
13	SEQ ID	<i>Trichoderma</i>	Fungus	15	506
	NO: 36	<i>parapiluliferum</i>			
14	SEQ ID	<i>Lecanicillium</i> sp.	Fungus	912	799
	NO: 39	WMM742			
15	SEQ ID	<i>Ascochyta fabae</i>	Fungus	54	639
	NO: 42				
16	SEQ ID	<i>Simplicillium</i>	Fungus	157	848
	NO: 45	<i>lamellicola</i>			
17	SEQ ID	<i>Penicillium</i>	Fungus	840	841
	NO: 48	<i>polonicum</i>			
18	SEQ ID	<i>Simplicillium</i>	Fungus	17	717
	NO: 51	<i>lamellicola</i>			
19	SEQ ID	<i>Setosphaeria</i>	Fungus	60	841
	NO: 54	<i>rostrata</i>			
20	SEQ ID	<i>Trichoderma</i>	Fungus	54	726
	NO: 57	<i>parapiluliferum</i>			
21	SEQ ID	<i>Trichoderma</i>	Fungus	13	721
	NO: 60	<i>atroviride</i>			
22	SEQ ID	<i>Acremonium</i> sp.	Fungus	733	827
	NO: 63	XZ2020			
23	SEQ ID	<i>Clathrosphaerina</i>	Fungus	675	815

	NO: 66	sp.			
24	SEQ ID	<i>Thermoascus</i>	Fungus	554	800
	NO: 69	<i>aurantiacus</i>			
25	SEQ ID	<i>Cadophora</i>	Fungus	218	798
	NO: 72	<i>malorum</i>			
26	SEQ ID	<i>Cladosporium</i> sp.	Fungus	14	247
	NO: 75				
27	SEQ ID	Glass-biofilm- enrichment E, pH 7	Metagenome	681	-
28	SEQ ID	<i>Cadophora</i>	Fungus	12	867
	NO: 90	<i>malorum</i>			
29	SEQ ID	<i>Trichoderma</i>	Fungus	21	624
	NO: 93	<i>atroviride</i>			
30	SEQ ID	<i>Trichoderma</i>	Fungus	11	670
	NO: 96	<i>parapiluliferum</i>			
31	SEQ ID	<i>Acremonium</i> sp.	Fungus	67	989
	NO: 99	XZ2020			
32	SEQ ID	<i>Clathrosphaerina</i>	Fungus	24	713
	NO: 102	sp.			
33	SEQ ID	<i>Trichoderma</i>	Fungus	31	587
	NO: 110	<i>harzianum</i>			

The results showed that the polypeptides had RNase activity. The data also showed that, for polypeptides tested in both water and the model detergent, that activity was generally higher in the detergent than in water.

5

Example 4. Removal of RNA from textiles by laundering using RNase

Some of the T2 RNase polypeptides tested their ability to remove RNA from soiled laundry. Twelve socks from twelve different pairs of socks (one sock per pair) were used (Warwick Equest). From each sock, four 1 cm diameter swatches were cut - two swatches from the sole and two swatches from the heel of the sock. Each sole swatch was paired with a heel swatch from the same sock, and the paired swatches were added together to a well of a 24-deep well plate (24 total wells, each containing a sole and heel swatch from the same sock). Six wells were laundered with a detergent

10

composition containing the polypeptide of SEQ ID NO: 6. Six wells were laundered with a detergent composition containing the polypeptide of SEQ ID NO: 72. Six wells were laundered with a detergent composition containing no polypeptide (negative control).

The laundering was performed as follows. Two mL of model detergent B wash liquor (EU, 3.3 g/L) was added to each well. For each experimental well, RNase polypeptide was also present at 1 ppm. For each control well, no RNase polypeptide was present. Therefore, EU conditions of 3.3 g/L detergent and water with a hardness of 15°dH (Ca:Mg:NaHCO₃ 4:1:1.5) were used. The 24-deep well plates were then incubated at 30°C with shaking (800 rpm) for 1 hour. Subsequently, the swatches were rinsed in water (hardness 15°dH) and dried.

To extract remaining RNA from the swatches, so the amount of RNA could be determined, 0.8 mL of an RNase-free buffer (0.1% v/v DEPC, 10 mM EDTA, 0.9% NaCl, pH 4.5) was added to each well, and the deep well plates were incubated for 1 hour at room temperature with shaking (800 rpm). Subsequently, 100 µl from each well was added to 100 µl of Quant-IT™ RiboGreen® (Thermofisher) reagent as recommended by the supplier protocol. After 3 min incubation at room temperature, endpoint fluorescence was measured at 22°C in a SpectraMax® microplate reader (Molecular Devices) using excitation at 500 nm and emission at 525nm. Fluorescence of the experimental samples (plus RNase) was subtracted from the control samples (no RNase) to determine the percent reduction in relative fluorescence units in the experimental samples as compared to the controls (% RFU reduction). The data are shown below.

Table 3. RNA removed from textiles by detergent containing RNases	
RNase	% RFU reduction
SEQ ID NO: 6	62
SEQ ID NO: 72	21

The results showed that the polypeptides having RNase activity reduced the amount of RNA on soiled textiles, when used as part of a detergent composition.

Example 5. RNase activity of enzymes with/without C-terminal domain

As described in Example 3 RNase activity was determined with RNaseAlert assay. To better discriminate enzymes activity was measured in model detergent B (EU, 3.3 g/L) at 1 ppm enzyme concentration, and in 100mM HEPES buffer pH 8 and model detergent A (EU, 3.3g/L) at 0.1 ppm enzyme concentration.

Model detergent B wash liquor (100%) was prepared as described in Example 3. by dissolving 3.33 g/l of model detergent B in water. Model detergent B contained 7.2% LAS, 6.6% AEO Biosoft N25-7 (NI), 4.2% AEOS (SLES), 6% MPG (mono propylene glycol), 3% ethanol, 3% TEA (triethanolamine), 2.75% cocoa soap, 2.75% soya soap, 2% glycerol, 1.2% sodium hydroxide, 2% sodium citrate, 1% sodium formiate, 0.2% DTMPA and 0.2% PCA (Propenoic acid). All percentages are w/w (weight/weight).

Model detergent A wash liquor (100%) was prepared by dissolving 3.33 g/l of model detergent A in water. Model A contained 12% LAS, 11% AEO Biosoft N25-7 (NI), 7% AEOS (SLES), 6% MPG, 3% ethanol, 3% TEA (triethanolamine), 2.75% cocoa soap, 2.75% soya soap, 2% glycerol, 2% sodium hydroxide, 2% sodium citrate, 1% sodium formiate, 0.2% DTMPA and 0.2% PCA (Propenoic acid). All percentages are w/w (weight/weight).

A kinetic curve was measured for 10 min at 22°C using a SpectraMax® microplate reader, Molecular Devices (excitation 490nm, emission at 520nm). Initial slopes (0-2 minutes) are given in Table 3. Initial slopes given as '>4' mean that reaction reached saturation before measurement.

It is clear from the data that lack of C-terminal domain results in lower, but not absent, RNase activity.

Table 4. RNase activity of polypeptides with/without C-terminal domain

Sample	RNase	C-terminal domain	Activity in 100mM HEPES, pH8, 0.1 ppm	Activity in Model B, 1 ppm (Initial slope)	Activity in Model A, 0.1 ppm (Initial slope)
1	SEQ ID NO: 110	NO	0,04	1,6	0,1
2	SEQ ID NO: 6	YES	0,1	>4	0,6
3	SEQ ID NO: 90	NO	0,008	2,6	0,2
4	SEQ ID NO: 72	YES	0,8	>4	>4
5	SEQ ID NO: 93	NO	0,03	1,4	0,1
6	SEQ ID NO: 60	YES	0,04	>4	2,2
7	SEQ ID NO: 96	NO	0,008	1,7	0,1
8	SEQ ID NO: 57	YES	0,3	>4	3,2
9	SEQ ID NO: 99	NO	0,2	>4	1,1
10	SEQ ID NO: 63	YES	3,5	>4	>4
11	SEQ ID NO: 102	NO	0,05	1,9	0,1

12	SEQ ID NO: 66	YES	2,0	>4	>4
----	---------------	-----	-----	----	----

Claims

1. A cleaning composition, preferably a laundry composition, comprising at least 0.001 ppm, such as at least 0.01 ppm or at least 0.1 ppm T2 RNase, preferably comprising the Pfam PF00445 domain, and at least one cleaning component.
2. A cleaning composition, preferably a laundry composition, wherein the T2 RNase is from E.C. class EC 3.1.27.1.
3. The cleaning composition according to any of claims 1 or 2, wherein the T2 RNase comprises at least one of the amino acid sequences [VL]HGLWP[QNEG] (SEQ ID NO: 105) or L[SFT]WX[PT]XXC (SEQ ID NO: 106), where X is any naturally-occurring amino acid.
4. The cleaning composition according to any of the preceding claims, wherein the T2 RNase comprises the amino acid sequence HGLWPD (SEQ ID NO: 103).
5. The cleaning composition according to any of the preceding claims wherein the T2 RNase is of microbial origin, preferably of bacterial or fungal origin.
6. The cleaning composition according to any of the preceding claims, wherein the T2 RNase comprises a C-terminal domain.
7. The cleaning composition according to any of the preceding claims, additionally comprising an enzyme selected from the group consisting of proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases, deoxyribonucleases and mannanases.
8. The cleaning composition according to any of the preceding claims, wherein the composition comprises from about 0.5 wt% to about 80 wt% of an anionic surfactant.
9. The cleaning composition according to claim 8, wherein the anionic surfactant is selected from the group consisting of 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), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodeceny/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.

10. The cleaning composition according to any of the preceding claims, wherein the composition comprises from about 0.1 wt% to about 40 wt% of a non-ionic surfactant.
11. The cleaning composition according to claim 10, wherein the nonionic surfactant is selected from the group consisting of 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) and combinations thereof.
12. The cleaning composition according to any of the preceding claims, wherein the composition comprises a fabric care component selected from the group consisting of cationic softening-compounds, silicone softening-compounds, paraffins, waxes, dispersible polyolefins and mixtures thereof.
13. The cleaning composition according to any of the preceding claims, wherein the T2 RNase comprises a polypeptide with RNase activity, selected from the group consisting of: an amino acid sequence having at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to
 - (a) SEQ ID NO: 3;
 - (b) SEQ ID NO: 6;

(c) SEQ ID NO: 9;
(d) SEQ ID NO: 12;
(e) SEQ ID NO: 15;
(f) SEQ ID NO: 18;
(g) SEQ ID NO: 21;
(h) SEQ ID NO: 24;
(i) SEQ ID NO: 27;
(j) SEQ ID NO: 30;
(k) SEQ ID NO: 33;
(l) SEQ ID NO: 36;
(m) SEQ ID NO: 39;
(n) SEQ ID NO: 42;
(o) SEQ ID NO: 45;
(p) SEQ ID NO: 48;
(q) SEQ ID NO: 51;
(r) SEQ ID NO: 54;
(s) SEQ ID NO: 57;
(t) SEQ ID NO: 60;
(u) SEQ ID NO: 63;
(v) SEQ ID NO: 66;
(w) SEQ ID NO: 69;
(x) SEQ ID NO: 72;
(y) SEQ ID NO: 75;
(z) SEQ ID NO: 78;
(aa)SEQ ID NO: 81;
(bb) SEQ ID NO: 84;
(cc)SEQ ID NO: 87;
(dd)SEQ ID NO: 90;
(ee)SEQ ID NO: 93;
(ff)SEQ ID NO: 96;
(gg)SEQ ID NO: 99;
(hh)SEQ ID NO: 102; and
(ii)SEQ ID NO: 110.

14. Use of a cleaning composition according to any of claims 1-13 for cleaning an item by:

- i. preventing, reducing or removing stickiness of the item;
- ii. preventing, reducing or removing biofilm or biofilm components from the item;
- iii. reducing or removing stains comprises RNA from the item;
- iv. preventing, reducing or removing redeposition of soil during cleaning of the item;
- v. preventing, reducing or removing adherence of soil to the item;
- vi. maintaining or improving whiteness of the item; or
- vii. preventing, reducing or removing malodor from the item,

wherein the item is a textile, a hard surface or a dish ware.

15. A method for laundering an item, comprising:

- (a) exposing the item to the composition of any of claims 1 to 13;
- (b) completing at least one wash cycle; and
- (c) optionally, rinsing the textile.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/106223

A. CLASSIFICATION OF SUBJECT MATTER

C11D 3/386(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C11D

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)

CNMED, CNABS, CPEA, DWPI, SIPOABS, AUABS, TWMED, ILABS, TWABS, HKABS, MOABS, SGABS, CNKI, NCBI, EMBL, GoogleScholar:T2 RNase, PF00445, cleaning composition, laundry, EC 3.1.27.1 Retrieving System for Biological Sequence of Chinese Patent: sequence search based on SEQ ID NO:103, 105, 106

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016176240 A1 (PROCTER & GAMBLE) 03 November 2016 (2016-11-03) see the whole document	1-15
A	WO 2016176282 A1 (PROCTER & GAMBLE) 03 November 2016 (2016-11-03) see the whole document	1-15
A	WO 2017214244 A1 (PROCTER & GAMBLE) 14 December 2017 (2017-12-14) see the whole document	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

“D” document cited by the applicant in the international application

“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

20 November 2019

Date of mailing of the international search report

19 December 2019

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088
China

Authorized officer

ZHANG, Yanxia

Facsimile No. (86-10)62019451

Telephone No. 62089438

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/106223

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2019/106223

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2016176240	A1	03 November 2016	US	2016319225	A1	03 November 2016
				ZA	201706457	B	29 May 2019
				CN	107624127	A	23 January 2018
				EP	3088505	A1	02 November 2016
WO	2016176282	A1	03 November 2016	ES	2683568	T3	26 September 2018
				ZA	201706665	B	31 July 2019
				HU	E043043	T2	29 July 2019
				US	2016319224	A1	03 November 2016
				PL	3088503	T3	31 October 2018
				EP	3088503	A1	02 November 2016
				JP	2018517803	A	05 July 2018
				CN	107532116	A	02 January 2018
				DK	3088503	T3	20 August 2018
WO	2017214244	A1	14 December 2017	EP	3088503	B1	23 May 2018
				CN	109312272	A	05 February 2019
				CA	3022951	A1	14 December 2017
				US	2017355933	A1	14 December 2017
				JP	2019522074	A	08 August 2019
				EP	3469055	A1	17 April 2019