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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
07 September 2023 (07.09.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/165950 A1

(51) International Patent Classification:
C12N 9/22 (2006.01)

(21) International Application Number:
PCT/EP2023/054907

(22) International Filing Date:
28 February 2023 (28.02.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
22160258.4 04 March 2022 (04.03.2022) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title:** DNASE VARIANTS AND COMPOSITIONS

(57) **Abstract:** The present invention relates to novel DNase variants exhibiting alterations relative to a parent DNase that have been found to provide improved stability, e.g. improved detergent stability. The invention also relates to detergent compositions comprising the DNase variants, and methods for using and producing the DNase variants, as well as isolated DNA sequences encoding the variants, expression vectors and host cells. The variants of the present invention are suitable for use in cleaning processes and detergent compositions.

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DNASE VARIANTS AND COMPOSITIONS

Reference to a Sequence Listing

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

Background of the Invention

Field of the Invention

The present invention relates to novel DNase (deoxyribonuclease) variants exhibiting alterations relative to a parent DNase that have been found to provide improved stability, e.g. improved detergent stability. The invention also relates to detergent compositions comprising the DNase variants, and methods for using and producing the DNase variants, as well as isolated DNA sequences encoding the variants, expression vectors and host cells. The variants of the present invention are suitable for use in cleaning processes and detergent compositions, such as laundry compositions and dishwashing compositions.

Description of the Related Art

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

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

When laundry items are used, for example T-shirts and sportswear as well as other clothing items, they are exposed to bacteria from the body of the user and from the rest of the environment in which they are used. Some of these bacteria are capable of adhering to the laundry item and form a biofilm on the item. The presence of bacteria implies that the laundry items become sticky and therefore soil adheres to the sticky areas. This soil has been shown to be difficult to remove by commercially available detergent compositions, leading to challenging problems such as malodor and yellowing and greying of the items. Malodor arising from bacteria

can be difficult to remove and may remain even after wash, since bacteria may adhere to the textile surface, thereby continuing to be a source of malodor. Further, when very dirty laundry items are washed together with less dirty laundry items, the dirt present in the wash liquor tends to stick to the biofilm. As a result, the laundry items may be subjected to "redeposition" of soil during wash.

WO 2014/087011 discloses bacterial DNase polypeptides and methods for biofilm disruption and prevention. WO 2015/155350 discloses detergent compositions comprising certain DNase polypeptides obtained from a fungal source. WO 2017/064269 discloses variants of a fungal DNase from *Aspergillus oryzae* with improved stability. In spite these disclosures, there is still a need to further improve DNase enzymes, for example to further improve stability of the enzymes in detergent compositions.

The present invention provides DNase variants with improved properties compared to the parent DNase, in particular improved stability, obtained by way of combining certain substitutions that have been found by the inventors to be particularly advantageous.

Summary of the Invention

The present invention relates to isolated DNase variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 having substitutions in two or more of positions 32, 35, 69, 102, 105, 111 and 181, wherein position numbers are based on SEQ ID NO: 1.

The present invention also relates to compositions such as detergent compositions comprising a DNase variant disclosed herein as well as isolated polynucleotides encoding the variants; nucleic acid constructs, vectors, and host cells comprising the polynucleotides, and methods of producing the variants. The invention further relates to methods of using the DNase variants and compositions for cleaning, e.g. for laundry.

Brief Description of the Figures

Figure 1 is an alignment of the polypeptides of SEQ ID NOs: 1, 2 and 3.

Overview of Sequence Listing

SEQ ID NO: 1 mature DNase polypeptide obtained from *Aspergillus oryzae*

SEQ ID NO: 2 mature DNase polypeptide obtained from *Aspergillus oryzae* with truncated N-terminal

SEQ ID NO: 3 mature DNase polypeptide obtained from *Aspergillus oryzae* with truncated N-terminal

Definitions

In accordance with this detailed description, the following definitions apply. Note that the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise.

Unless defined otherwise or clearly indicated by context, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

DNase: The term "DNase" means a polypeptide having DNase (deoxyribonuclease) activity that catalyzes the hydrolytic cleavage of phosphodiester linkages in DNA, thus degrading DNA. DNases belong to the esterases (EC number 3.1), a subgroup of the hydrolases. The DNases are classified in EC 3.1.21. For purposes of the present invention, DNase activity may be determined according to the procedure described in Assay I. The terms "DNase" and "a polypeptide with DNase activity" may be used interchangeably throughout the application.

DNase variant: A "DNase variant" is a variant of the DNase of SEQ ID NO: 1, 2 or 3 having one or more individual mutations, typically substitutions, or combinations of substitutions.

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

Expression vector: An "expression vector" refers to a linear or circular DNA construct comprising a DNA sequence encoding a variant, which coding sequence is operably linked to a suitable control sequence capable of effecting expression of the DNA in a suitable host. Such control sequences may include a promoter to effect transcription, an optional operator sequence to control transcription, a sequence encoding suitable ribosome binding sites on the mRNA, enhancers and sequences which control termination of transcription and translation.

Extension: The term "extension" means an addition of one or more amino acids to the amino and/or carboxyl terminus of a variant, wherein the "extended" variant has DNase activity.

Fragment: The term "fragment" means a variant having one or more amino acids absent from the amino and/or carboxyl terminus of the variant; wherein the fragment has DNase activity. A fragment in the context of the present invention includes variants of SEQ ID NO: 1 in which one or more amino acids are absent from the amino terminus, for example SEQ ID NO: 2 in which 15 N-terminal amino acids are absent or SEQ ID NO: 3 in which 17 N-terminal amino acids are absent.

Host cell: A "host cell" is an organism into which an expression vector, phage, virus, or other DNA construct, including a polynucleotide encoding a variant, has been introduced. Exemplary host strains are microorganism cells (*e.g.*, bacteria, filamentous fung, and yeast) capable of expressing the polypeptide of interest.

Improved property: The term "improved property" means a characteristic associated with a variant that is improved compared to the parent. Such improved properties include, but are not limited to, catalytic efficiency, catalytic rate, chemical stability, oxidation stability, pH activity, pH stability, specific activity, stability under storage conditions, substrate binding, substrate cleavage, substrate specificity, substrate stability, surface properties, thermal activity, and thermostability. In the context of the present invention, the improved property is in particular

improved stability, for example improved storage stability, including improved storage stability in a detergent composition, improved stability in use in a detergent composition, and/or improved thermostability. Additionally, variants of the invention may also have other improved properties such as improved DNase activity, e.g. improved specific activity and/or improved wash performance.

Isolated: The term “isolated” means a polypeptide variant, nucleic acid, cell, or other specified material or component that is separated from at least one other material or component, including but not limited to, other proteins, nucleic acids, cells, etc. An isolated polypeptide, nucleic acid, cell or other material is thus in a form that does not occur in nature. An isolated polypeptide includes, but is not limited to, a culture broth containing the secreted variant expressed in a host cell. Typically, an isolated variant of the invention will be separated from other components in a culture broth using known protein purification methods.

Mature polypeptide: The term “mature polypeptide” means a polypeptide in its mature form following N-terminal processing and/or C-terminal processing (*e.g.*, removal of signal peptide).

It is known in the art that a host cell may produce a mixture of two of more different mature polypeptides (i.e., with a different C terminal and/or N terminal amino acid) expressed by the same polynucleotide. It is also known that different host cells process polypeptides differently, and thus, one host cell expressing a polynucleotide may produce a different mature polypeptide (e.g., having a different C terminal and/or N terminal amino acid) as compared to another host cell expressing the same polynucleotide. In the context of the present invention, the mature polypeptide is typically N-terminally processed by the host cell, so that the mature polypeptide may have fewer than the 221 amino acid residues of the mature polypeptide shown in SEQ ID NO: 1, for example 206 amino acids as shown in SEQ ID NO: 2 or 204 amino acids as shown in SEQ ID NO: 3.

Mature polypeptide coding sequence: The term “mature polypeptide coding sequence” means a polynucleotide that encodes a mature polypeptide having DNase activity.

Mutant: The term “mutant” means a polynucleotide encoding a variant.

Native: The term "native" means a nucleic acid or polypeptide naturally occurring in a host cell.

Nucleic acid: The term "nucleic acid" encompasses DNA, RNA, heteroduplexes, and synthetic molecules capable of encoding a variant. Nucleic acids may be single stranded or double stranded, and may be chemical modifications. The terms "nucleic acid" and "polynucleotide" are used interchangeably. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and the present compositions and methods encompass nucleotide sequences that encode a particular amino acid sequence. Unless otherwise indicated, nucleic acid sequences are presented in 5'-to-3' orientation.

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

Operably linked: The term "operably linked" means that specified components are in a relationship (including but not limited to juxtaposition) permitting them to function in an intended manner. For example, a regulatory sequence is operably linked to a coding sequence such that expression of the coding sequence is under control of the regulatory sequence.

Parent or parent DNase: The term “parent” or “parent DNase” means a DNase polypeptide to which an alteration is made to produce the DNase variants of the present invention. Thus, the parent is a DNase polypeptide having the identical amino acid sequence of a variant disclosed herein but not having the specified alterations, typically substitutions, disclosed herein.

In a particular embodiment the DNase parent is a DNase with 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 72%, at least 73%, at least 74%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% or 100% identity to a polypeptide having SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

The parent DNase may e.g. be obtained from a fungal species, e.g. an *Aspergillus* species such as *Aspergillus oryzae*.

In one embodiment, the parent DNase is a DNase having the sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. In a preferred embodiment, the parent DNase is the polypeptide of SEQ ID NO: 1.

Recombinant: The term "recombinant" is used in its conventional meaning to refer to the manipulation, *e.g.*, cutting and rejoining, of nucleic acid sequences to form constellations different from those found in nature. The term recombinant refers to a cell, nucleic acid, variant or vector that has been modified from its native state. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell, or express native genes at different levels or under different conditions than found in nature. The term “recombinant” is synonymous with “genetically modified” and “transgenic”.

Sequence identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter “sequence identity”.

For purposes of the present invention, the sequence identity between two amino acid sequences is determined as the output of “longest identity” using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open

Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 6.6.0 or later. The parameters used are a gap open penalty of 10, a gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. In order for the Needle program to report the longest identity, the -nobrief option must be specified in the command line. The output of Needle labeled "longest identity" is calculated as follows:

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

Sequence identity percentages may be referred to herein with reference to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. As explained above, it is known that a host cell may produce a mixture of two of more different mature polypeptides, and that different host cells process polypeptides differently. In the case of the mature polypeptide of SEQ ID NO: 1, it has been found that this may be expressed with different N-terminal truncations, for example as the polypeptide of SEQ ID NO: 2 (206 amino acid residues, corresponding to amino acids 16 to 221 of SEQ ID NO: 1) or SEQ ID NO: 3 (204 amino acid residues, corresponding to amino acids 18 to 221 of SEQ ID NO: 1). Since SEQ ID NOs: 1, 2 and 3 are identical other than truncation at the N-terminal, any reference herein to a polypeptide having a given percent sequence identity to SEQ ID NO: 1 should be understood as also including polypeptides having that percent sequence identity to SEQ ID NO: 2 or to SEQ ID NO: 3.

Signal peptide: A "signal peptide" is a sequence of amino acids attached to the N-terminal portion of a protein, which facilitates the secretion of the protein outside the cell. The mature form of an extracellular protein lacks the signal peptide, which is cleaved off during the secretion process.

Variant: The term "variant" means a polypeptide having DNase activity comprising a substitution, an insertion (including extension), and/or a deletion (*e.g.*, truncation), at one or more 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 1-5 amino acids (*e.g.*, 1-3 amino acids, in particular one amino acid) adjacent to and immediately following the amino acid occupying a position. The variants of the present invention include various substitititions compared to SEQ ID NO: 1 as described in detail herein.

Wild-type: The term "wild-type" in reference to an amino acid sequence or nucleic acid sequence means that the amino acid sequence or nucleic acid sequence is a native or naturally occurring sequence. As used herein, the term "naturally occurring" refers to anything (*e.g.*, proteins, amino acids, or nucleic acid sequences) that is found in nature. Conversely, the term "non naturally occurring" refers to anything that is not found in nature (*e.g.*, recombinant nucleic acids and protein sequences produced in the laboratory or by modification of the wild- type sequence).

Biofilm: A biofilm is any group of microorganisms in which cells stick to each other on a surface, such as a textile or a dishware or other hard surface. These adherent cells are frequently

embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS is a polymeric conglomeration generally composed of extracellular DNA, proteins and polysaccharides. Biofilms may form on living or non-living surfaces. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single cells that may float or swim in a liquid medium. Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. On laundry, biofilm producing bacteria can be found among the following species: *Acinetobacter sp., Aeromicrobium sp., Brevundimonas sp., Microbacterium sp., Micrococcus luteus, Pseudomonas sp., Staphylococcus epidermidis,* and *Stenotrophomonas sp.*

Improved DNase activity: The term "improved DNase activity" is defined herein as an altered DNase activity, e.g. by increased catalysis of hydrolytic cleavage of phosphodiester linkages in the DNA, the DNase variant displaying an alteration of the activity relative to the activity of the parent DNase, such as compared to a DNase with SEQ ID NO: 1. The improved activity may e.g. be improved specific activity.

Stability: The term "stability" includes storage stability and stability during use, e.g. during a wash process, and reflects the stability of the DNase variant according to the invention as a function of time, e.g. how much activity is retained when the DNase variant is kept in solution, in particular in a detergent solution. The stability is influenced by many factors such as pH, temperature, and the detergent composition, e.g. amount of builder, surfactants etc. The DNase stability may be measured as described in the examples and expressed e.g. as a melting temperature (Tm) or a half-life improvement factor (HIF) compared to the parent DNase or a reference sequence such as SEQ ID NO: 1. The term "improved stability" or "increased stability" is defined herein as a variant DNase displaying an increased stability in solutions, relative to the stability of the parent DNase without the substitutions in the variant and/or relative to SEQ ID NO: 1. The terms "improved stability" and "increased stability" include detergent stability.

Improved wash performance: The term "improved wash performance" may be defined as improved deep cleaning effect (where "deep cleaning" refers to the disruption or removal of a biofilm or components thereof) of a DNase variant according to the invention compared to the DNase parent or the DNase with SEQ ID NO: 1. The DNase variants may also have improved malodor removal. 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. Another example is unpleasant smells caused by sweat or body odor adhered to an item which has been in contact with a human or animal. Another example of malodor can be the odor from spices which stick to items, for example curry or other spices which smell

strongly. One way of measuring the ability of an item to adhere malodor is by using Assay II disclosed herein.

Wash performance may be expressed as a remission value of the stained swatches. After washing and rinsing the swatches are spread out flat and allowed to air dry at room temperature overnight. All washed swatches are evaluated the day after washing. Light reflectance evaluations of the swatches are done using a Macbeth Color Eye 7000 reflectance spectrophotometer with very small aperture. The measurements are made without UV in the incident light and remission value at 460 nm is extracted.

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

Detergent composition: The term "detergent composition" (or “cleaning composition”) includes unless otherwise indicated any form of detergent or cleaning composition. These include granular or powder-form all-purpose or heavy-duty washing agents, especially cleaning detergents; liquid, gel or paste-form all-purpose washing agents, especially the so-called heavy-duty liquid (HDL) types; single unit dose (SUD) compositions such as pods, capsules, tabs, etc. with one or more chambers; liquid fine-fabric detergents; hand dishwashing agents or light duty dishwashing agents, especially those of the high-foaming type; machine dishwashing agents, including the various tablet, granular, liquid and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, including antibacterial hand-wash types, cleaning bars, soap bars, mouthwashes, denture cleaners, car or carpet shampoos, bathroom cleaners; hair shampoos and hair-rinses; shower gels, foam baths; metal cleaners; as well as cleaning auxiliaries such as bleach additives and "stain-stick" or pre-treat types. The terms "detergent composition" and "detergent formulation" are used in reference to mixtures which are intended for use in a wash medium for the cleaning of soiled objects. In some embodiments, the term is used in reference to laundering fabrics and/or garments (e.g., "laundry detergents"). In alternative embodiments, the term refers to other detergents, such as those used to clean dishes, cutlery, etc. (e.g., "dishwashing detergents"). It is not intended that the present invention be limited to any particular detergent formulation or composition. The term “detergent composition” is not intended to be limited to compositions that contain surfactants. It is intended that in addition to the variants according to the invention, the term encompasses detergents that may contain, e.g., surfactants, builders, chelators or chelating agents, bleach system or bleach components, polymers, fabric conditioners, foam boosters, suds suppressors, dyes, perfume, tannish inhibitors, optical brighteners, bactericides, fungicides, soil suspending agents, anti-corrosion agents, enzyme inhibitors or stabilizers, enzyme activators, transferases, hydrolytic enzymes, oxido reductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers.

Fabric: The term "fabric" encompasses any textile material. Thus, it is intended that the term encompass garments, as well as fabrics, yarns, fibers, non-woven materials, natural materials, synthetic materials, and any other textile material.

Textile: The term "textile" refers to woven fabrics, as well as staple fibers and filaments suitable for conversion to or use as yarns, woven, knit, and non-woven fabrics. The term encompasses yarns made from natural, as well as synthetic (e.g., manufactured) fibers. The term "textile materials" is a general term for fibers, yarn intermediates, yarn, fabrics, and products made from fabrics (e.g., garments and other articles).

Non-fabric detergent compositions: The term "non-fabric detergent compositions" include non-textile surface detergent compositions, including but not limited to compositions for hard surface cleaning, such as dishwashing detergent compositions including manual dishwashing compositions, oral detergent compositions, denture detergent compositions, and personal cleansing compositions.

Effective amount of enzyme: The term "effective amount of enzyme" refers to the quantity of enzyme necessary to achieve the enzymatic activity required in the specific application, e.g., in a defined detergent composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and are based on many factors, such as the particular enzyme used, the cleaning application, the specific composition of the detergent composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. The term "effective amount" of a DNase variant refers to the quantity of DNase variant described hereinbefore that achieves a desired level of enzymatic activity, e.g., in a defined detergent composition.

Relevant washing conditions: The term "relevant washing conditions" is used herein to indicate the conditions, particularly washing temperature, time, washing mechanics, detergent concentration, type of detergent and water hardness, actually used in households in a detergent market segment.

Wash liquor: The term “wash liquor” (or “wash water”) refers to an aqueous solution comprising a DNase variant of the invention. A wash liquor is a solution, e.g. found in a washing machine or dishwasher, containing water and a detergent composition comprising the DNase variant. The detergent composition, prior to being mixed with water to form a wash liquor, may be in any suitable form as described elsewhere herein, for example a liquid or powder.

Water hardness: The term “water hardness” or “degree of hardness” or “dH” or “°dH” as used herein refers to German degrees of hardness. One degree is defined as 10 milligrams of calcium oxide per liter of water.

Adjunct materials: The term "adjunct materials" or “adjunct ingredients” means any liquid, solid or gaseous material selected for the particular type of detergent composition desired and the form of the product (e.g., liquid, granule, powder, bar, paste, spray, tablet, gel, or foam composition), which materials are also preferably compatible with the DNase variant enzyme used in the composition. More detailed information on adjunct materials is provided further below.

Low detergent concentration: The term "low detergent concentration" system includes detergents where less than about 800 ppm of detergent components is present in the wash water. Asian, e.g., Japanese detergents are typically considered low detergent concentration systems.

Medium detergent concentration: The term "medium detergent concentration" system includes detergents wherein between about 800 ppm and about 2000 ppm of detergent components is present in the wash water. North American detergents are generally considered to be medium detergent concentration systems.

High detergent concentration: The term "high detergent concentration" system includes detergents wherein greater than about 2000 ppm of detergent components is present in the wash water. European detergents are generally considered to be high detergent concentration systems.

Conventions for Designation of Variants

For purposes of the present invention, the polypeptide disclosed in SEQ ID NO: 1 is used to determine the corresponding amino acid positions in another DNase. The amino acid sequence of another DNase is aligned with the polypeptide disclosed in SEQ ID NO: 1, and based on the alignment, the amino acid position number corresponding to any amino acid residue in the polypeptide disclosed in SEQ ID NO: 1 is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix.

In describing the variants of the present invention, the nomenclature described below is adapted for ease of reference. The accepted IUPAC single letter or three letter amino acid abbreviation is employed.

Substitutions. For an amino acid substitution, the following nomenclature is used: Original amino acid, position, substituted amino acid. Accordingly, the substitution of threonine at position 226 with alanine is designated as "T226A" (or "Thr226Ala" using the three-letter code). Multiple mutations may be separated by addition marks ("+"), *e.g.*, "G205R + S411F", representing substitutions at positions 205 and 411 of glycine (G) with arginine (R) and serine (S) with phenylalanine (F), respectively. Multiple mutations may alternatively be indicated by a space, a comma, or a plus sign, e.g. "G205R S411F", "G205R, S411F" or "G205R+S411F".

Deletions. For an amino acid deletion, the following nomenclature is used: Original amino acid, position, *. Accordingly, the deletion of glycine at position 195 is designated as "G195*". Multiple deletions are separated by addition marks ("+"), *e.g.*, "G195* + S411*".

Insertions. For an amino acid insertion, the following nomenclature is used: Original amino acid, position, original amino acid, inserted amino acid. Accordingly, the insertion of lysine after glycine at position 195 is designated "G195GK". An insertion of multiple amino acids is

designated [Original amino acid, position, original amino acid, inserted amino acid #1, inserted amino acid #2; etc.]. For example, the insertion of lysine and alanine after glycine at position 195 is indicated as "G195GKA".

In such cases the inserted amino acid residue(s) are numbered by the addition of lower-case letters to the position number of the amino acid residue preceding the inserted amino acid residue(s). In the above example, the sequence would thus be:

Parent:	Variant:
195	195 195a 195b
G	G - K - A

Multiple alterations. Variants comprising multiple alterations are separated by addition marks ("+") as explained above, *e.g.*, "R170Y+G195E" representing a substitution of arginine and glycine at positions 170 and 195 with tyrosine and glutamic acid, respectively. Alternatively, the multiple alterations may be separated by a space or comma as mentioned above.

Different alterations. Where different alterations can be introduced at a position, the different alterations are separated by a comma, *e.g.*, "R170Y,E" represents a substitution of arginine at position 170 with tyrosine or glutamic acid. Thus, "Y167G,A + R170G,A" designates the following variants: "Y167G+R170G", "Y167G+R170A", "Y167A+R170G" and "Y167A+R170A".

Detailed Description of the Invention

The present invention provides novel DNase variants, in particular variants of DNases obtained from *Aspergillus*, including *Aspergillus oryzae*. The DNases of the present invention have at least 60% sequence identity to a polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and comprise at least two subsitutions, such as at least three substitutions, at positions selected from the group consisting of 111, 32, 35, 69, 102, 105 and 181 in SEQ ID NO: 1 as described herein.

In one embodiment, the invention provides DNase variants comprising three or more substitutions at positions selected from the group consisting of 26, 32, 35, 65, 67, 69, 98, 102, 105, 111, 115, 150, 157, 159, 161, 172, 181, 182, 185, 187, 192, 206, 208 and 212, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60% but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity.

More particularly, the variant may comprise three or more substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60% but less than 100% to the

polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity. In one aspect, the variant comprises two or more substitutions, such as three or more substitutions, selected from the group consisting of A111P, D32E, V35I, S69V, Q102E, K105N and G181N, and optionally one or more of the other substitutions listed above.

The invention also provides compositions, in particular detergent compositions such as laundry compositions, comprising the DNase variants, and methods for using the DNase variants. Further provided are isolated DNA sequences encoding the variants, expression vectors and host cells, as well as methods for producing the DNase variants.

Variants

As mentioned above, in one embodiment the DNase variants of the invention comprise three or more substitutions at positions selected from the group consisting of 26, 32, 35, 65, 67, 69, 98, 102, 105, 111, 115, 150, 157, 159, 161, 172, 181, 182, 185, 187, 192, 206, 208 and 212, in particular three or more substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, wherein the numbering is based on SEQ ID NO: 1. In some embodiments, the variant may comprise four, five or more of said substitutions.

More particularly, the invention relates in one aspect to a DNase variant comprising at least two substitutions, such as at least three substitutions, at positions selected from the group consisting of 111, 32, 35, 102, 105 and 181, in particular at least two substitutions selected from the group consisting of A111P, D32E, V35I, S69V, Q102E, K105N and G181N, for example at least three of said substitutions, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60% but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity.

In certain preferred embodiments, the variant comprises the substitution A111P. Preferably, variants of this embodiment comprise the substitution A111P and two or more substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

Among such DNase variants with the substitution A111P, preferred variants include those that also comprise the substitutions D32E+V35I and/or the substitution G181N. Other preferred embodiments of variants with the substitution A111P include those comprising the substitution S69V and/or Q102E, as well as those comprising the substitution K105N.

In other embodiments, the variant comprises the substitution K105N, preferably in combination with the substitution A111P. Variants of this embodiment may further comprise at least one additional substitution among those listed above, i.e. one or more of S26H, D32E, V35I,

K65E, K67A, S69E, S69V, S98R, Q102E, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, for example two or more of said additional substitutions.

In still other embodiments, the variant comprises the substitution S69V and/or Q102E, preferably S69V+Q102E. Variants of this embodiment may further comprise at least one additional substitution among those listed above, i.e. one or more of S26H, D32E, V35I, K65E, K67A, S98R, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, for example two or more of said additional substitutions.

In one preferred embodiment, the variant comprises the substitutions D32E+V35I+A111P. Variants of this embodiment may further comprise at least one additional substitution among those listed above, i.e. one or more of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, for example two or more of said additional substitutions.

In another preferred embodiment, the variant comprises the substitutions A111P+G181N. Variants of this embodiment may further comprise at least one additional substitution among those listed above, i.e. one or more of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, for example two or more of said additional substitutions.

In a further preferred embodiment, the variant comprises the substitutions D32E+V35I+A111P+G181N. Variants of this embodiment may further comprise at least one additional substitution among those listed above, i.e. one or more of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions D32E+V35I+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions D32E+V35I+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions D32E+V35I+S69V+Q102E+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions D32E+V35I+S69V+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions S69V+Q102E+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions S69V+Q102E+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions S69V+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions Q102E+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions S69V+Q102E+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

In another embodiment, the variant comprises the substitutions D32E+V35I+K105N+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

Examples of preferred DNase variants of the invention include those comprising one of the following sets of substitutions, and optionally one or more additional substitutions disclosed herein:

- D32E+V35I+A111P
- D32E+V35I+A111P+G181N
- D32E+V35I+Q102E+A111P+G181N
- D32E+V35I+K105N+A111P+G181N

- D32E+V35I+S69V+Q102E+A111P
- D32E+V35I+S69V+Q102E+A111P+G181N
- S69V+Q102E+A111P
- S69V+Q102E+G181N
- S69V+Q102E+A111P+G181N
- Q102E+K105N+A111P+G181N
- S69V+Q102E+K105N+A111P+G181N
- D32E+V35I+K105N+A111P

The variant may for example comprise or consist of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with one of these sets of substitutions.

In any of the embodiments disclosed above or elsewhere herein, the DNase variant may further comprise one or more additional alterations, e.g. one or more additional substitutions other than those disclosed above. Non-limiting examples of such additional substitutions include any one or more of K18S, T19P, D32P, K86E, A101E, A101T, K105D, K105E, K105T, G137R, N146H, K147N, K155E, A172D, K178S, K192A, N214D and N217A.

Examples of specific DNase variants of the invention include variants comprising a set of substitutions selected from the group consisting of:

- S69V+K86E+Q102E+K147N+K155E+A172D+K192A+Q208V;
- G20C+K30C+D32E+V35I+A111P;
- S69V+K86E+Q102E+K147N+K155E+A172D+G181N+K192A+Q208V;
- D32E+V35I+N77E+A111P+G181N+S182V;
- D32E+E34N+V35I+K86E+A111P+G181N;
- S69V+K86E+Q102E+K105T+K147N+K155E+A172D+K192A+Q208V;
- S69V+Q102E+Q157E+T159Q+N214D+N217A;
- S26H+D32E+V35I+A111P+K155L;
- K18S+D32E+V35I+A111P+K185I+K215T;
- D32E+V35I+A111P+K185I+K215T;
- D32E+V35I+K82G+A111P+G181N+S182V;
- D32E+V35I+A111P+K178M+K212T;
- K18S+D32E+V35I+A111P+K178S+K212L;
- S69V+Q102E+V138A+S144R+Q157E+T159Q+A172E+G181N;
- D32E+V35I+A111P+K204L+K215I;
- K18F+D32E+V35I+A111P+K185Q;
- D32E+V35I+A111P+K147N+K204L;
- S69V+K86E+Q102E+K105D+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+S69V+K86E+Q102E+A111P+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;

- D32E+V35I+A111P+K147N;
- S69V+K86E+Q102E+S115T+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P+N140H;
- D32E+V35I+K65Q+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+K178S+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P;
- E6D+A101E+Q150E+G181N+Q208V;
- S26H+D32E+V35I+A111P;
- K18Q+D32E+V35I+K105E+A111P+K155F;
- D32E+V35I+A111P+G199E;
- S69V+Q102E+Q157E+T159Q+G181N;
- D32E+V35I+K67A+A111P+P175N+S182V;
- D32E+V35I+A111P+N214D;
- S69V+Q102E+Q157E+T159Q+A172E;
- E6D+A101E+Q150E+G181N+Q208V;
- D32E+V35I+S69E+A111P;
- D32E+V35I+Q85E+A111P;
- K18Q+D32E+V35I+A111P;
- D32E+V35I+A101E+K105T+A111P+G181N+K212E+Y218E;
- D32E+V35I+A111P+G181N+S182V+K185E;
- D32E+V35I+S98R+A111P+G181N+S182Y;
- D32E+V35I+A101E+Q102E+A111P+G181N;
- T19P+D32E+V35I+Q102E+K105E+A111P;
- D32E+V35I+D72V+A111P;
- S69V+K86E+Q102E+F112W+K147N+K155E+A172D+K192A+Q208V;
- S69V+Q102E+A111P+Q157E+T159Q+A172E;
- K67A+S69V+Q102E+K147N+Q157E+T159Q+A172E+D197S;
- S69V+Q102E+S115T+Q157E+T159Q+A172E;
- S69V+K86E+Q102E+G136E+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+K67A+A111P+K204G;
- A101E+A111P+Q150E+Q208V;
- D32E+V35I+A101E+K105E+A111P+S182V;
- D32E+V35I+A101E+K105E+A111P+S182V;

- D32E+V35I+A101T+K105N+A111P+K147E+G181N+S182Y;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+Q102E+A111P;
- S26H+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+K67A+K105T+A111P+K178L+K185T;
- K67A+K105T+G181N;
- T19P+D32E+V35I+A101E+K105T+A111P+K160A+G181N;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+Q102E+A111P;
- K18S+D32E+V35I+Q102E+K105E+A111P;
- D32E+V35I+K65E+A101E+K105T+A111P+N217A;
- D32E+V35I+K105T+A111P+G181N+K204W+K215L;
- D32E+V35I+K105T+A111P+K178L+G181N+K212T;
- S26H+S69V+Q102E+A111P+N146H+Q150E+Q157E+T159Q+A172E;
- K18Q+D32E+V35I+K67A+K105T+A111P+K215V;
- D32E+V35I+Q85E+A111P;
- K18S+T19P+D32E+V35I+A101E+K105T+A111P+G181N;
- D32E+V35I+A101E+K105N+A111P;
- K18M+D32E+V35I+K67A+K105T+A111P+K178S;
- D32E+V35I+K105T+A111P+G181N;
- D32E+V35I+K67A+K105T+A111P+K185G+K212M;
- D32E+V35I+A101T+K105N+A111P+G181N+S182Y+N214D;
- T19P+D32E+V35I+A101E+K105T+A111P+K178S+G181N;
- D32E+V35I+K105T+A111P+G181N+K185L+K192V+K204W;
- D32E+V35I+S98R+K105N+A111P+K147N+G181N+S182Y;
- D32E+V35I+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+K105T+A111P;
- D32E+V35I+K53F+K105N+A111P+G181N+S182Y+K212E;
- K18S+D32E+V35I+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+S98R+K105N+A111P+Q158D+G181N+S182Y;
- D32E+V35I+K67A+K105T+A111P+K190V+K204G;
- N4K+D32E+V35I+S98R+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+K67A+K105T+A111P+K147N+K190N;
- D32E+V35I+S98R+K105N+A111P+G181N+S182Y+Q208V;
- D32E+V35I+A101E+K105T+A111P+G181N+K212E+N214D;
- D32E+V35I+K82T+A101E+K105T+A111P+G181N+K212E;

- D32E+V35I+A111P+S115T;
- S26H+S69V+Q102E+A111P+K147N+A149S+Q150E+Q157E+T159Q+A172E;
- K18S+D32E+V35I+K67A+K105T+A111P+K185L+K192F;
- V12L+D32E+V35I+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+S69E+K105N+A111P+K147E+G181N+S182Y;
- K18S+D32E+V35I+A101E+K105T+A111P+G181N+K212E;
- D32E+V35I+K65E+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+S98R+K105N+A111P+G181N+S182Y+D189N+N214D;
- D32E+V35I+S98R+A101T+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+A111P+Q157E+T159Q+G181N+A206G+Q208V;
- D32E+V35I+K67A+S69R+A111P+G181N+K212E;
- D32E+V35I+K67A+A111P+G181N+A206G+Q208V;
- D32E+V35I+A101E+K105T+A111P+G181N;
- D32E+V35I+K67A+A101E+K105D+A111P+N217A;
- D32E+V35I+K67A+A111P+Q157E+G181N+Q208V;
- D32E+V35I+K67A+A111P+Q157E+A172E+G181N+A206G+Q208V;
- S26H+D32E+V35I+K67A+S69R+A111P+G181N+K212E;
- D32E+V35I+S69E+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+K105D+A111P+S115T;
- D32E+V35I+S69V+K86E+Q102E+K105N+A111P+S115T+K147N+Q150E+K155E+T159Q+G161R+A172D+G181N+V187N+K192A+Q208V+N217A;
- D32E+V35I+K65E+K67A+K105N+A111P+G181N+S182Y;
- S69V+Q102E+K105N+A111P+S115T+V138M+Q150E+G161R+G181N+V187N+K192I;
- D32E+V35I+K67A+S69V+K105T+A111P+Q157E+G181N+A206G+Q208V;
- S69V+Q102E+K105N+A111P+S115T+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+Q150E+K160T+G161R+G181N+V187N+K192I;
- S69V+D72S+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32E+V35I+S69V+K86E+Q102E+K105N+A111P+S115T+V127T+G137R+K147N+Q150E+K155E+T159Q+G161R+A172D+G181N+V187N+K192A+Q208V+N217A;
- S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+S134H+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- D32P+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;

- D32P+S69V+Q102E+K105N+A111P+S115T+T120H+N146H+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+Q150E+Q157E+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- S26H+D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- K65Q+S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- S69A+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+A172D+G181N+V187N+K192I;
- D32P+S69A+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+K192I;
- D32P+S69V+Q102E+K105D+A111P+S115T+Q150E+G161R+G181N+V187N+K192I; and
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I.

The variant may for example comprise or consist of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with one of these sets of substitutions.

In a preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions D32E+V35I+A111P.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions D32E+V35I+K105N+A111P+G181N+S182Y.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions S26H+S69V+Q102E+A111P+Q157E+T159Q+A172E.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions D32E+V35I+K67A+A111P+G181N+A206G+Q208V.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions D32E+V35I+A111P+G181N+S182V+K185E.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions S69V+Q102E+ K105N+A111P+S115T+G161R+G181N+V187N+K192I.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions D32E+V35I+ S69E+S98R+K105N+A111P+G181N+S182Y.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions D32E+V35I+ K65E+K105N+A111P+G181N+S182Y+K212E.

In another preferred embodiment, the DNase variant of the invention comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with the substitutions D32E+V35I+ K65E+K67A+K105N+A111P+G181N+S182Y.

The DNase variants disclosed herein have a sequence identity to the parent DNase without the substitutions in the variant of at least 60%. The DNase variants may *e.g.* have a sequence identity to the parent DNase of at least 65%, at least 70%, at least 75% or at least 80%. The DNase variants may for example have a sequence identity to the parent DNase of at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94% or at least 95%, for example at least 96%, at least 97% or at least 98%, but less than 100%.

In particular, any of the DNase variants disclosed herein have a sequence identity to SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 of at least 60%. The DNase variants may *e.g.* have a sequence identity to any of SEQ ID NOs: 1, 2 or 3 of at least 65%, at least 70%, at least 75% or at least 80%. The DNase variants may for example have a sequence identity to any of SEQ ID NOs: 1, 2 or 3 of at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94% or at least 95%, for example at least 96%, at least 97% or at least 98%, but less than 100%.

In one aspect, the number of alterations in the variants of the present invention compared to SEQ ID NO: 1, 2 or 3 is 1-20, *e.g.*, 1-10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 alterations.

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

Examples of conservative substitutions are within the groups of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter

specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, in *The Proteins*, Academic Press, New York. Common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

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

Essential amino acids in a polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant molecules are tested for DNase activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identity of essential amino acids can also be inferred from an alignment with a related polypeptide, and/or be inferred from sequence homology and conserved catalytic machinery with a related polypeptide or within a polypeptide or protein family with polypeptides/proteins descending from a common ancestor, typically having similar three-dimensional structures, functions, and significant sequence similarity. Additionally or alternatively, protein structure prediction tools can be used for protein structure modelling to identify essential amino acids and/or active sites of polypeptides. See, for example, Jumper *et al.*, 2021, "Highly accurate protein structure prediction with AlphaFold", *Nature* 596: 583-589.

Variants of the invention may e.g. consist of 195 to 230 amino acids, preferably 200 to 225 amino acids, such as 204 to 221 amino acids. The variants may optionally comprise an extension of one or more amino acids at the N-terminal and/or C-terminal ends, or a truncation of one or more amino acids at the N-terminal and/or C-terminal ends. For example, the variants may include fragments in which a number of N-terminal amino acids are absent compared to SEQ ID NO: 1, for example variants of SEQ ID NO: 2 or SEQ ID NO: 3.

In an embodiment, the variant has improved stability, in particular improved storage stability, i.e. improved stabilty under storage in a detergent composition compared to the parent enzyme. As mentioned above, the improved stability may be expressed e.g. by way of a melting temperature (Tm) or a half-life improvement factor (HIF) as described in the examples herein.

In an embodiment, the DNase variant has improved stability expressed as thermostability compared to the parent DNase. Preferably, the variant has a melting temperature (Tm), e.g.

determined as described in the examples herein, which is at least 1°C higher than that of the parent, for example at least 2°C, at least 3°C or at least 4°C higher than the Tm of the parent. In preferred embodiments, the variant may have a melting temperature which is at least 5°C, at least 6°C, at least 7°C, at least 8°C, at least 9°C or at least 10°C higher than the Tm of the parent.

In an embodiment, the DNase variant has an improved stability expressed as half-life improvement factor (HIF) compared to the parent DNase, i.e. an HIF value greater than 1. Preferably, the variant has half-life improvement factor compared to the parent, e.g. determined as described in the examples herein, of at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9 or at least 2.0. In preferred embodiments, the variant may have a half-life improvement factor compared to the parent of at least 2.5, at least 3.0, at least 3.5 or at least 4.0.

The DNase variants of the invention are preferably isolated, more preferably purified, using standard protein purification methods known in the art.

Preparation of Variants

The present invention also relates to methods for obtaining a variant having DNase activity with the substitutions disclosed herein. In one embodiment the method comprises (a) introducing into a parent DNase at least two subsitutions, such as at least three substitutions, at positions selected from the group consisting of 111, 32, 35, 69, 102, 105 and 181, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60%, e.g. at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity; and (b) recovering the variant.

In another embodiment the method comprises (a) introducing into a parent DNase three or more substitutions at positions selected from the group consisting of 26, 32, 35, 65, 67, 69, 98, 102, 105, 111, 115, 150, 157, 159, 161, 172, 181, 182, 185, 187, 192, 206, 208 and 212, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60%, e.g. at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity; and (b) recovering the variant.

In another embodiment the method comprises (a) introducing into a parent DNase three or more substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60%, e.g. at least 65%, at least 70%, at least 75%, at least 80%, at least 85%,

at least 90% or at least 95%, but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity; and (b) recovering the variant.

In another embodiment the method comprises (a) introducing into a parent DNase at least two substitutions, such as at least three substitutions, selected from the group consisting of A111P, D32E, V35I, S69V, Q102E, K105N and G181N, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60%, e.g. at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity; and (b) recovering the variant.

In other embodiments, the method comprises (a) introducing into a parent DNase any of the sets of substitutions listed above under the heading “Variants”, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60%, e.g. at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity; and (b) recovering the variant.

The variants can be prepared using any mutagenesis procedure known in the art, such as site-directed mutagenesis, synthetic gene construction, semi-synthetic gene construction, random mutagenesis, DNA shuffling, etc.

Polynucleotides

The present invention also relates to polynucleotides encoding a variant of the present invention.

The polynucleotide may be a genomic DNA, a cDNA, a synthetic DNA, a synthetic RNA, a mRNA, or a combination thereof.

In an aspect, the polynucleotide is isolated, preferably purified.

Nucleic Acid Constructs

The present invention also relates to nucleic acid constructs comprising a polynucleotide encoding a variant 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. Examples of control sequences that may be used are promoters, terminators, mRNA stabilizers, leader sequences, polyadenylation sequences, signal peptides, propeptides, regulatory sequences and transcription factors, all of which are well known in the art.

The polynucleotide may be manipulated in a variety of ways to provide for expression of a variant. 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.

Expression Vectors

The present invention also relates to recombinant expression vectors comprising a polynucleotide encoding a variant 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 variant 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. Expression vectors suitable for recombinant expression are well known in the art, as are e.g. methods for introducing them into a host cell.

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 variant 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 choice of a host cell will to a large extent depend upon the gene encoding the variant and its source. The recombinant host cell may comprise a single copy, or at least two copies, *e.g.*, three, four, five, or more copies of the polynucleotide of the present invention.

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

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

The bacterial host cell may be any *Bacillus* cell including, but not limited to, *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus*

megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, and *Bacillus thuringiensis* cells.

A fungal host cell may be a yeast cell or a filamentous fungal cell.

The filamentous fungal host cell may e.g. be an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Bjerkandera*, *Ceriporiopsis*, *Chrysosporium*, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trametes*, or *Trichoderma* cell. In a preferred embodiment, the filamentous fungal host cell is an *Aspergillus*, *Trichoderma* or *Fusarium* cell. In a further preferred embodiment, the filamentous fungal host cell is an *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei,* or *Fusarium venenatum* cell.

In an aspect, the host cell is isolated, preferably purified.

Methods of Production

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

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

The variant may be detected using methods known in the art that are specific for the variant, including, but not limited to, the use of specific antibodies, formation of an enzyme product, disappearance of an enzyme substrate, or an enzyme assay determining the relative or specific activity of the variant.

The variant may be recovered from the medium using methods known in the art, including, but not limited to, collection, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation. In one aspect, the whole fermentation broth is recovered. In another aspect, a cell-free fermentation broth comprising the polypeptide is recovered.

The variant may be purified by a variety of procedures known in the art to obtain substantially pure variants and/or fragments (see, *e.g.*, Wingfield, 2015, *Current Protocols in Protein Science*; 80(1): 6.1.1-6.1.35; Labrou, 2014, *Protein Downstream Processing*, 1129: 3-10).

In an alternative aspect, the variant is not recovered.

Compositions

The present invention further relates to cleaning compositions comprising at least one DNase variant according to the invention and at least one cleaning adjunct ingredient. The cleaning composition may be used for improving deep-cleaning effect, including but not limited to deep cleaning of an item, for preventing and/or reducing the stickiness of an item, for pretreating stains on the item, for preventing and/or reducing redeposition of soil during a wash cycle, for preventing and/or reducing adherence of soil to an item, for maintaining or improving the whiteness of an item and for preventing and/or reducing malodor from an item. The DNase variants of the invention are useful in powder and liquid cleaning compositions as well as in e.g. single unit dose compositions.

The composition may contain one or more cleaning adjunct ingredients selected from the group consisting of surfactants, builders, flocculating aid, chelating agents, dye transfer inhibitors, enzymes, enzyme stabilizers, enzyme inhibitors, catalytic materials, bleach activators, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, builders and co-builders, fabric hueing agents, anti-foaming agents, dispersants, processing aids, and/or pigments.

The cleaning composition will typically contain a surfactant and normally other cleaning adjunct ingredients such as a builder or a clay/soil removal/anti-redeposition agent.

The cleaning adjunct ingredient may be one or more enzymes other than a DNase. The one or more enzymes may e.g. be selected from the group consisting of proteases, amylases, lipases, cutinases, cellulases, endoglucanases, xyloglucanases, pectinases, pectin lyases, xanthanases, peroxidases, haloperoxygenases, catalases and mannanases. Specific enzymes suitable for the detergent compositions of the invention are described below.

The cleaning composition may be formulated in any suitable form, such as a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid. The cleaning composition can thus e.g. be a liquid detergent or a powder or granular detergent, optionally in “concentrated” or "compact" form. It may also be in the form of a single unit dose composition.

The amount of DNase in the cleaning composition may vary depending on factors such as the degree of concentration or compactness of the composition and the desired DNase concentration in the wash liquor. The DNase will normally be included in the cleaning composition in an amount of up to about 10,000 ppm, typically up to about 5000 ppm or up to about 2000 ppm. The DNase can e.g. be included in the cleaning composition at a level of from 1 ppm to 10,000 ppm, such as from 10 ppm to 5000 ppm, from 20 ppm to 2000 ppm, from 50 ppm to 1000

ppm, from 80 ppm to 600 ppm, or from 100 ppm to 500 ppm. The unit "ppm" in this context is intended to refer to mg/l for an enzyme added to a liquid composition (e.g. liquid, gel, etc.), or mg/kg for an enzyme added to a solid composition (e.g. powder, granulate, tablet, etc.)

In some aspects, the detergent composition is a liquid or powder laundry detergent, suitable for e.g. washing at high temperature and/or pH, such as at or above 40ºC and/or at or above pH 8. In some aspects, the detergent composition is a liquid or powder laundry detergent, suitable for e.g. washing at low temperature and/or pH, such as at or below 20ºC and/or pH 6. The detergent may also be formulated as a unit dose detergent and/or compact detergent optionally with minimum or no water. The detergent may also be a dish wash detergent. The laundry and dish wash detergents may be phosphate-free.

Surfactants

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

The anionic surfactants are preferably added to the detergent in the form of salts. Suitable cations in these salts are alkali metal ions, such as sodium, potassium and lithium and ammonium salts, for example (2-hydroxyethyl) ammonium, bis(2-hydroxyethyl) ammonium and tris(2-hydroxyethyl) ammonium salts. Non-limiting examples of nonionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxylated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxylated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxyalkyl fatty acid amides, or N-acyl N-alkyl derivatives of glucosamine (glucamides, GA,

or fatty acid glucamides, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof. Commercially available nonionic surfactants include Plurafac™, Lutensol™ and Pluronic™ from BASF, Dehypon™ series from Cognis and Genapol™ series from Clariant.

Builders

A builder is preferably selected among phosphates, sodium citrate builders, sodium carbonate, sodium silicate, sodium aluminosilicate (zeolite). Suitable builders are alkali metal or ammonium phosphates, polyphosphates, phosphonates, polyphosphates, carbonates, bicarbonates, borates, citrates, and polycarboxylates. Citrate builders, e.g., citric acid and soluble salts thereof (particularly sodium salt), are polycarboxylate builders. Citrates can be used in combination with zeolite, silicates like the BRITESIL types, and/or layered silicate builders. The builder is preferably added in an amount of about 0-65% by weight, such as about 5% to about 50% by weight. In a laundry detergent, the level of builder is typically about 40-65% by weight, particularly about 50-65% by weight, particularly from 20% to 50% by weight. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder known in the art for use in cleaning detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (e.g., SKS-6 from Hoechst), and (carboxymethyl)inulin (CMI), and combinations thereof. Further non-limiting examples of builders include citrate, chelators such as aminocarboxylates, aminopolycarboxylates and phosphonates, and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2"-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-N,N'-disuccinic acid (EDDS), methylglycine-N,N-diacetic acid (MGDA), glutamic acid-N,N-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid, N-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-N-monoacetic acid (ASMA), aspartic acid-N,N-diacetic acid (ASDA), aspartic acid-N-monopropionic acid (ASMP), iminodisuccinic acid (IDA), N-(sulfomethyl)aspartic acid (SMAS), N-(2-sulfoethyl)-aspartic acid (SEAS), N-(sulfomethylglutamic acid (SMGL), N-(2-sulfoethyl)-glutamic acid (SEGL), N-methyliminodiacetic acid (MIDA), serine-N,N-diacetic acid (SEDA), isoserine-N,N-diacetic acid (ISDA), phenylalanine-N,N-diacetic acid (PHDA), anthranilic acid-N,N-diacetic acid (ANDA), sulfanilic acid-N,N-diacetic acid (SLDA) , taurine-N,N-diacetic acid (TUDA) and N´-(2-hydroxyethyl)ethylenediamine-N,N,N'-triacetic acid (HEDTA), diethanolglycine (DEG), and combinations and salts thereof. Phosphonates suitable for use herein include 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetrakis (methylenephosphonicacid) (EDTMPA), diethylenetriaminepentakis (methylenephosphonic acid) (DTMPA or DTPMPA or DTPMP), nitrilotris (methylenephosphonic acid) (ATMP or NTMP), 2-phosphonobutane-1,2,4-tricarboxylic acid

(PBTC), hexamethylenediaminetetrakis (methylenephosphonic acid) (HDTMP). The composition may also contain 0-50% by weight, such as about 5% to about 30%, of a detergent co-builder. The detergent composition may include a co-builder alone, or in combination with a builder, for example a zeolite builder. Non-limiting examples of co-builders include homopolymers of polyacrylates or copolymers thereof, such as poly (acrylic acid) (PAA) or copoly (acrylic acid/maleic acid) (PAA/PMA) or polyaspartic acid. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053. In some aspects, the builder is a non-phosphorus based builder such as citric acid and/or methylglycine-N, N-diacetic acid (MGDA) and/or glutamic-N, N-diacetic acid (GLDA) and/or salts thereof. The liquid composition may also be phosphate free in that instance the preferred builders includes citrate and/or methylglycine-N, N-diacetic acid (MGDA) and/or glutamic-N, N-diacetic acid (GLDA) and / or salts thereof.

Bleach components

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

Sources of hydrogen peroxide: Suitable sources of hydrogen peroxide are inorganic persalts, including alkali metal salts such as sodium percarbonate and sodium perborates (usually mono- or tetrahydrate), and hydrogen peroxide—urea (1/1).

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

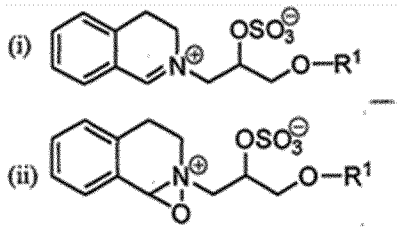
a) Suitable preformed peracids include, but are not limited to, peroxycarboxylic acids such as peroxybenzoic acid and its ring-substituted derivatives, peroxy-α-naphthoic acid, peroxyphthalic acid, peroxylauric acid, peroxystearic acid, ε-phthalimidoperoxycaproic acid [phthalimidoperoxyhexanoic acid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

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

(decanoyloxy)benzene-1-sulfonate, 4-(decanoyloxy)benzoic acid (DOBA), sodium 4-(nonanoyloxy)benzene-1-sulfonate (NOBS), and/or those disclosed in WO98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particularly preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that they are environmentally friendly. Furthermore, acetyl triethyl citrate and triacetin have good hydrolytical stability in the product upon storage and are efficient bleach activators. Finally, ATC is multifunctional, as the citrate released in the perhydrolysis reaction may function as a builder.

Bleach catalysts and boosters: The bleaching system may also include a bleach catalyst or booster. Some non-limiting examples of bleach catalysts that may be used in the compositions of the present invention include manganese oxalate, manganese acetate, manganese-collagen, cobalt-amine catalysts and manganese triazacyclononane (MnTACN) catalysts; particularly preferred are complexes of manganese with 1,4,7-trimethyl-1,4,7-triazacyclononane (Me3-TACN) or 1,2,4,7-tetramethyl-1,4,7-triazacyclononane (Me4-TACN), in particular Me3-TACN, such as the dinuclear manganese complex [(Me3-TACN)Mn(O)3Mn(Me3-TACN)](PF6)2, and [2,2',2''-nitrilotris(ethane-1,2-diylazanylylidene-κN-methanylylidene)triphenolato-κ3O]manganese(III). The bleach catalysts may also be other metal compounds, such as iron or cobalt complexes. Other suitable bleach catalysts are acylhydrazone catalysts such as those disclosed in US 2014/0323381.

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



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

Other exemplary bleaching systems are described, e.g. in WO 2007/087258, WO 2007/087244, WO 2007/087259, EP 1867708 (Vitamin K) and WO 2007/087242. Suitable photobleaches may for example be sulfonated zinc or aluminium phthalocyanines.

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

are categorized by general header according to a 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, including the exemplary non-limiting components shown in below.

Hydrotropes

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

Polymers

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

Fabric hueing agents

The detergent composition of the present invention may also include fabric hueing agents such as dyes or pigments, which when formulated in detergent compositions can deposit onto a fabric when said fabric is contacted with a wash liquor comprising said detergent compositions and thus altering the tint of said fabric through absorption/reflection of visible light. Fluorescent whitening agents emit at least some visible light. In contrast, fabric hueing agents alter the tint of a surface as they absorb at least a portion of the visible light spectrum. Suitable fabric hueing

agents include dyes and dye-clay conjugates, and may also include pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct Blue, Direct Red, Direct Violet, Acid Blue, Acid Red, Acid Violet, Basic Blue, Basic Violet and Basic Red, or mixtures thereof, for example as described in WO 2005/03274, WO 2005/03275, WO 2005/03276 and EP 1876226 (hereby incorporated by reference). The detergent composition preferably comprises from about 0.00003 wt% to about 0.2 wt%, from about 0.00008 wt% to about 0.05 wt%, or even from about 0.0001 wt% to about 0.04 wt% fabric hueing agent. The composition may comprise from 0.0001 wt% to 0.2 wt% fabric hueing agent, this may be especially preferred when the composition is in the form of a unit dose pouch. Suitable hueing agents are also disclosed in, e.g. WO 2007/087257 and WO 2007/087243.

Enzymes

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

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

Cellulases

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

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and WO 99/001544.

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

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S) Carezyme Premium™ (Novozymes A/S), Celluclean™ (Novozymes A/S), Celluclean Classic™ (Novozymes A/S), Cellusoft™ (Novozymes A/S), Whitezyme™ (Novozymes A/S),

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

Proteases

Suitable proteases may be of any origin, but are preferably of bacterial or fungal origin, optionally in the form of protein engineered or chemically modified mutants. The protease 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 a subtilisin. A metalloprotease may for example be a thermolysin, e.g. from the M4 family, or another metalloprotease such as those from the M5, M7 or M35 families.

The term "subtilases" refers to a sub-group of serine proteases according to Siezen et al., *Protein Eng.* 4 (1991) 719-737 and Siezen et al., *Protein Sci.* 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 six subdivisions, the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

Although proteases suitable for detergent use may be obtained from a variety of organisms, including fungi such as *Aspergillus*, detergent proteases have generally been obtained from bacteria and in particular from *Bacillus*. Examples of *Bacillus* species from which subtilases have been derived include *Bacillus lentus, Bacillus alkalophilus, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus pumilus* and *Bacillus gibsonii*. Particular subtilisins include *subtilisin lentus*, *subtilisin* Novo, *subtilisin* Carlsberg, *subtilisin* BPN', *subtilisin* 309, *subtilisin* 147 and *subtilisin* 168 and e.g. protease PD138 (described in WO 93/18140). Other useful proteases are e.g. those described in WO 01/16285 and WO 02/16547.

Examples of trypsin-like proteases include the *Fusarium* protease described in WO 94/25583 and WO 2005/040372, and the chymotrypsin proteases derived from *Cellumonas* described in WO 2005/052161 and WO 2005/052146.

Examples of metalloproteases include the neutral metalloproteases described in WO 2007/044993 such as those derived from *Bacillus amyloliquefaciens*, as well as e.g. the metalloproteases described in WO 2015/158723 and WO 2016/075078.

Examples of useful proteases are the protease variants described in WO 89/06279 WO 92/19729, WO 96/34946, WO 98/20115, WO 98/20116, WO 99/11768, WO 01/44452, WO 03/006602, WO 2004/003186, WO 2004/041979, WO 2007/006305, WO 2011/036263, WO 2014/207227, WO 2016/087617 and WO 2016/174234. Preferred protease variants may, for example, comprise one or more of the mutations selected from the group consisting of: S3T, V4I, S9R, S9E, A15T, S24G, S24R, K27R, N42R, S55P, G59E, G59D, N60D, N60E, V66A, N74D, S85R, A96S, S97G, S97D, S97A, S97SD, S99E, S99D, S99G, S99M, S99N, S99R, S99H, S101A, V102I, V102Y, V102N, S104A, G116V, G116R, H118D, H118N, A120S, S126L, P127Q,

S128A, S154D, A156E, G157D, G157P, S158E, Y161A, R164S, Q176E, N179E, S182E, Q185N, A188P, G189E, V193M, N198D, V199I, Q200L, Y203W, S206G, L211Q, L211D, N212D, N212S, M216S, A226V, K229L, Q230H, Q239R, N246K, S253D, N255W, N255D, N255E, L256E, L256D T268A and R269H, wherein position numbers correspond to positions of the *Bacillus lentus* protease shown in SEQ ID NO: 1 of WO 2016/001449. Protease variants having one or more of these mutations are preferably variants of the *Bacillus lentus* protease (Savinase®, also known as subtilisin 309) shown in SEQ ID NO: 1 of WO 2016/001449 or of the *Bacillus amyloliquefaciens* protease (BPN') shown in SEQ ID NO: 2 of WO 2016/001449. Such protease variants preferably have at least 80% sequence identity to SEQ ID NO: 1 or to SEQ ID NO: 2 of WO 2016/001449.

Another protease of interest is the alkaline protease from *Bacillus lentus* DSM 5483, as described for example in WO 91/02792, and variants thereof which are described for example in WO 92/21760, WO 95/23221, EP 1921147, EP 1921148 and WO 2016/096711.

The protease may alternatively be a variant of the TY145 protease having SEQ ID NO: 1 of WO 2004/067737, for example a variant comprising a substitution at one or more positions corresponding to positions 27, 109, 111, 171, 173, 174, 175, 180, 182, 184, 198, 199 and 297 of SEQ ID NO: 1 of WO 2004/067737, wherein said protease variant has a sequence identity of at least 75% but less than 100% to SEQ ID NO: 1 of WO 2004/067737. TY145 variants of interest are described in e.g. WO 2015/014790, WO 2015/014803, WO 2015/014804, WO 2016/097350, WO 2016/097352, WO 2016/097357 and WO 2016/097354.

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, Blaze Evity® 200T, Neutrase®, Everlase®, Esperase®, Progress® Uno, Progress® In, Progress® Key and Progress® Excel (Novozymes A/S), those sold under the tradename Maxatase™, Maxacal™, Maxapem®, Purafect® Ox, Purafect® OxP, Puramax®, FN2™, FN3™, FN4^{ex}™, Excellase®, Excellenz™ P1000, Excellenz™ P1250, Eraser™, Preferenz® P100, Preferenz® P300, Purafect Prime, Preferenz P110™, Effectenz P1000™, Purafect®, Effectenz P1050™, Purafect® Ox, Effectenz ™ P2000, Purafast™, Properase®, Opticlean™ and Optimase® (Danisco/DuPont), BLAP (sequence shown in Figure 29 of US 5352604) and variants hereof (Henkel AG), and KAP (*Bacillus alkalophilus* subtilisin) from Kao.

Lipases and Cutinases

Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutant enzymes are included. Examples include lipase from *Thermomyces*, e.g. from *T. lanuginosus* (previously named *Humicola lanuginosa*) as described in EP258068 and EP305216, cutinase from *Humicola*, e.g. *H. insolens* (WO96/13580), lipase

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

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

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

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

Amylases

Suitable amylases which can be used together with the DNases of the invention may be an alpha-amylase or a glucoamylase and may be of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus*, *e.g.*, a special strain of *Bacillus licheniformis*, described in more detail in GB 1,296,839.

Suitable amylases include amylases having SEQ ID NO: 2 in WO 95/10603 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants are described in WO 94/02597, WO 94/18314, WO 97/43424 and SEQ ID NO: 4 of WO 99/019467, such as variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and 444.

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

Other amylases which are suitable are hybrid alpha-amylase comprising residues 1-33

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

M197T;

H156Y+A181T+N190F+A209V+Q264S; or

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

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

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

Other amylases which can be used are amylases having SEQ ID NO: 2 of WO 08/153815, SEQ ID NO: 10 in WO 01/66712 or variants thereof having 90% sequence identity to SEQ ID NO: 2 of WO 08/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 01/66712. Preferred variants of SEQ ID NO: 10 in WO 01/66712 are those having a substitution, a deletion or an insertion in one of more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264.

Further suitable amylases are amylases having SEQ ID NO: 2 of WO 09/061380 or variants having 90% sequence identity to SEQ ID NO: 2 thereof. Preferred variants of SEQ ID NO: 2 are those having a truncation of the C-terminus and/or a substitution, a deletion or an insertion in one of more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320,

Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one of more of the following positions: Q87E,R, Q98R, S125A, N128C, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:
N128C+K178L+T182G+Y305R+G475K;
N128C+K178L+T182G+F202Y+Y305R+D319T+G475K;
S125A+N128C+K178L+T182G+Y305R+G475K; or
S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K wherein the variants are C-terminally truncated and optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO 01/66712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one of more of the following positions of SEQ ID NO: 12 in WO01/66712: R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, N484. Particular preferred amylases include variants having a deletion of D183 and G184 and having the substitutions R118K, N195F, R320K and R458K, and a variant additionally having substitutions in one or more position selected from the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions.

Other examples are amylase variants such as those described in WO 2011/098531, WO 2013/001078 and WO 2013/001087.

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

Hexosaminidases

Detergent compositions comprising a DNase of the invention may also include one or more hexosaminidases. The term hexosaminidase includes “dispersin” and the abbreviation “Dsp”, which means a polypeptide having hexosaminidase activity, EC 3.2.1.-, that catalyzes the hydrolysis of β-1,6-glycosidic linkages of N-acetyl-glucosamine polymers found e.g. in biofilm. The term hexosaminidase includes polypeptides having N-acetylglucosaminidase activity and β-N-acetylglucosaminidase activity.

A polypeptide having hexosaminidase activity may be obtained from microorganisms of any genus, in particular from bacteria or fungi. Preferably the hexosaminidase, e.g. a dispersin, is obtained from *Terribacillus, Curtobacterium, Aggregatibacter, Haemophilus* or *Actinobacillus*,

preferably *Terribacillus*. The hexosaminidase may also be a variant of a polypeptide obtained from any of these or other organisms.

Suitable hexosaminidases include those disclosed in WO2017186936, WO2017186937, WO2017186943, WO2017207770, WO2018184873, WO2019086520, WO2019086528, WO2019086530, WO2019086532, WO2019086521, WO2019086526, WO2020002604, WO2020002608, WO2020007863, WO2020007875, WO2020008024, WO2020070063, WO2020070249, WO2020088957, WO2020088958 and WO2020207944.

Peroxidases/Oxidases

A peroxidase may be comprised by the enzyme classification EC 1.11.1.7, as set out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), or any fragment obtained therefrom, exhibiting peroxidase activity. Suitable peroxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinopsis*, *e.g.*, from *C. cinerea* (EP 179,486), and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. A peroxidase may also include a haloperoxidase enzyme, such as chloroperoxidase, bromoperoxidase and compounds exhibiting chloroperoxidase or bromoperoxidase activity. Haloperoxidases are classified according to their specificity for halide ions. Chloroperoxidases (E.C. 1.11.1.10) catalyze formation of hypochlorite from chloride ions. In an aspect, the haloperoxidase is a chloroperoxidase. Preferably, the haloperoxidase is a vanadium haloperoxidase, *i.e.*, a vanadate-containing haloperoxidase. In a preferred method the vanadate-containing haloperoxidase is combined with a source of chloride ion. Haloperoxidases have been isolated from many different fungi, in particular from the fungus group dematiaceous hyphomycetes, such as *Caldariomyces*, *e.g.*, *C. fumago*, *Alternaria*, *Curvularia*, *e.g.*, *C. verruculosa* and *C. inaequalis*, *Drechslera*, *Ulocladium* and *Botrytis*. Haloperoxidases have also been isolated from bacteria such as *Pseudomonas*, *e.g.*, *P. pyrrocinia* and *Streptomyces*, *e.g.*, *S. aureofaciens*. In a preferred aspect, the haloperoxidase is derivable from *Curvularia* sp., in particular *Curvularia verruculosa* or *Curvularia inaequalis*, such as *C. inaequalis* CBS 102.42 as described in WO 95/27046; or *C. verruculosa* CBS 147.63 or *C. verruculosa* CBS 444.70 as described in WO 97/04102; or from *Drechslera hartlebii* as described in WO 01/79459, *Dendryphiella salina* as described in WO 01/79458, *Phaeotrichoconis crotalarie* as described in WO 01/79461, or *Geniculosporium* sp. as described in WO 01/79460.

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

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

Suitable examples from fungi include a laccase derivable from a strain of *Bacillus, 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* (WO 92/01046), or *Coriolus*, *e.g.*, *C. hirsutus* (JP 2238885).

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

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

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

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

Dispersants

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

Dye Transfer Inhibiting Agents

The cleaning compositions of the present invention may also include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not

limited to, polyvinylpyrrolidone polymers, polyamine *N*-oxide polymers, copolymers of *N*-vinylpyrrolidone and *N*-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. When present in a subject composition, the dye transfer inhibiting agents may be present at levels from about 0.0001 % to about 10%, from about 0.01% to about 5% or even from about 0.1% to about 3% by weight of the composition.

Fluorescent whitening agents

The detergent composition may preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agent or optical brighteners. Where present the brightener is preferably at a level of about 0.01% to about 0.5%. Any fluorescent whitening agent suitable for use in a laundry detergent composition may be used in the composition of the present invention. The most commonly used fluorescent whitening agents are those belonging to the classes of diaminostilbene-sulfonic acid derivatives, diarylpyrazoline derivatives and bisphenyl-distyryl derivatives. Examples of the diaminostilbene-sulfonic acid derivative type of fluorescent whitening agents include the sodium salts of: 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino) stilbene-2.2'-disulfonate, 4,4'-bis-(2-anilino-4-(*N*-methyl-*N*-2-hydroxy-ethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(4-phenyl-1,2,3-triazol-2-yl)stilbene-2,2'-disulfonate and sodium 5-(2*H*-naphtho[1,2-*d*][1,2,3]triazol-2-yl)-2-[(*E*)-2-phenylvinyl]benzenesulfonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt of 4,4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenyl-styryl)-disulfonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Tinopal CBS-X is a 4.4'-bis-(sulfostyryl)-biphenyl disodium salt also known as Disodium Distyrylbiphenyl Disulfonate. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins.

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

Soil release polymers

The detergent compositions may also include one or more soil release polymers which aid the removal of soils from fabrics such as cotton and polyester based fabrics, the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalte based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides see for example Chapter 7 in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc. Another type of soil release polymers is amphiphilic alkoxylated grease cleaning polymers comprising a core structure and a plurality

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

Anti-redeposition agents

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

Rheology Modifiers

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

Other suitable adjunct materials include, but are not limited to, 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.

Other materials

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

Formulation of detergent products

The detergent composition may be in any convenient form, e.g., a bar, a homogenous tablet, a tablet having two or more layers, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid. Other detergent formulation forms include single unit dose forms such as layered forms and pouches.

Pouches can be configured as single or multicompartments. They can be of any form, shape and material which is suitable for hold the composition, e.g. without allowing release of the composition from the pouch prior to water contact. The pouch is made from water soluble film which encloses an inner volume, which can be divided into compartments. Preferred films are polymeric materials, preferably polymers which are formed into a film or sheet. Preferred polymers, copolymers or derivates thereof are selected from polyacrylates and water-soluble acrylate copolymers, methyl cellulose, carboxy methyl cellulose, sodium dextrin, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, maltodextrin, polymethacrylates, most preferably polyvinyl alcohol copolymers and hydroxypropyl methyl cellulose (HPMC). Preferably the level of polymer in a film such as is at least about 60%. Preferred average molecular weight will typically be about 20,000 to about 150,000. Films can also be of blend compositions comprising hydrolytically degradable and water-soluble polymer blends such as polylactide and polyvinyl alcohol plus plasticisers such as 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. Compartments for liquid components can be different in composition than compartments containing solids; see e.g. US 2009/0011970 A1.

Detergent ingredients can be separated physically from each other by compartments in water dissolvable pouches or in different layers of tablets, thereby avoiding negative storage interaction between components. 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, or up to about 35% water. Concentrated liquid detergents may have lower water contents, for example not more than about 30% or not more than about 20%, e.g. in the range of about 1% to about 20%, such as from about 2% to about 15%. 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 alternatively be non-aqueous.

Liquid detergent compositions may be formulated to have a moderate pH of e.g. from about 6 to about 10, such as about pH 7, about pH 8 or about pH 9, or they may be formulated to have a higher pH of e.g. from about 10 to about 12, such as about pH 10, about pH 11 or about pH 12.

Unless indicated otherwise, the term “liquid” as used herein should be understood to encompass any kind of liquid detergent composition, for example concentrated liquids, gels, or the liquid or gel part of e.g. a pouch with one or more compartments.

Granular detergent formulations

Enzymes in the form of granules, comprising an enzyme-containing core and optionally one or more coatings, are commonly used in granular (powder) detergents. Various methods for preparing the core are well-known in the art and include, for example, a) spray drying of a liquid enzyme-containing solution, b) production of layered products with an enzyme coated as a layer around a pre-formed inert core particle, e.g. using a fluid bed apparatus, c) absorbing an enzyme onto and/or into the surface of a pre-formed core, d) extrusion of an enzyme-containing paste, e) suspending an enzyme-containing powder in molten wax and atomization to result in prilled products, f) mixer granulation by adding an enzyme-containing liquid to a dry powder composition of granulation components, g) size reduction of enzyme-containing cores by milling or crushing of larger particles, pellets, etc., and h) fluid bed granulation. The enzyme-containing cores may be dried, e.g. using a fluid bed drier or other known methods for drying granules in the feed or enzyme industry, to result in a water content of typically 0.1 -10% w/w water.

The enzyme-containing cores are optionally provided with a coating to improve storage stability and/or to reduce dust formation. One type of coating that is often used for enzyme granulates for detergents is a salt coating, typically an inorganic salt coating, which may e.g. be applied as a solution of the salt using a fluid bed. Other coating materials that may be used are, for example, polyethylene glycol (PEG), methyl hydroxy-propyl cellulose (MHPC) and polyvinyl alcohol (PVA). The granules may contain more than one coating, for example a salt coating followed by an additional coating of a material such as PEG, MHPC or PVA.

For further information on enzyme granules and production thereof, see WO 2013/007594 as well as e.g. WO 2009/092699, EP 1705241, EP 1382668, WO 2007/001262, US 6,472,364, WO 2004/074419 and WO 2009/102854.

Formulation of enzyme in co-granule

The DNase may be formulated as a granule for example as a co-granule that combines one or more enzymes. Each enzyme will then be present in more granules securing a more uniform distribution of enzymes in the detergent. This also reduces the physical segregation of different enzymes due to different particle sizes. Methods for producing multi-enzyme co-granulate for the detergent industry is disclosed in the IP.com disclosure IPCOM000200739D.

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

Liquid Formulations

The present invention also relates to liquid compositions comprising a DNase variant of the invention. The composition may comprise an enzyme stabilizer (examples of which include polyols such as propylene glycol or glycerol, sugar or sugar alcohol, lactic acid, reversible protease inhibitor, boric acid, or a boric acid derivative, *e.g.*, an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid).

In some embodiments, fillers or carrier materials are included to increase the volume of such compositions. Suitable filler or carrier materials include, but are not limited to, various salts of sulfate, carbonate and silicate as well as talc, clay and the like. Suitable filler or carrier materials for liquid compositions include, but are not limited to, water or low molecular weight primary and secondary alcohols including polyols and diols. Examples of such alcohols include, but are not limited to, methanol, ethanol, propanol and isopropanol. In some embodiments, the compositions contain from about 5% to about 90% of such materials.

In an aspect, the liquid formulation comprises 20-80% w/w of polyol. In one embodiment, the liquid formulation comprises 0.001-2% w/w preservative.

In another embodiment, the invention relates to liquid formulations comprising:

(A) 0.001-25% w/w of a variant of the present invention;

(B) 20-80% w/w of polyol;

(C) optionally 0.001-2% w/w preservative; and

(D) water.

In another embodiment, the invention relates to liquid formulations comprising:

(A) 0.001-25% w/w of a variant of the present invention;

(B) 0.001-2% w/w preservative;

(C) optionally 20-80% w/w of polyol; and

(D) water.

In another embodiment, the liquid formulation comprises one or more formulating agents, such as a formulating agent selected from the group consisting of polyol, sodium chloride, sodium benzoate, potassium sorbate, sodium sulfate, potassium sulfate, magnesium sulfate, sodium thiosulfate, calcium carbonate, sodium citrate, dextrin, glucose, sucrose, sorbitol, lactose, starch, PVA, acetate and phosphate, preferably selected from the group consisting of sodium sulfate, dextrin, cellulose, sodium thiosulfate, kaolin and calcium carbonate. In one embodiment, the polyols is selected from the group consisting of glycerol, sorbitol, propylene glycol (MPG), ethylene glycol, diethylene glycol, triethylene glycol, 1,2-propylene glycol or 1,3-propylene glycol, dipropylene glycol, polyethylene glycol (PEG) having an average molecular weight below about 600 and polypropylene glycol (PPG) having an average molecular weight below about 600, more preferably selected from the group consisting of glycerol, sorbitol and propylene glycol (MPG) or any combination thereof.

In another embodiment, the liquid formulation comprises 20-80% polyol (*i.e.*, total amount of polyol), *e.g.*, 25-75% polyol, 30-70% polyol, 35-65% polyol, or 40-60% polyol. In one embodiment, the liquid formulation comprises 20-80% polyol, *e.g.*, 25-75% polyol, 30-70% polyol, 35-65% polyol, or 40-60% polyol, wherein the polyol is selected from the group consisting of glycerol, sorbitol, propylene glycol (MPG), ethylene glycol, diethylene glycol, triethylene glycol, 1,2-propylene glycol or 1,3-propylene glycol, dipropylene glycol, polyethylene glycol (PEG) having an average molecular weight below about 600 and polypropylene glycol (PPG) having an average molecular weight below about 600. In one embodiment, the liquid formulation comprises 20-80% polyol (*i.e.*, total amount of polyol), *e.g.*, 25-75% polyol, 30-70% polyol, 35-65% polyol, or 40-60% polyol, wherein the polyol is selected from the group consisting of glycerol, sorbitol and propylene glycol (MPG).

In another embodiment, the preservative is selected from the group consisting of sodium sorbate, potassium sorbate, sodium benzoate and potassium benzoate or any combination thereof. In one embodiment, the liquid formulation comprises 0.02-1.5% w/w preservative, *e.g.*, 0.05-1% w/w preservative or 0.1-0.5% w/w preservative. In one embodiment, the liquid formulation comprises 0.001-2% w/w preservative (*i.e.*, total amount of preservative), *e.g.*, 0.02-1.5% w/w preservative, 0.05-1% w/w preservative, or 0.1-0.5% w/w preservative, wherein the preservative is selected from the group consisting of sodium sorbate, potassium sorbate, sodium benzoate and potassium benzoate or any combination thereof.

In another embodiment, the liquid formulation further comprises one or more additional enzymes, *e.g.* as described above.

Use of DNase variants

The DNase variants of the invention are suitable for use in a cleaning process such as laundry or hard surface cleaning, in particular for laundry. Thus, one aspect of the invention relates a method for laundering an item, wherein the item is a textile, the method comprising:

a) exposing the item to a wash liquor comprising a DNase variant of the invention;

b) completing at least one wash cycle; and optionally

c) rinsing the item.

The pH of the liquid wash liquor solution is typically in the range about 5.5 to about 10, more typically in the range of about 7 to about 9, such as in the range of about 7 to about 8.5 or about 7 to about 8.

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

The concentration of the DNase variant enzyme in the wash liquor is typically in the range of from 0.0001 mg/l to 10 mg/l enzyme protein, from 0.0002 mg/l to 10 mg/l, from 0.001 mg/l to 10 mg/l, from 0.002 mg/l to 10 mg/l, from 0.01 mg/l to 10 mg/l, from 0.02 mg/l to 10 mg/l, from 0.1 mg/l to 10 mg/l, from 0.2 mg/l to 10 mg/l, or from 0.2 mg/l to 5 mg/l.

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

In one embodiment, the DNase variants of the present invention have improved malodor removal properties compared to the parent or a reference DNase such as SEQ ID NO: 1, wherein the malodor is measured as described in Assay II.

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

Examples

Assays and detergent compositions

Model detergent A2 (liquid)

Ingredients: 12% LAS, 12% AEO, 4% AEOS/SLES, 2% MPG (monopropylene glycol), 3% ethanol, 2% TEA, 3% soap, 0.5% NaOH, 3.9% sodium citrate, 1.5% DTMPA Na7, 0.5% phenoxyethanol, and water to 100% (all percentages are w/w); pH adjusted to 8.4 with NaOH

Assay I: Testing of DNase activity

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

Assay II: Analysis of E-2-nonenal on textile using an electronic nose.

One way of testing for the presence of malodor on textiles is by using E-2-Nonenal as a marker for the malodor, as this compound contributes to malodor on laundry. A solution of E-2-nonenal is added to a 5 cm x 5 cm textile swatch and the swatch is placed in a 20 mL glass vial for gas chromatography (GC) analysis, and the vial is capped. 5 mL headspace from the capped vials is analyzed in a Heracles II Electronic nose from Alpha M.O.S., France (double column gas chromatograph with 2 FIDs, column 1: MXT5 and column 2: MXT1701) after 20 minutes incubation at 40°C.

Methods

General methods of PCR, cloning, ligation nucleotides etc. are well-known to a person skilled in the art and may for example be found in in "Molecular cloning: A laboratory manual", Sambrook *et al.* (1989), Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. *et al.* (eds.); *"Current protocols in Molecular Biology"*, John Wiley and Sons, (1995); Harwood, C. R., and Cutting, S. M. (eds.); "DNA Cloning: A Practical Approach, Volumes I and II", D.N. Glover ed. (1985); "Oligonucleotide Synthesis", M.J. Gait ed. (1984); "Nucleic Acid Hybridization", B.D. Hames & S.J. Higgins eds (1985); "A Practical Guide To Molecular Cloning", B. Perbal, (1984).

Example 1: Preparation and initial screening of DNase variants

Site-directed variants of the DNase of SEQ ID NO: 1 were generated using specific primers that contained the desired new mutation. The new codon selected was the codon with

the highest natural abundance for the specific amino acid in *Aspergillus oryzae*. The transformation substrate was made using PCR:

1) Separate amplification of N-terminal and C-terminal fragment, relative to the mutation site

The N-terminal fragment was amplified using a universal forward primer and a position specific reverse primer. The C-terminal fragment was amplified using a mutation specific forward primer and universal reverse primer. Both universal primers are complementary to sequences necessary for homologous integration in the *Aspergillus* genome.

2) Assembly of the N-terminal and C-terminal fragments by *in vivo* recombination in the cells.

The resulting transformation substrate was transformed into *Aspergillus oryzae* and transformants were selected for by growth on nitrate as the sole nitrogen source. Three single colonies of each type were picked into microtiter plates and grown for 4 days at 30°C in broth specific for *Aspergillus*.

The supernatants were used for initial stability screening. Based on the initial screening, substitutions of interest were combined in new variants containing multiple substitutions, which were purified and subjected to further stability testing as described below.

Example 2: Purification of DNase variants

Purification of *A. oryzae* DNase culture supernatants containing multiple substitutions compared to SEQ ID NO: 1 was performed by CaptoMMC as follows: The culture broth was filtered through a Nalgene 0.2 µm filtration unit to remove the host cells. The filtered supernatant was applied to a 25mL CaptoMMC column (Cytiva) equilibrated in 20mM MES (2-(N-morpholino)ethanesulfonic acid), pH 6.0. The supernatant was applied with a 1:3 in-line dilution using 20mM MES, pH 6.0 buffer as the diluent. The column was washed with 20mM MES, pH 6.0 before elution with a step gradient to 100% 50mM Tris, 1M NaCl, pH 9.0 held for five column volumes. Fractions were analyzed by SDS-PAGE and pooled according to purity to be used as the purified enzyme preparation for further experiments.

Example 3: Determination of melting temperature (Tm) of purified DNase variants by nDSF

Purified DNase variants were diluted with 0.01% Triton X-100 to 0.6 mg/ml, with the concentration calculated from absorbance at 280 nm. 40 µl diluted sample was mixed with either 40 µl buffer (0.2 M Hepes, 20 mM $MgCl_2$, pH 8.5) or 40 µl 20% Model detergent A2.

The thermal stability of purified DNase variants was estimated by differential scanning fluorimetry (nanoDSF or nDSF) using a Prometheus NT.48 or NT.Plex instrument (NanoTemper Technologies GmbH, Germany), which measures the change in intrinsic tryptophan and tyrosine

fluorescence due to unfolding upon temperature increase (20°C – 95°C, ramp-up rate 3.3°C/min). Melting temperatures are calculated from the inflection points in the melting curve using the first and second derivative. The variants were generally tested in triplicate.

Example 4: Storage stability assay for purified DNase variants

The storage stability of purified DNase variants of the invention having multiple substitutions compared to SEQ ID NO: 1 was determined using an accelerated storage stability assay in which the variants were stored in a detergent composition at an elevated temperature of either 40°C or 45°C for 7 days. Residual DNase activity was determined after various incubation times, and the results were compared with the result for the wildtype DNase of SEQ ID NO: 1 stored at the same temperature.

Purified DNase variants were diluted with 0.01% Triton X-100 to to concentrations ranging from 10 ppm to 150 ppm in the final reaction, with the enzyme concentration calculated from absorbance at 280 nm. 15 µl diluted DNase sample was mixed with 285 µl concentrated Model detergent A2 and 0.5% of a protease (Progress Uno 101L, Novozymes A/S) and added to the well of a microtiter plate (detergent plate, Nunc U96 PP 0.5 ml) using a magnetic bar. After mixing, the detergent plate was incubated at 40°C or 45°C in a Biosan PST-100HL thermomixer.

After various incubation times (e.g. 0, 3, 24, 96 and 168 hours) residual DNase activity was measured. 5 µl from the detergent plate was mixed with 195 µl DNA substrate solution (3.3 mg DNA (Sigma D1626) in 50 mM Tris pH 7, 20 mM $MgCl_2$, 20 mM $CaCl_2$, 1.3 mM EDTA). Viscosity was measured every minute for 30 minutes using pressure sensing during aspiration with a Hamilton Microlab STAR, and an activity was calculated from the measured reduction in viscosity. Each variant was tested in duplicate at each of two different enzyme concentrations.

The decrease in activity during incubation with detergent was assumed to be exponential. Half-lives (T½) were found from linear regression of Log(Activity) versus incubation time, and half-life improvement factors (HIF or T½ IF) were calculated as half-life of the DNase variant relative to the half-life of a reference, in this case the DNase of SEQ ID NO: 1. Variants assayed at either 40°C or 45°C were compared with the DNase of SEQ ID NO: 1 assayed at the same temperature, with the HIF value of the DNase of SEQ ID NO: 1 defined as 1.

Example 5: Melting temperature and storage stability results

The results for melting temperature (Tm) and half-life improvement factor (HIF) of DNase variants of the invention, determined as described above, are provided in Tables 1 and 2 below. In Table 1, data is provided for the melting temperature (Tm) determined by nDSF in both buffer and Model detergent A2 as described in Example 3. In Table 2, data is provided for the half-life improvement factor (HIF) compared to the DNase of SEQ ID NO: 1, determined in Model detergent A2 at the indicated temperature as described in Example 4.

Table 1: Melting temperature (Tm) of variants of SEQ ID NO: 1

Substitutions compared to SEQ ID NO: 1	Tm buffer	Tm Model det. A2
Wildtype (SEQ ID NO: 1)	76.0	64.0
D32E V35I A111P	80.7	70.2
D32E V35I Q102E A111P	82.7	73.6
D32E V35I A111P S115T	83.9	74.9
D32E V35I Q85E A111P	81.4	72.6
D32E V35I A111P N214D	80.9	72.6
K18Q D32E V35I A111P	81.4	72.2
D32E V35I A111P G199E	80.7	
D32E V35I D72V A111P	82.0	
S69V K86E Q102E S115T K147N K155E A172D K192A Q208V	79.9	70.4
S69V K86E Q102E K105T K147N K155E A172D K192A Q208V	78.9	70.9
S69V K86E Q102E K105D K147N K155E A172D K192A Q208V	79.8	72.0
S69V K86E Q102E K147N K155E A172D G181N K192A Q208V	78.1	69.0
G20C K30C D32E V35I A111P	77.5	67.2
S69V Q102E Q157E T159Q G181N	80.8	70.2
A101E K105N N146H V148I N214D Y218E	77.1	71.3
D32E V35I A111P K147N	79.9	68.5
S26H D32E V35I A111P	80.5	69.1
D32E V35I S69E A111P	81.4	70.6
D32E V35I K105D A111P S115T	85.8	71.9
D32E V35I A101E K105T A111P G181N	84.6	74.6
D32E V35I K105N A111P G181N S182Y	83.5	72.4
S69V K86E Q102E F112W K147N K155E A172D K192A Q208V	82.1	73.6
S69V K86E Q102E G136E K147N K155E A172D K192A Q208V	82.3	72.7
A101E A111P Q150E Q208V	82.3	68.2
S69V Q102E A111P Q157E T159Q A172E	82.1	70.0
S69V Q102E S115T Q157E T159Q A172E	82.2	69.9
D32E V35I K105T A111P G181N	82.3	68.1
D32E V35I A101E K105N A111P	82.4	68.1
S26H S69V Q102E A111P Q157E T159Q A172E	82.7	70.2
D32E V35I S69V Q102E A111P Q157E T159Q A172E	82.4	70.3
S69V Q102E K105N A111P S115T Q150E G161R A172D G181N V187N K192I	91.4	80.9
D32P S69V Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	91.0	76.7
S69V Q102E K105N A111P S115T Q150E K160T G161R G181N V187N K192I	89.6	80.3
K65Q S69V Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	90.9	80.9
S69V Q102E K105N A111P S115T V138M Q150E G161R G181N V187N K192I	87.3	74.6
S26H D32E V35I A111P K155L	79.0	67.4
D32E V35I K82G A111P G181N S182V	79.3	63.3
D32E V35I N77E A111P G181N S182V	78.2	63.1
K18S D32E V35I Q102E K105E A111P	82.8	70.5
T19P D32E V35I Q102E K105E A111P	82.0	66.3
K67A S69V Q102E K147N Q157E T159Q A172E D197S	82.1	71.3

Substitutions compared to SEQ ID NO: 1	Tm buffer	Tm Model det. A2
S69V Q102E V138A S144R Q157E T159Q A172E G181N	79.6	64.7
S69V Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	90.6	75.2
E6D A101E Q150E G181N Q208V	80.2	70.5
S69V Q102E K105N A111P S115T G137R Q150E G161R G181N V187N K192I	90.1	75.7
D32E V35I S69V K86E Q102E K105N A111P S115T V127T G137R K147N Q150E K155E T159Q G161R A172D G181N V187N K192A Q208V N217A	89.8	78.8
D32E V35I S69V K86E Q102E K105N A111P S115T K147N Q150E K155E T159Q G161R A172D G181N V187N K192A Q208V N217A	86.3	79.8
D32E V35I A111P N140H	80.0	69.5
D32E V35I A111P G181N S182V K185E	81.6	69.9
D32E E34N V35I K86E A111P G181N	78.7	67.6
D32E V35I K67A S69R A111P G181N K212E	84.4	69.1
D32E V35I K67A A111P P175N S182V	80.9	64.4
D32E V35I K67A A111P G181N A206G Q208V	84.5	69.9
D32E V35I A101E Q102E A111P G181N	81.8	68.5
D32E V35I S69V K86E Q102E A111P K147N K155E T159Q A172D K178S G181N K192A Q208V N217A	80.0	68.6
D32E V35I K65Q S69V K86E Q102E A111P K147N K155E T159Q A172D G181N K192A Q208V N217A	80.0	70.5
D32E V35I S69V K86E Q102E A111P K155E T159Q A172D G181N K192A Q208V N217A	79.8	69.0
D32E V35I S69V K86E Q102E A111P K147N K155E T159Q A172D G181N K192A Q208V N217A	79.9	68.8
S69V Q102E K105N A111P S115T G161R G181N V187N K192I	89.3	73.0
S69A Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	91.0	76.0
S69V D72S Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	89.6	74.0
N4K D32E V35I S98R K105N A111P G181N S182Y K212E	83.7	70.4
D32E V35I S98R A111P G181N S182Y	81.7	66.8
D32E V35I S98R K105N A111P Q158D G181N S182Y	83.7	69.9
D32E V35I S69E S98R K105N A111P G181N S182Y	85.2	72.6
K18S D32E V35I S98R K105N A111P G181N S182Y	83.6	69.9
D32E V35I S98R A101T K105N A111P G181N S182Y	84.2	70.1
D32E V35I S98R K105N A111P G181N S182Y D189N N214D	84.2	70.1
D32E V35I S98R K105N A111P K147N G181N S182Y	83.5	69.6
V12L D32E V35I S98R K105N A111P G181N S182Y	83.9	69.7
D32E V35I S98R K105N A111P G181N S182Y Q208V	83.8	71.4
D32P S69V Q102E K105D A111P S115T Q150E G161R G181N V187N K192I	91.6	77.4
D32E V35I K67A A101E K105D A111P N217A	84.8	72.7
D32E V35I K65E A101E K105T A111P N217A	82.8	72.9
D32E V35I A101T K105N A111P K147E G181N S182Y	82.4	72.1
D32E V35I S69E K105N A111P K147E G181N S182Y	83.9	75.9
D32E V35I K65E K105N A111P G181N S182Y K212E	84.1	74.3
D32E V35I K67A K105N A111P G181N S182Y K212E	85.6	73.3

Substitutions compared to SEQ ID NO: 1	Tm buffer	Tm Model det. A2
D32E V35I K65E K67A K105N A111P G181N S182Y	87.2	78.3
D32E V35I A101T K105N A111P G181N S182Y N214D	83.3	71.1
T19P D32E V35I A101E K105T A111P K160A G181N	82.6	71.5
T19P D32E V35I A101E K105T A111P K178S G181N	83.4	70.6
K18S T19P D32E V35I A101E K105T A111P G181N	83.1	69.9
K18S D32E V35I A101E K105T A111P G181N K212E	84.0	72.8
D32E V35I A101E K105T A111P G181N K212E N214D	83.8	71.5
D32P S69V Q102E K105N A111P S115T N146H Q150E G161R G181N V187N K192I	90.8	75.4
D32P S69V Q102E K105N A111P S115T S134H Q150E G161R G181N V187N K192I	90.3	72.7
S26H S69V Q102E A111P K147N A149S Q150E Q157E T159Q A172E	83.9	74.0
S26H S69V Q102E A111P N146H Q150E Q157E T159Q A172E	83.0	71.2
D32P S69A Q102E K105N A111P S115T N146H Q150E G161R G181N K192I	91.4	77.5
D32E V35I K53F K105N A111P G181N S182Y K212E	83.6	72.6
K18Q D32E V35I K105E A111P K155F	80.5	67.6
D32E V35I A101E K105T A111P G181N K212E Y218E	81.5	70.0
D32E V35I K82T A101E K105T A111P G181N K212E	83.8	72.4
D32E V35I A111P K147N K204L	79.8	70.2
D32E V35I A111P K185I K215T	79.2	69.3
K18F D32E V35I A111P K185Q	79.8	68.7
D32E V35I A111P K204L K215I	79.6	69.4
D32E V35I K67A A111P K204G	82.3	70.1
D32E V35I A111P K178M K212T	79.5	68.7
D32E V35I K67A K105T A111P K185G K212M	83.3	71.0
D32E V35I K67A K105T A111P K147N K190N	83.8	72.7
D32E V35I K67A K105T A111P K178L K185T	82.6	72.1
D32E V35I K67A K105T A111P K190V K204G	83.7	70.6
K18M D32E V35I K67A K105T A111P K178S	83.2	71.2
K18Q D32E V35I K67A K105T A111P K215V	83.0	70.4
S26H D32P S69V Q102E K105N A111P S115T N146H Q150E G161R G181N V187N K192I	90.9	78.7
D32P S69V Q102E K105N A111P S115T T120H N146H Q150E G161R G181N V187N K192I	90.5	78.7
D32P Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	90.5	77.2
D32P S69V Q102E K105N A111P S115T Q150E Q157E G181N V187N K192I	90.8	82.6
D32E V35I K67A S69V K105T A111P Q157E G181N A206G Q208V	87.4	75.7
D32E V35I K105T A111P G181N K204W K215L	83.0	72.4
D32E V35I K105T A111P G181N K185L K192V K204W	83.5	72.7
D32E V35I K105T A111P K178L G181N K212T	83.0	72.1
S26H D32E V35I K67A S69R A111P G181N K212E	85.1	73.0
D32E V35I K67A A111P Q157E G181N Q208V	85.0	72.8
D32E V35I K67A A111P Q157E A172E G181N A206G Q208V	85.1	71.4
K18S D32E V35I K67A K105T A111P K185L K192F	83.9	76.6

Substitutions compared to SEQ ID NO: 1	Tm buffer	Tm Model det. A2
K18S D32E V35I A111P K178S K212L	79.5	69.4
D32E V35I K67A A111P Q157E T159Q G181N A206G Q208V	84.4	70.8
K18S D32E V35I A111P K185I K215T	79.0	69.5

Table 2: Storage stability (half-life improvement factor, HIF) of variants of SEQ ID NO: 1

Substitutions compared to SEQ ID NO: 1	HIF avg.	Stress temp. °C
Wildtype (SEQ ID NO: 1)	1.0	40/45
D32E V35I A111P	2.0	40
D32E V35I Q102E A111P	1.6	40
D32E V35I A111P S115T	1.5	40
D32E V35I Q85E A111P	1.5	40
D32E V35I A111P N214D	1.4	40
K18Q D32E V35I A111P	1.4	40
D32E V35I A111P G199E	1.4	40
D32E V35I D72V A111P	2.4	40
G20C K30C D32E V35I A111P	1.4	40
D32E V35I A111P K147N	1.8	40
S26H D32E V35I A111P	3.4	40
D32E V35I S69E A111P	2.8	40
D32E V35I K105D A111P S115T	1.6	40
D32E V35I A101E K105T A111P G181N	2.3	40
D32E V35I K105N A111P G181N S182Y	2.2	40
A101E A111P Q150E Q208V	1.3	40
S69V Q102E A111P Q157E T159Q A172E	1.3	40
D32E V35I A101E K105E A111P S182V	1.7	40
D32E V35I K67A K105T A111P	3.8	40
D32E V35I K105T A111P G181N	2.9	40
D32E V35I A101E K105N A111P	1.9	40
S26H S69V Q102E A111P Q157E T159Q A172E	2.3	45
D32E V35I S69V Q102E A111P Q157E T159Q A172E	1.2	40
S26H D32E V35I A111P K155L	1.2	40
D32E V35I K82G A111P G181N S182V	2.3	40
D32E V35I N77E A111P G181N S182V	2.5	40
K18S D32E V35I Q102E K105E A111P	1.7	40
T19P D32E V35I Q102E K105E A111P	2.2	40
K67A S69V Q102E K147N Q157E T159Q A172E D197S	1.2	40
S69V Q102E V138A S144R Q157E T159Q A172E G181N	1.6	40
S69V Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	3.6	40
S69V Q102E K105N A111P S115T G137R Q150E G161R G181N V187N K192I	3.5	40
D32E V35I A111P N140H	2.6	40
D32E V35I A111P G181N S182V K185E	2.1	40
D32E E34N V35I K86E A111P G181N	1.6	40

Substitutions compared to SEQ ID NO: 1	HIF avg.	Stress temp. °C
D32E V35I K67A S69R A111P G181N K212E	3.1	40
D32E V35I K67A A111P P175N S182V	2.9	40
D32E V35I K67A A111P G181N A206G Q208V	3.0	40
D32E V35I A101E Q102E A111P G181N	1.7	40
S69V Q102E K105N A111P S115T G161R G181N V187N K192I	4.8	45
S69A Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	5.2	40
S69V D72S Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	4.3	40
N4K D32E V35I S98R K105N A111P G181N S182Y K212E	2.5	40
D32E V35I S98R A111P G181N S182Y	2.3	40
D32E V35I S98R K105N A111P Q158D G181N S182Y	2.4	40
D32E V35I S69E S98R K105N A111P G181N S182Y	2.4	40
K18S D32E V35I S98R K105N A111P G181N S182Y	2.1	40
D32E V35I S98R A101T K105N A111P G181N S182Y	2.5	40
D32E V35I S98R K105N A111P G181N S182Y D189N N214D	2.4	40
D32E V35I S98R K105N A111P K147N G181N S182Y	2.4	40
V12L D32E V35I S98R K105N A111P G181N S182Y	2.3	40
D32E V35I S98R K105N A111P G181N S182Y Q208V	2.4	40
D32P S69V Q102E K105D A111P S115T Q150E G161R G181N V187N K192I	1.5	45
D32E V35I K67A A101E K105D A111P N217A	2.6	45
D32E V35I K65E A101E K105T A111P N217A	1.8	45
D32E V35I A101T K105N A111P K147E G181N S182Y	2.4	45
D32E V35I S69E K105N A111P K147E G181N S182Y	2.5	45
D32E V35I K65E K105N A111P G181N S182Y K212E	2.5	45
D32E V35I K67A K105N A111P G181N S182Y K212E	3.2	45
D32E V35I K65E K67A K105N A111P G181N S182Y	3.1	45
D32E V35I A101T K105N A111P G181N S182Y N214D	2.7	45
T19P D32E V35I A101E K105T A111P K160A G181N	1.2	45
T19P D32E V35I A101E K105T A111P K178S G181N	2.1	45
K18S T19P D32E V35I A101E K105T A111P G181N	1.9	45
K18S D32E V35I A101E K105T A111P G181N K212E	1.9	45
D32E V35I A101E K105T A111P G181N K212E N214D	2.3	45
D32P S69V Q102E K105N A111P S115T N146H Q150E G161R G181N V187N K192I	4.5	45
S26H S69V Q102E A111P K147N A149S Q150E Q157E T159Q A172E	3.5	45
S26H S69V Q102E A111P N146H Q150E Q157E T159Q A172E	1.8	45
D32P S69A Q102E K105N A111P S115T N146H Q150E G161R G181N K192I	4.5	45
D32E V35I K53F K105N A111P G181N S182Y K212E	2.7	45
K18Q D32E V35I K105E A111P K155F	1.2	45
D32E V35I A101E K105T A111P G181N K212E Y218E	2.2	45
D32E V35I K82T A101E K105T A111P G181N K212E	2.3	45
D32E V35I A111P K147N K204L	2.2	45
D32E V35I A111P K185I K215T	2.3	45
K18F D32E V35I A111P K185Q	1.2	45

Substitutions compared to SEQ ID NO: 1	HIF avg.	Stress temp. °C
D32E V35I A111P K204L K215I	2.1	45
D32E V35I K67A A111P K204G	2.8	45
D32E V35I A111P K178M K212T	2.1	45
D32E V35I K67A K105T A111P K185G K212M	2.8	45
D32E V35I K67A K105T A111P K147N K190N	2.8	45
D32E V35I K67A K105T A111P K178L K185T	4.6	45
D32E V35I K67A K105T A111P K190V K204G	3.0	45
K18M D32E V35I K67A K105T A111P K178S	1.8	45
K18Q D32E V35I K67A K105T A111P K215V	2.0	45
S26H D32P S69V Q102E K105N A111P S115T N146H Q150E G161R G181N V187N K192I	4.8	45
D32P S69V Q102E K105N A111P S115T T120H N146H Q150E G161R G181N V187N K192I	5.2	45
D32P Q102E K105N A111P S115T Q150E G161R G181N V187N K192I	5.1	45
D32P S69V Q102E K105N A111P S115T Q150E Q157E G181N V187N K192I	3.8	45
D32E V35I K67A S69V K105T A111P Q157E G181N A206G Q208V	4.6	45
D32E V35I K105T A111P G181N K204W K215L	2.3	45
D32E V35I K105T A111P G181N K185L K192V K204W	2.9	45
D32E V35I K105T A111P K178L G181N K212T	2.8	45
S26H D32E V35I K67A S69R A111P G181N K212E	5.8	45
D32E V35I K67A A111P Q157E G181N Q208V	4.4	45
D32E V35I K67A A111P Q157E A172E G181N A206G Q208V	4.0	45
K18S D32E V35I K67A K105T A111P K185L K192F	2.5	45
K18S D32E V35I A111P K178S K212L	1.9	45
D32E V35I K67A A111P Q157E T159Q G181N A206G Q208V	2.7	45
K18S D32E V35I A111P K185I K215T	2.1	45

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

The invention is further defined by the following numbered paragraphs:

1. A DNase variant, comprising at least two subsitutions, such as at least three substitutions, at positions selected from the group consisting of 111, 32, 35, 69, 102, 105 and 181, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a

sequence identity of at least 60% but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity.

2. A DNase variant, comprising three or more substitutions at positions selected from the group consisting of 26, 32, 35, 65, 67, 69, 98, 102, 105, 111, 115, 150, 157, 159, 161, 172, 181, 182, 185, 187, 192, 206, 208 and 212, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60% but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity.

3. A DNase variant, comprising three or more substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60% but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity.

4. A DNase variant, comprising at least two substitutions, such as at least three substitutions, selected from the group consisting of A111P, D32E, V35I, S69V, Q102E, K105N and G181N, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60% but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity.

5. The variant of any of the preceding paragraphs, wherein the variant comprises the substitution A111P.

6. The variant of paragraph 5, wherein the variant comprises two or more substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

7. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I and/or the substitution G181N.

8. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+A111P.

9. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions A111P+G181N.

10. The variant of any of the preceding paragraphs, wherein the variant comprises the substitution S69V and/or Q102E.

11. The variant of any of the preceding paragraphs, wherein the variant comprises the substitution K105N.

12. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

13. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

14. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

15. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

16. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+S69V+Q102E+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

17. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+S69V+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

18. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions S69V+Q102E+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

19. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions S69V+Q102E+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

20. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions S69V+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

21. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions Q102E+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

22. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions S69V+Q102E+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

23. The variant of any of the preceding paragraphs, wherein the variant comprises the substitutions D32E+V35I+K105N+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

24. The variant of any of the preceding paragraphs, wherein the variant comprises at least one further substitution selected from the group consisting of K18S, T19P, D32P, K86E, A101E, A101T, K105D, K105E, K105T, G137R, N146H, K147N, K155E, A172D, K178S, K192A, N214D and N217A.

25. The variant of any of the preceding paragraphs, wherein the variant has at least 65%, e.g. at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, but less than 100% sequence identity to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

26. The variant of any one of the preceding paragraphs, wherein the number of substitutions is 1-20, e.g. 1-10 or 1-5, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 substitutions.

27. The variant of any of the preceding paragraphs, wherein the variant consists of 195 to 230 amino acids, preferably 200 to 225 amino acids, such as 204 to 221 amino acids

28. The variant of any of the preceding paragraphs, wherein the variant comprises a set of substitutions selected from the group consisting of:

- S69V+K86E+Q102E+K147N+K155E+A172D+K192A+Q208V;
- G20C+K30C+D32E+V35I+A111P;
- S69V+K86E+Q102E+K147N+K155E+A172D+G181N+K192A+Q208V;
- D32E+V35I+N77E+A111P+G181N+S182V;
- D32E+E34N+V35I+K86E+A111P+G181N;
- S69V+K86E+Q102E+K105T+K147N+K155E+A172D+K192A+Q208V;
- S69V+Q102E+Q157E+T159Q+N214D+N217A;
- S26H+D32E+V35I+A111P+K155L;
- K18S+D32E+V35I+A111P+K185I+K215T;
- D32E+V35I+A111P+K185I+K215T;
- D32E+V35I+K82G+A111P+G181N+S182V;
- D32E+V35I+A111P+K178M+K212T;
- K18S+D32E+V35I+A111P+K178S+K212L;
- S69V+Q102E+V138A+S144R+Q157E+T159Q+A172E+G181N;

- D32E+V35I+A111P+K204L+K215I;
- K18F+D32E+V35I+A111P+K185Q;
- D32E+V35I+A111P+K147N+K204L;
- S69V+K86E+Q102E+K105D+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+S69V+K86E+Q102E+A111P+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P+K147N;
- S69V+K86E+Q102E+S115T+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P+N140H;
- D32E+V35I+K65Q+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+K178S+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P;
- E6D+A101E+Q150E+G181N+Q208V;
- S26H+D32E+V35I+A111P;
- K18Q+D32E+V35I+K105E+A111P+K155F;
- D32E+V35I+A111P+G199E;
- S69V+Q102E+Q157E+T159Q+G181N;
- D32E+V35I+K67A+A111P+P175N+S182V;
- D32E+V35I+A111P+N214D;
- S69V+Q102E+Q157E+T159Q+A172E;
- E6D+A101E+Q150E+G181N+Q208V;
- D32E+V35I+S69E+A111P;
- D32E+V35I+Q85E+A111P;
- K18Q+D32E+V35I+A111P;
- D32E+V35I+A101E+K105T+A111P+G181N+K212E+Y218E;
- D32E+V35I+A111P+G181N+S182V+K185E;
- D32E+V35I+S98R+A111P+G181N+S182Y;
- D32E+V35I+A101E+Q102E+A111P+G181N;
- T19P+D32E+V35I+Q102E+K105E+A111P;
- D32E+V35I+D72V+A111P;
- S69V+K86E+Q102E+F112W+K147N+K155E+A172D+K192A+Q208V;
- S69V+Q102E+A111P+Q157E+T159Q+A172E;
- K67A+S69V+Q102E+K147N+Q157E+T159Q+A172E+D197S;

- S69V+Q102E+S115T+Q157E+T159Q+A172E;
- S69V+K86E+Q102E+G136E+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+K67A+A111P+K204G;
- A101E+A111P+Q150E+Q208V;
- D32E+V35I+A101E+K105E+A111P+S182V;
- D32E+V35I+A101E+K105E+A111P+S182V;
- D32E+V35I+A101T+K105N+A111P+K147E+G181N+S182Y;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+Q102E+A111P;
- S26H+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+K67A+K105T+A111P+K178L+K185T;
- K67A+K105T+G181N;
- T19P+D32E+V35I+A101E+K105T+A111P+K160A+G181N;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+Q102E+A111P;
- K18S+D32E+V35I+Q102E+K105E+A111P;
- D32E+V35I+K65E+A101E+K105T+A111P+N217A;
- D32E+V35I+K105T+A111P+G181N+K204W+K215L;
- D32E+V35I+K105T+A111P+K178L+G181N+K212T;
- S26H+S69V+Q102E+A111P+N146H+Q150E+Q157E+T159Q+A172E;
- K18Q+D32E+V35I+K67A+K105T+A111P+K215V;
- D32E+V35I+Q85E+A111P;
- K18S+T19P+D32E+V35I+A101E+K105T+A111P+G181N;
- D32E+V35I+A101E+K105N+A111P;
- K18M+D32E+V35I+K67A+K105T+A111P+K178S;
- D32E+V35I+K105T+A111P+G181N;
- D32E+V35I+K67A+K105T+A111P+K185G+K212M;
- D32E+V35I+A101T+K105N+A111P+G181N+S182Y+N214D;
- T19P+D32E+V35I+A101E+K105T+A111P+K178S+G181N;
- D32E+V35I+K105T+A111P+G181N+K185L+K192V+K204W;
- D32E+V35I+S98R+K105N+A111P+K147N+G181N+S182Y;
- D32E+V35I+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+K105T+A111P;
- D32E+V35I+K53F+K105N+A111P+G181N+S182Y+K212E;
- K18S+D32E+V35I+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+S98R+K105N+A111P+Q158D+G181N+S182Y;

- D32E+V35I+K67A+K105T+A111P+K190V+K204G;
- N4K+D32E+V35I+S98R+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+K67A+K105T+A111P+K147N+K190N;
- D32E+V35I+S98R+K105N+A111P+G181N+S182Y+Q208V;
- D32E+V35I+A101E+K105T+A111P+G181N+K212E+N214D;
- D32E+V35I+K82T+A101E+K105T+A111P+G181N+K212E;
- D32E+V35I+A111P+S115T;
- S26H+S69V+Q102E+A111P+K147N+A149S+Q150E+Q157E+T159Q+A172E;
- K18S+D32E+V35I+K67A+K105T+A111P+K185L+K192F;
- V12L+D32E+V35I+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+S69E+K105N+A111P+K147E+G181N+S182Y;
- K18S+D32E+V35I+A101E+K105T+A111P+G181N+K212E;
- D32E+V35I+K65E+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+S98R+K105N+A111P+G181N+S182Y+D189N+N214D;
- D32E+V35I+S98R+A101T+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+A111P+Q157E+T159Q+G181N+A206G+Q208V;
- D32E+V35I+K67A+S69R+A111P+G181N+K212E;
- D32E+V35I+K67A+A111P+G181N+A206G+Q208V;
- D32E+V35I+A101E+K105T+A111P+G181N;
- D32E+V35I+K67A+A101E+K105D+A111P+N217A;
- D32E+V35I+K67A+A111P+Q157E+G181N+Q208V;
- D32E+V35I+K67A+A111P+Q157E+A172E+G181N+A206G+Q208V;
- S26H+D32E+V35I+K67A+S69R+A111P+G181N+K212E;
- D32E+V35I+S69E+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+K105D+A111P+S115T;
- D32E+V35I+S69V+K86E+Q102E+K105N+A111P+S115T+K147N+Q150E+K155E+T159Q+G161R+A172D+G181N+V187N+K192A+Q208V+N217A;
- D32E+V35I+K65E+K67A+K105N+A111P+G181N+S182Y;
- S69V+Q102E+K105N+A111P+S115T+V138M+Q150E+G161R+G181N+V187N+K192I;
- D32E+V35I+K67A+S69V+K105T+A111P+Q157E+G181N+A206G+Q208V;
- S69V+Q102E+K105N+A111P+S115T+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+Q150E+K160T+G161R+G181N+V187N+K192I;
- S69V+D72S+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32E+V35I+S69V+K86E+Q102E+K105N+A111P+S115T+V127T+G137R+K147N+Q150E+K155E+T159Q+G161R+A172D+G181N+V187N+K192A+Q208V+N217A;
- S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;

- D32P+S69V+Q102E+K105N+A111P+S115T+S134H+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- D32P+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+T120H+N146H+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+Q150E+Q157E+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- S26H+D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- K65Q+S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- S69A+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+A172D+G181N+V187N+K192I;
- D32P+S69A+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+K192I;
- D32P+S69V+Q102E+K105D+A111P+S115T+Q150E+G161R+G181N+V187N+K192I; and
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I.

29. The variant of paragraph 28, wherein the variant comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with one of said sets of substitutions.

30. The variant of any of the preceding paragraphs, wherein the variant has improved stability compared to the polypeptide of SEQ ID NO: 1 or to a parent polypeptide having the same amino acid sequence as the variant without said substitutions.

31. The variant of paragraph 30, wherein the improved stability is selected from the group consisting of detergent stability and thermostability.

32. A detergent composition comprising the DNase variant of any of paragraphs 1-31 and at least one detergent adjunct ingredient.

33. Use of the DNase variant of any of paragraphs 1-31 or the detergent composition of paragraph 32 in a cleaning process, such as laundry or hard surface cleaning such as dishwashing.

34. A method of cleaning an item, comprising exposing the item to a wash liquor comprising the DNase variant of any of paragraphs 1-31 or the detergent composition of paragraph 32.

35. The method of paragraph 34, wherein the item is a textile or a hard surface, for example dishware or a surface such as a tabletop, wall or floor or an interior surface of a machine such as a washing machine or a dishwasher.

36. A method for laundering a textile, comprising:

a) exposing the textile to a wash liquor comprising the DNase variant of any of paragraphs 1-31 or the detergent composition of paragraph 32;

b) completing at least one wash cycle; and optionally

c) rinsing the item.

37. A granule comprising:

(a) a core comprising the DNase variant of any one of paragraphs 1-31, and, optionally

(b) a coating consisting of one or more layers surrounding the core.

38. A liquid composition comprising the DNase variant of any one of paragraphs 1-31 and an enzyme stabilizer, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, a reversible protease inhibitor, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid.

39. A polynucleotide encoding the DNase variant of any of paragraphs 1-31, a nucleic acid construct or expression vector comprising the polynucleotide, or a recombinant host cell transformed with the polynucleotide.

40. A method of producing a DNase variant, comprising:;

a. cultivating the recombinant host cell of paragraph 39 under conditions suitable for expression of the variant; and

b. recovering the variant.

Claims

1. A DNase variant, comprising at least two substitutions, such as at least three substitutions, selected from the group consisting of A111P, D32E, V35I, S69V, Q102E, K105N and G181N, wherein position numbers are based on the numbering of SEQ ID NO: 1, wherein the variant has a sequence identity of at least 60% but less than 100% to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and wherein the variant has DNase activity.

2. The variant of claim 1, wherein the variant comprises the substitution A111P.

3. The variant of claim 2, wherein the variant further comprises two or more substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

4. The variant of any of the preceding claims, wherein the variant comprises:
D32E+V35I, for example D32E+V35I+A111P; and/or
G181N, for example A111P+G181N; and/or
S69V+Q102E.

5. The variant of any of the preceding claims, wherein the variant comprises:
D32E+V35I+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;
D32E+V35I+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;
D32E+V35I+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;
D32E+V35I+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;

D32E+V35I+S69V+Q102E+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;

D32E+V35I+S69V+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;

S69V+Q102E+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;

S69V+Q102E+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, A111P, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;

S69V+Q102E+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, K105N, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;

Q102E+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S69E, S69V, S98R, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E;

S69V+Q102E+K105N+A111P+G181N, and optionally one or more additional substitutions selected from the group consisting of S26H, D32E, V35I, K65E, K67A, S98R, S115T, Q150E, Q157E, T159Q, G161R, A172E, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E; or

D32E+V35I+K105N+A111P, and optionally one or more additional substitutions selected from the group consisting of S26H, K65E, K67A, S69E, S69V, S98R, Q102E, S115T, Q150E, Q157E, T159Q, G161R, A172E, G181N, S182Y, S182V, K185E, V187N, K192I, A206G, Q208V and K212E.

6. The variant of any of the preceding claims, wherein the variant has at least 65%, e.g. at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, but less than 100% sequence identity to the polypeptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

7. The variant of any of the preceding claims, wherein the variant comprises a set of substitutions selected from the group consisting of:

- S69V+K86E+Q102E+K147N+K155E+A172D+K192A+Q208V;
- G20C+K30C+D32E+V35I+A111P;
- S69V+K86E+Q102E+K147N+K155E+A172D+G181N+K192A+Q208V;
- D32E+V35I+N77E+A111P+G181N+S182V;
- D32E+E34N+V35I+K86E+A111P+G181N;
- S69V+K86E+Q102E+K105T+K147N+K155E+A172D+K192A+Q208V;
- S69V+Q102E+Q157E+T159Q+N214D+N217A;
- S26H+D32E+V35I+A111P+K155L;
- K18S+D32E+V35I+A111P+K185I+K215T;
- D32E+V35I+A111P+K185I+K215T;
- D32E+V35I+K82G+A111P+G181N+S182V;
- D32E+V35I+A111P+K178M+K212T;
- K18S+D32E+V35I+A111P+K178S+K212L;
- S69V+Q102E+V138A+S144R+Q157E+T159Q+A172E+G181N;
- D32E+V35I+A111P+K204L+K215I;
- K18F+D32E+V35I+A111P+K185Q;
- D32E+V35I+A111P+K147N+K204L;
- S69V+K86E+Q102E+K105D+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+S69V+K86E+Q102E+A111P+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P+K147N;
- S69V+K86E+Q102E+S115T+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P+N140H;
- D32E+V35I+K65Q+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+G181N+K192A+Q208V+N217A;
- D32E+V35I+S69V+K86E+Q102E+A111P+K147N+K155E+T159Q+A172D+K178S+G181N+K192A+Q208V+N217A;
- D32E+V35I+A111P;
- E6D+A101E+Q150E+G181N+Q208V;
- S26H+D32E+V35I+A111P;
- K18Q+D32E+V35I+K105E+A111P+K155F;
- D32E+V35I+A111P+G199E;
- S69V+Q102E+Q157E+T159Q+G181N;

- D32E+V35I+K67A+A111P+P175N+S182V;
- D32E+V35I+A111P+N214D;
- S69V+Q102E+Q157E+T159Q+A172E;
- E6D+A101E+Q150E+G181N+Q208V;
- D32E+V35I+S69E+A111P;
- D32E+V35I+Q85E+A111P;
- K18Q+D32E+V35I+A111P;
- D32E+V35I+A101E+K105T+A111P+G181N+K212E+Y218E;
- D32E+V35I+A111P+G181N+S182V+K185E;
- D32E+V35I+S98R+A111P+G181N+S182Y;
- D32E+V35I+A101E+Q102E+A111P+G181N;
- T19P+D32E+V35I+Q102E+K105E+A111P;
- D32E+V35I+D72V+A111P;
- S69V+K86E+Q102E+F112W+K147N+K155E+A172D+K192A+Q208V;
- S69V+Q102E+A111P+Q157E+T159Q+A172E;
- K67A+S69V+Q102E+K147N+Q157E+T159Q+A172E+D197S;
- S69V+Q102E+S115T+Q157E+T159Q+A172E;
- S69V+K86E+Q102E+G136E+K147N+K155E+A172D+K192A+Q208V;
- D32E+V35I+K67A+A111P+K204G;
- A101E+A111P+Q150E+Q208V;
- D32E+V35I+A101E+K105E+A111P+S182V;
- D32E+V35I+A101E+K105E+A111P+S182V;
- D32E+V35I+A101T+K105N+A111P+K147E+G181N+S182Y;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+Q102E+A111P;
- S26H+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+K67A+K105T+A111P+K178L+K185T;
- K67A+K105T+G181N;
- T19P+D32E+V35I+A101E+K105T+A111P+K160A+G181N;
- D32E+V35I+S69V+Q102E+A111P+Q157E+T159Q+A172E;
- D32E+V35I+Q102E+A111P;
- K18S+D32E+V35I+Q102E+K105E+A111P;
- D32E+V35I+K65E+A101E+K105T+A111P+N217A;
- D32E+V35I+K105T+A111P+G181N+K204W+K215L;
- D32E+V35I+K105T+A111P+K178L+G181N+K212T;
- S26H+S69V+Q102E+A111P+N146H+Q150E+Q157E+T159Q+A172E;

- K18Q+D32E+V35I+K67A+K105T+A111P+K215V;
- D32E+V35I+Q85E+A111P;
- K18S+T19P+D32E+V35I+A101E+K105T+A111P+G181N;
- D32E+V35I+A101E+K105N+A111P;
- K18M+D32E+V35I+K67A+K105T+A111P+K178S;
- D32E+V35I+K105T+A111P+G181N;
- D32E+V35I+K67A+K105T+A111P+K185G+K212M;
- D32E+V35I+A101T+K105N+A111P+G181N+S182Y+N214D;
- T19P+D32E+V35I+A101E+K105T+A111P+K178S+G181N;
- D32E+V35I+K105T+A111P+G181N+K185L+K192V+K204W;
- D32E+V35I+S98R+K105N+A111P+K147N+G181N+S182Y;
- D32E+V35I+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+K105T+A111P;
- D32E+V35I+K53F+K105N+A111P+G181N+S182Y+K212E;
- K18S+D32E+V35I+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+S98R+K105N+A111P+Q158D+G181N+S182Y;
- D32E+V35I+K67A+K105T+A111P+K190V+K204G;
- N4K+D32E+V35I+S98R+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+K67A+K105T+A111P+K147N+K190N;
- D32E+V35I+S98R+K105N+A111P+G181N+S182Y+Q208V;
- D32E+V35I+A101E+K105T+A111P+G181N+K212E+N214D;
- D32E+V35I+K82T+A101E+K105T+A111P+G181N+K212E;
- D32E+V35I+A111P+S115T;
- S26H+S69V+Q102E+A111P+K147N+A149S+Q150E+Q157E+T159Q+A172E;
- K18S+D32E+V35I+K67A+K105T+A111P+K185L+K192F;
- V12L+D32E+V35I+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+S69E+K105N+A111P+K147E+G181N+S182Y;
- K18S+D32E+V35I+A101E+K105T+A111P+G181N+K212E;
- D32E+V35I+K65E+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+S98R+K105N+A111P+G181N+S182Y+D189N+N214D;
- D32E+V35I+S98R+A101T+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+A111P+Q157E+T159Q+G181N+A206G+Q208V;
- D32E+V35I+K67A+S69R+A111P+G181N+K212E;
- D32E+V35I+K67A+A111P+G181N+A206G+Q208V;
- D32E+V35I+A101E+K105T+A111P+G181N;
- D32E+V35I+K67A+A101E+K105D+A111P+N217A;
- D32E+V35I+K67A+A111P+Q157E+G181N+Q208V;

- D32E+V35I+K67A+A111P+Q157E+A172E+G181N+A206G+Q208V;
- S26H+D32E+V35I+K67A+S69R+A111P+G181N+K212E;
- D32E+V35I+S69E+S98R+K105N+A111P+G181N+S182Y;
- D32E+V35I+K67A+K105N+A111P+G181N+S182Y+K212E;
- D32E+V35I+K105D+A111P+S115T;
- D32E+V35I+S69V+K86E+Q102E+K105N+A111P+S115T+K147N+Q150E+K155E+T159Q+G161R+A172D+G181N+V187N+K192A+Q208V+N217A;
- D32E+V35I+K65E+K67A+K105N+A111P+G181N+S182Y;
- S69V+Q102E+K105N+A111P+S115T+V138M+Q150E+G161R+G181N+V187N+K192I;
- D32E+V35I+K67A+S69V+K105T+A111P+Q157E+G181N+A206G+Q208V;
- S69V+Q102E+K105N+A111P+S115T+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+Q150E+K160T+G161R+G181N+V187N+K192I;
- S69V+D72S+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32E+V35I+S69V+K86E+Q102E+K105N+A111P+S115T+V127T+G137R+K147N+Q150E+K155E+T159Q+G161R+A172D+G181N+V187N+K192A+Q208V+N217A;
- S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+S134H+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- D32P+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+T120H+N146H+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+Q150E+Q157E+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- S26H+D32P+S69V+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+V187N+K192I;
- K65Q+S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- S69A+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- D32P+S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I;
- S69V+Q102E+K105N+A111P+S115T+Q150E+G161R+A172D+G181N+V187N+K192I;
- D32P+S69A+Q102E+K105N+A111P+S115T+N146H+Q150E+G161R+G181N+K192I;
- D32P+S69V+Q102E+K105D+A111P+S115T+Q150E+G161R+G181N+V187N+K192I; and

- S69V+Q102E+K105N+A111P+S115T+G137R+Q150E+G161R+G181N+V187N+K192I.

8. The variant of claim 7, wherein the variant comprises or consists of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 with one of said sets of substitutions.

9. The variant of any of the preceding claims, wherein the variant has improved stability compared to the polypeptide of SEQ ID NO: 1 or to a parent polypeptide having the same amino acid sequence as the variant without said substitutions, e.g. wherein the improved stability is selected from the group consisting of detergent stability and thermostability.

10. A detergent composition comprising the DNase variant of any of claims 1-9 and at least one detergent adjunct ingredient.

11. Use of the DNase variant of any of claims 1-9 or the detergent composition of claim 10 in a cleaning process, such as laundry or hard surface cleaning such as dishwashing.

12. A method of cleaning an item, comprising exposing the item to a wash liquor comprising the DNase variant of any of claims 1-9 or the detergent composition of claim 10, e.g. wherein the item is a textile or a hard surface.

13. A method for laundering a textile, comprising:

a) exposing the textile to a wash liquor comprising the DNase variant of any of claims 1-9 or the detergent composition of claim 10;

b) completing at least one wash cycle; and optionally

c) rinsing the item.

14. A polynucleotide encoding the variant of any one of claims 1-9, a nucleic acid construct or expression vector comprising the polynucleotide, or a recombinant host cell transformed with the polynucleotide.

15. A method of producing a DNase variant, comprising:

a) cultivating the recombinant host cell of claim 14 under conditions suitable for expression of the variant; and

b) recovering the variant.

```
SEQ1    VPVNPEPDATSVENVALKTGSGDSQSDPIKADLEVKGQSALPFDVDCWAILCKGAPNVLQ
SEQ2    ---------------ALKTGSGDSQSDPIKADLEVKGQSALPFDVDCWAILCKGAPNVLQ
SEQ3    -----------------KTGSGDSQSDPIKADLEVKGQSALPFDVDCWAILCKGAPNVLQ
                         ****************************************

SEQ1    RVNEKTKNSNRDRSGANKGPFKDPQKWGIKALPPKNPSWSAQDFKSPEEYAFASSLQGGT
SEQ2    RVNEKTKNSNRDRSGANKGPFKDPQKWGIKALPPKNPSWSAQDFKSPEEYAFASSLQGGT
SEQ3    RVNEKTKNSNRDRSGANKGPFKDPQKWGIKALPPKNPSWSAQDFKSPEEYAFASSLQGGT
        ************************************************************

SEQ1    NAILAPVNLASQNSQGGVLNGFYSANKVAQFDPSKPQQTKGTWFQITKFTGAAGPYCKAL
SEQ2    NAILAPVNLASQNSQGGVLNGFYSANKVAQFDPSKPQQTKGTWFQITKFTGAAGPYCKAL
SEQ3    NAILAPVNLASQNSQGGVLNGFYSANKVAQFDPSKPQQTKGTWFQITKFTGAAGPYCKAL
        ************************************************************

SEQ1    GSNDKSVCDKNKNIAGDWGFDPAKWAYQYDEKNNKFNYVGK
SEQ2    GSNDKSVCDKNKNIAGDWGFDPAKWAYQYDEKNNKFNYVGK
SEQ3    GSNDKSVCDKNKNIAGDWGFDPAKWAYQYDEKNNKFNYVGK
        ******************************************
```

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/054907

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/22
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE NCBI Ref Seq [Online] NCBI; 24 January 2020 (2020-01-24), Kjaerbolling T ET AL: "uncharacterized protein BDW43draft_322506 [Aspergillus alliaceus]", XP055948978, Database accession no. XP_031895814 the whole document -----	1
A	WO 2017/064269 A1 (NOVOZYMES AS [DK]) 20 April 2017 (2017-04-20) 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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2023	22/05/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Strobel, Andreas

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2023/054907

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. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2023/054907

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