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LAUNDRY WASHING COMPOSITION

(57)

The invention relates to a composition comprising a serine protease, in particular subtilisin, a nuclease and specific surfactants, for the for the breakdown of hard-to-remove fixed skin sebum and regulation of kinetics of sweat crystal formation on fabrics of various materials. The composition can be included in a laundry washing detergent and can be used in a method for cleaning fabrics and/or for fabric care.

Microscopic assessment of the tissue surface

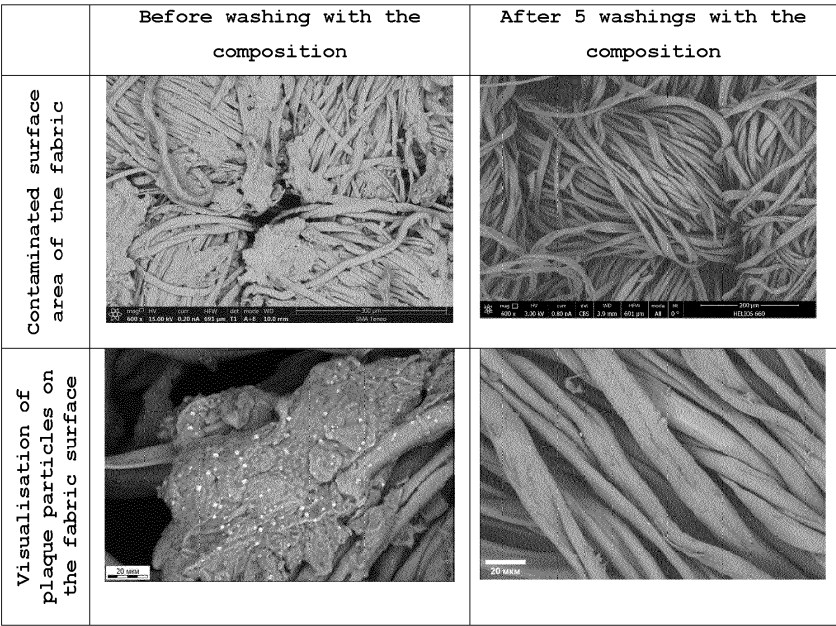


Fig. 1

Description**TECHNICAL FIELD**

[0001] The invention relates to a biodegradable deoxyribonuclease-based composition, particularly a laundry washing composition, for the breakdown of hard-to-remove fixed skin sebum and regulation of kinetics of sweat crystal formation on fabrics of various materials. The composition is intended to be included in detergents for fabric care, application of which increases efficiency of removal of fixed biocontaminations and human biostains enriched with nucleic acids, as well as regulation of sweat crystallization and decreases intensity of unpleasant smells. The composition is biodegradable, has a safe effect on the skin and can be used to prepare household detergents for sensitive skin, such as dry, liquid, concentrated laundry detergents, surface washes, with lasting cleanliness and a pleasant fragrance. The use of the composition may help to reduce the allergen load on the consumer caused by the specific effect on the bacterial flora.

THE PRIOR ART AND THE GIST THE INVENTION AS DEFINED IN THE CLAIMS

[0002] The development of household care products with improved performance for the consumer remains one of the most promising trends in the home care sector. According to analysts, consumers in developed European countries (France, UK, Germany, Italy) have become 39% more likely to pay attention to the composition of the laundry detergent they buy and carefully examine the ingredients for safety for humans and the environment [survey of 1,933 people using the Kantar Profiles/Mintel resource; A year of innovation in fabric and dish care, 2021, Mintel]. More than 65% of Chinese consumers are willing to pay more for household detergents containing natural substances that are as effective as traditional synthetic laundry detergents [KuRunData/Mintel survey of 2,100 people; A year of innovation in fabric and dish care, 2021, Mintel]. 31% of Canadian consumers are looking for household cleaning products without harmful chemical additives [Kantar Profiles/Mintel survey of 1,429 people; A year of innovation in fabric and dish care, 2021, Mintel]. 49% of consumers in Peru and other Latin American countries would like to see more ethical and effective household cleaning products with clear environmental initiatives by brands at various points of sale. [survey of 1,000 people by Offerwise/Mintel; A year of innovation in fabric and dish care, 2021, Mintel].

[0003] The activity of various compounds, in particular proteases and nucleases, has been investigated to achieve a high cleaning capacity of household cleaners. However, their combined use to date has presented a number of challenges of a biochemical nature, as proteases cleave peptide bonds in proteins and nucleases cleave phosphodiester bonds in nucleic acids (Veluchamy et al., 2011; Morales-Garcia et al., 2020).

[0004] Subtilisin is a proteolytic enzyme of the serine proteinase class. It is produced by bacteria mainly of the genus *Bacillus*, particularly *Bacillus subtilis*, to protect against other microorganisms. Subtilisin is involved in the cleavage of proteins through the hydrolysis of peptide bonds. It initiates a nucleophilic attack on the peptide bond via a serine residue that is located in its active site (Marget et al., 2022). The enzyme has antimicrobial and fungicidal properties and inhibits the formation of microbial biofilms. Subtilisin is widely used in household chemicals, cosmetics, food processing and pharmaceuticals (Veluchamy et al., 2011).

[0005] As subtilisin is a protease that cleaves proteins, including nucleases, it is used in stability experiments. Nucleases are a broad group of enzymes that cleave phosphodiester bonds between nucleic acid subunits. A distinction is made between ribonucleases (RNases) and deoxyribonucleases (DNases). The first cleaves ribonucleic acids (RNA), the latter cleaves deoxyribonucleic acids (DNA).

[0006] Taniuchi et al. investigated the stability of the staphylococcal nuclease and used subtilisin along with other nucleases. The authors showed that subtilisin cleaves the N-terminus of the nuclease (residues 1 to 5) (Taniuchi et al., 1969). Subtilisin can not only cleave nucleases but also inhibit the nuclease activity of enzymes. Yu et al. investigated the activity of the enzyme RecB, which has helicase and nuclease activities. Although scientists had previously shown that the 30 kDa domain at the C-terminus of RecB has nuclease activity, no such activity was detected after treatment with subtilisin (Yu et al., 1998). Hauser and Gray Jr. showed that subtilisin cleaves proteobacterium *Alteromonas espejiana* F and S nucleases and critically reduces their exonuclease activities against duplex DNA (Hauser et al., 1990). All the mentioned nucleases have DNAase activity, as does the nuclease described in the present patent application.

[0007] Despite the conflict between subtilisin and deoxyribonuclease described in the literature, the authors of the invention unexpectedly found a synergistic effect between subtilisin and bionuclease derived from fungi and yeast-like fungi if, and only if, a surfactant as defined as per component C) in claim 1 of the attached claim set is added. When these three components A, B and C are used together as defined in claim 1, a high cleaning performance of the composition is observed. It is important to note that the applicants' own studies and experiments also showed that the addition of EDTA or replacement of component C) of claim 1 with EDTA produced no success, which appears to be due to digestion and/or inhibition of the nuclease proteinase, as indicated with reference to prior art studies (no experimental data are provided). In addition, on the other hand, additional data of the applicants (not presented in the present application) show that the advantageous technical and synergistic effect provided by the combination of the claimed protease

and nuclease can be further enhanced by adding glycerol to the said composition as an additional component. The technical result of the innovative combination or complex based on nuclease and protease (subtilisin) consists in effective removal of biostains and regulation of kinetics of crystal formation of sweat enriched with nucleic acids, removal of unpleasant smells and keeping enzymatic stability of enzymes in combined presence in laundry detergents compositions. The complex is active over a wide pH range, in particular at a pH of 6.0-11.0, and over a wide temperature range, in particular from +15 to +60 °C, which broadens the scope of application in eco-friendly products to preserve the planet's resources.

[0008] The combination of nuclease and protease (subtilisin) was found to increase the removal efficiency of complex biological and protein soiling on various fabrics, in particular cotton, synthetic, mixed, delicate fabrics, as well as to increase the detergent efficiency by more than 15% on various fabrics while maintaining surfactant content based on components of natural origin. Nuclease and protease (subtilisin) are understood to be raw materials containing the respective pure active ingredients as well as technical impurities that may have formed during production of the target raw materials.

[0009] Combining the components allows for synergistic action and maintains efficacy at a lower percentage of input of the individual components. Nuclease, produced by modern non-GMO biotechnology methods, is an active enzyme that cleaves insoluble nucleic substrates with phosphorus diether bonds. Protease (subtilisin) acts as an additive enzyme to break down peptide bonds in the most common proteinaceous contaminants, as well as in exfoliated or peeled epidermal cells. An additional property is the regulation of the kinetics of nucleic acid enriched sweat crystal formation, for deep hygienic cleansing and whitening of clothes. It also neutralises odorous aromatic substances represented by different classes of organic substances: aldehydes and ketones, terpenes, amines, indoles, sulphur-containing components (mercaptans), organic acids and their esters, phenols and cresols.

[0010] The innovative complex according to claim 1, comprising in particular nuclease and protease (subtilisin), aims at effective cleaning and removal of biocontamination through regulation of reaction kinetics of nucleic acids breakdown and sweat crystals formation, increasing washing efficiency of household cleaning products and neutralization of unpleasant odours. The complex based on natural and biodegradable components is effective in a pH range of 6.0-11.0 in the presence of different synthetic and natural components, that allows its use as a part of wide range of household chemistry products for washing of different fabrics. The components target complex biological contaminations on various fabrics and also bind excreted metabolic products with an unpleasant aroma. Thus, the combined use of the components of the claimed composition in a single agent leads to an increase in the kinetics of the enzymatic cleavage reaction of complex biological contaminants by increasing the activity of nuclease due to protease (subtilisin), which allows for fast spot removal even in conditions of cold water. A distinctive feature is that the components in the stated concentrations act only against difficult soiling and do not affect the consumer appearance of most fabrics, in particular cotton, synthetic, mixed and delicate. Aggressive inorganic surfactants and organic solvents, occlusive film-formers and alcohols, quaternary ammonium bases, silicones, optical brighteners, chlorine and phosphorus derivatives are absent in the composition, so it is possible to use detergents and cleaners with this composition on a regular basis without any harm to human skin. The combined use of these ingredients has a synergistic effect, providing complete care for different fabrics in a single household detergent for everyday use.

[0011] Thus, the invention as a whole relates to the composition and its application, allowing to achieve technical results such as effective enzymatic biostain cleavage with simultaneous effective neutralisation of unpleasant odours on various types of fabrics and regulation of kinetics of crystallisation of sweat enriched with nucleic acids, with maintenance of long-term cleanliness and colour of fabrics in coordinates ΔL^*a^*b , which are not achieved or not sufficiently achieved by modern commercially available means in the present technical field.

ESSENCE OF INVENTION

[0012] In a first aspect, the invention relates to a composition intended for use in laundry detergents and is active at pH of 5.5-10.5 and a water hardness of 0-15.0 °dH, corresponding to "soft" and "medium" hardness (while higher water hardness is not excluded, even though less desired), consisting of:

(A) a bionuclease obtained biotechnologically from fungi or yeast-like fungi resistant to the presence of a protease, wherein the said bionuclease has a biological activity greater than 100 U/g, a pH of a 1-10% solution of 4.0-7.0 at 25 °C, a viscosity of a 1-10% solution of 0-1000 sPas at 25 °C;

(B) a bioprotease obtained biotechnologically from microorganisms selected from the group of serine endopeptidases with activity of at least 80 U/g, preferably at least 100 U/g, a pH of a 1-10% solution of 4.0-7.0 at 25 °C, a viscosity of a 1-10% solution of 0-1000 sPas at 25 °C;

(C) an aerobically rapidly biodegradable surfactant selected from an alkyl carboxylate and/or alkyl sulfate and/or alkyl polyethylene glycol sulfate with the general formula $R^1-O(-CH_2-CH_2-O)^{n^1}(SO_3)^{n^2}X^1$, wherein n^1 takes a value from 0 to 10 and denotes the number of polyethylene groups, R^1 is an alkyl and/or alkenyl group with a

hydrocarbon chain length of 5 to 22 carbon atoms, n^2 takes a value from 0 to 1 and denotes the number of sulfate groups, X^1 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid, wherein the mass ratio of components A, B and C is (0.00125-0.25):(0.0005-0.25):(0.50-25.00) respectively.

[0013] In a second aspect, the invention relates to the use of the composition according to the invention in a household detergent for washing laundry. The household detergent according to the invention may contain 0.50 to 25.50 wt.% of the composition according to the invention.

[0014] The composition may differ in that said nuclease is in a water-glycerol or water-sorbitol or water-glycerol-sorbitol or water-propylene glycol solution, or in a powdered or granulated form with various excipients. The dry nuclease is a commercially available product Pristine® 100T (<https://biosolutions.novozymes.com/en/pristine/products/pristine-100-t>).

[0015] The composition may be characterized in that said aqueous-glycerol nuclease solution is the commercially available product Pristine® 100L. Pristine® 100L is available in particular from Novozymes and can be identified, for example, by <https://biosolutions.novozymes.com/en/pristine/products/pristine-100-1>.

[0016] The composition may be characterized in that said aqueous-sorbitol nuclease solution is a commercially available Pristine® 100L product modified with an additional amount of sorbitol.

[0017] The composition may be characterized in that it additionally contains an alkyl polyethylene glycol sulfate with the general formula $R^1-O(-CH_2-CH_2-O)n^1(SO_3)n^2X^1$, wherein n^1 takes a value from 0 to 10 and denotes the number of polyethylene groups, R^1 represents an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 22 carbon atoms, n^2 takes a value from 0 to 1 and denotes the number of sulfate groups, X^1 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid.

[0018] The composition may be characterized in that said alkyl polyethylene glycol sulfate with the general formula $R^1-O(-CH_2-CH_2-O)n^1(SO_3)n^2X^1$ is the commercially available product EMAL 270D or СУЛЬФАНОР™ (SULFANOR™) Sulfoethoxylates of fatty alcohols. EMAL 270D is available in particular from KAO and can be identified for example by <https://chemical.kao.com/en/products/B0001816en/?region=gl>. СУЛЬФАНОР™ (SULFANOR™) Sulfoethoxylates of fatty alcohols is available from Norkem, in particular, and can be identified, for example, by <https://www.norchem.ru/products/anionnye-pav/sulfanor-sulfoetoxilaty-zhirnyh-spirov-sles-marki-b1-b2-b2k-b3.html>.

[0019] The composition may be characterized in that the mass ratio of Pristine® 100L/ Pristine® 100T and EMAL 270D/ СУЛЬФАНОР™ (SULFANOR™) is (0.00125-0.25):(0.50-25.00).

[0020] The composition may be characterized in that the activity of said nuclease is at least 100 U/g at pH 4.0-7.0.

[0021] The composition may differ in that the activity of the said protease is at least 80 U/g, preferably at least 100 U/g, at pH 4.0-7.0.

[0022] The composition may be characterized in that said household detergent is selected from laundry detergent, including delicate laundry detergent or baby and child laundry detergent, laundry conditioner, stain remover for pre-treating and washing laundry, laundry gel and laundry conditioner.

[0023] In another aspect, the invention relates to a laundry detergent containing 0.50-25.50 wt.% of the composition according to the invention.

[0024] The laundry detergent may be characterized in that the detergent in question is selected from a delicate laundry detergent and a baby or child laundry detergent.

[0025] The laundry detergent may be characterized in that the detergent in question is selected from a detergent for membrane fabrics and a detergent for sportswear.

[0026] The laundry detergent may be characterized in that the detergent in question is a powder laundry detergent.

[0027] In another aspect, the invention relates to the use of a composition according to the invention for enzymatic degradation of biostains, regulation of the kinetics of nucleic acid-rich sweat crystallisation and neutralisation of unpleasant odours on various fabrics, maintaining long-lasting cleanliness and pleasant fragrance.

[0028] The use may be characterized in that said fabric is selected from cotton, synthetic, blended, delicate (wool, silk, cashmere, merino, down, feather and their mixtures), membrane.

[0029] The invention will be further explained in detail by way of specific embodiments and illustrated by exemplary realisations.

DETAILED DESCRIPTION OF THE INVENTION

[0030] In a first aspect, the invention relates to a composition intended for use in laundry detergents and active at a pH of 5.5-10.5 and a water hardness of 0-15 °dH, consisting of:

(A) a bionuclease obtained biotechnologically from fungi or yeast-like fungi resistant to the presence of a protease, wherein the said bionuclease has a biological activity greater than 100 U/g, a pH of a 1-10% solution in the range 4.0-7.0 at 25 °C, a viscosity of a 1-10% solution in the range of 0-1000 sPas at 25 °C;

(B) a bioprotease obtained biotechnologically from microorganisms selected from the group of serine endopeptidases with an activity of at least 80 U/g, preferably of at least 100 U/g, a pH of a 1-10% solution in the range of 4.0-7.0 at 25 °C, a viscosity of a 1-10% solution in the range of 0-1000 sPas at 25 °C;

(C) an aerobically rapidly biodegradable surfactant selected from an alkyl carboxylate and/or alkyl sulfate and/or alkyl polyethylene glycol sulfate with the general formula $R^1-O(-CH_2-CH_2-O)n^1(SO_3)n^2X^1$, wherein n^1 takes a value from 0 to 10 and denotes the number of polyethylene groups, R^1 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 22 carbon atoms, n^2 takes a value from 0 to 1 and denotes the number of sulfate groups, X^1 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid,

wherein the mass ratio of components A, B and C is (0.00125-0.25):(0.0005-0.25):(0.50-25.00) respectively.

[0031] The mass content of component A in said mass ratio may be 0.00125, 0.01, 0.05, 0.1, 0.2 or 0.25 or any value in between.

[0032] The mass content of component B in said mass ratio may be 0.0005, 0.001, 0.01, 0.1, 0.2 or 0.25 or any value in between.

[0033] The mass content of component C in the said mass ratio may be 0.5, 1, 5, 10, 15, 20 or 25 or any value in between.

[0034] The composition may be characterized in that the bionuclease is a nuclease or deoxyribonuclease (DNAase) obtained biotechnologically from microorganisms, in particular fungi or yeast-like fungi. The nuclease can be a substance or a commercially available product with registration number CAS 9003-98-9.

[0035] The composition may be characterized in that the bioprotease is a protease from the class of serine proteases obtained biotechnologically from microorganisms, in particular bacteria. The protease can be a substance or a commercially available product with the registration number CAS 9014-01-1.

[0036] The composition may be characterized in that the alkyl and/or alkyl sulfate and/or alkyl polyethylene glycol sulfate may be a C12-C18 monoalkyl sulfate, such as Sulfopon G1218 or Sulfopon G1218 MB, C10-16 alkyl polyethylene glycol sulfate 1-3 EO, such as EMAL 270D or СУЛЬФАНОР™ (SULFANOR™) Sulfoethoxylates of fatty alcohols, potassium cocoate such as Mackadet 40K or Eurasol KPZ SG or C12-C18 fatty acids sodium salts such as sodium palmitate or another commercially available components. These products specified are commercially available products and are suitable for incorporation into the formulations.

[0037] The present invention also relates to the use of the composition according to the invention in a household detergent.

[0038] The household detergent according to the invention may contain 0.50-50.25 wt.% of the composition according to the invention. For example, the household detergent according to the invention may contain 0.50, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 or 50.25 wt.% or any value in between of said compositions according to the invention.

[0039] The present invention also relates to a laundry agent, a laundry agent for sportswear or a laundry agent for baby's or children's laundry containing 0.50-50.25 wt.% of the composition according to the invention.

[0040] The present invention also relates to a powder detergent for washing white and coloured laundry containing 0.50-50.25 wt.% of the composition according to the invention.

[0041] In the composition according to the invention, the acceptable excipients may be selected from the following categories of components.

[0042] The remedy or composition according to the invention may contain, wt. %:

nuclease <i>Nuclease or Deoxyribonuclease or DNAse</i>	0.00125-0.25%,
protease <i>Protease or Subtilisin</i>	0.00050-0.25%,
alkyl and/or alkyl sulphate and/or alkyl polyethylene glycol sulphate with the general formula	
$R^1-O(-CH_2-CH_2-O)n^1(SO_3)n^2X^1$	0.5-25.00%.

[0043] The present invention also relates to the use of a composition according to the invention to remove biostains and regulate the kinetics of nucleic acid enriched sweat crystallisation on various fabrics, including cotton, synthetic, mixed, membrane fabrics, to maintain long lasting cleanliness and whiteness.

[0044] The present invention also relates to a high performance blend intended for use in household cleaning products, consisting of: 1) the nuclease Pristine® 100L or Pristine® 100T; 2) a protease (subtilisin) as part of the enzyme complex Medley Brilliant 300L, and 3) an anionic surfactant selected from Eurasol KPZ SG, EMAL 270D, СУЛЬФАНОР™ (SULFANOR™) Sulfoethoxylates of fatty alcohols, Sulfopon 1216G, MASCID 2012 or a mixture of two or more of these,

wherein the specified ingredients of the blend are taken in the ratio (0.00125-0.25):(0.0005-0.25):(0.5-25) respectively. The above products are commercially available products and are intended for inclusion in formulations of household chemicals. Such a blend has all the advantages of the composition described herein and is intended for use in household chemicals such as, laundry detergent, sports laundry detergent, baby or child laundry detergent, powdered laundry detergent.

[0045] The mass content of component (1) in said mass ratio may be 0.00125, 0.01, 0.05, 0.1, 0.2 or 0.25 or any value in between said values. The mass content of component (2) in said mass ratio may be 0.0005, 0.001, 0.01, 0.1, 0.2 or 0.25, or any value in between said values. The mass content of component (3) in said mass ratio may be 0.5, 1, 5, 10, 15, 20 or 25 or any values in between said values.

[0046] The composition preferably does not contain other active agents and/or excipients, such as washing active agents and/or acceptable excipients, but may contain them. Such agents may represent or represent agents traditionally used in the art and which are known to the skilled person in the art. The addition of said agents to the composition of the complex according to the invention does not invalidate the achievement of the claimed technical results, but can improve them.

[0047] Within the scope of the invention are also household chemicals such as laundry detergent, laundry detergent for sportswear, laundry detergent for baby's or children's laundry, powdered laundry detergent.

[0048] All mass fractions, mass parts, mass percentages as well as volume fractions, volume parts, volume percentages in the present disclosure are given in relation to the preparation, agent, composition or product to which they relate in the context specified.

[0049] In a household detergent according to the invention, the acceptable excipients may be selected from the following categories of components.

Anionic surfactants:

[0050]

Amide salt of a higher fatty acid and methylglycine with the general formula $R_4-C(O)-N(-CH_3)-CH_2-CO_2X_4$, wherein R_4 is an alkyl and/or alkenyl group with a hydrocarbon chain length from 5 to 21 carbon atoms and X_4 is an alkali and/or alkaline earth metal cation, ammonium, alkanolammonium, glucoammonium;

An alkyl polyethylene glycol carboxylate with the general formula: $R_5-O(-CH_2-CH_2-O)_n-CH_2-CO_2X_5$, wherein n can take values from 1 to 15 and denotes the number of polyethylene glycol groups, R_5 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms and X_5 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

A bisubstituted salt of 2-sulfo carboxylic acid with the general formula: $R_6-CH(-SO_3X_6)-CO_2X_6$, wherein R_6 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 4 to 20 carbon atoms and X_6 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

A mono- or divalent amide salt of a higher carboxylic acid and glutamic acid with the general formula: $R_7-C(O)-NH-CH(-CH_2-CH_2-CO_2X_7)-CO_2X_7$, wherein R_7 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms and X_7 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium or hydrogen;

An amide salt of a higher fatty acid and glycine with the general formula: $R_8-C(O)-NH-CH_2-CO_2X_8$, wherein R_8 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, and X_8 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An amide salt of a higher fatty acid and alanine with the general formula: $R_9-C(O)-NH-CH(-CH_3)-CO_2X_9$, wherein R_9 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms and X_9 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An amide salt of a higher fatty acid and 2-aminomethylethanesulfonic acid with the general formula: $R_{10}-C(O)-N(-CH_3)-CH_2-CH_2-SO_3X_{10}$, wherein R_{10} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, and X_{10} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An alkylpolyglucoside hydroxypropyl sulfonate with the general formula: $R_{11}-O-[G]p_1-O-CH_2-CH(-OH)-CH_2-SO_3X_{11}$, wherein R_{11} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms, G is a saccharide fragment containing 5 or 6 carbon atoms, p_1 can take values from 1 to 4, and X_{11} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An alkylpolyglucoside carboxylate with the general formula: $R_{12}-O-[G]p_2-O-CH_2-CO_2X_{12}$, wherein R_{12} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms, G is a saccharide fragment containing 5 or 6 carbon atoms, p_2 can take values from 1 to 4, and X_{12} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An amide salt of a higher fatty acid and a threonine with the general formula: $R_{13}-C(O)-NH-CH(-CH(-OH)-CH_3)-CO_2X_{13}$, wherein R_{13} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, and X_{13} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An amide salt of a higher fatty acid and an amino acid obtained by hydrolysis of proteins from vegetable raw materials, with the general formula: $R_{14}-C(O)-AAX_{14}$, wherein R_{14} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, AA is an amino acid or peptide obtained by hydrolysis of plant protein (possible protein sources: apple, soybean, wheat, cotton etc.), and X_{14} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium.

Amphoteric surfactants:

[0051]

A divalent salt of acylamphodiacetate with the general formula: $R_{15}-C(O)-NH-CH_2-CH_2-N(-CH_2-CO_2X_{15})-CH_2-CH_2-O-CH_2-CO_2X_{15}$, wherein R_{15} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms and X_{15} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An acylamphoacetate salt with the general formula: $R_{16}-C(O)-NH-CH_2-CH_2-N(-CH_2-CO_2X_{16})-CH_2-CH_2-OH$, wherein R_{16} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms and X_{16} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An alkylamphoacetate salt with the general formula: $R_{17}-C(=N-CH_2-CH_2-N(-CH_2-CH_2-OH)-CH_2-CO_2X_{17})-$, wherein R_{17} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms and X_{17} is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium;

An acylamidoalkylbetaine with the general formula: $R_{18}-C(O)-NH-R_{19}-N(-CH_3)_2-CH_2-CO_2$, wherein R_{18} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, R_{19} is an alkyl group with a hydrocarbon chain length of 1 to 4 carbon atoms;

An acylamidoalkylhydroxysultaine with the general formula: $R_{20}-C(O)-NH-R_{21}-N(-CH_3)_2-CH_2-CH(-OH)-CH_2-SO_3$, wherein R_{20} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, R_{21} is an alkyl group with a hydrocarbon chain length of 1 to 4 carbon atoms;

An acylamidoalkylamine oxide with the general formula: $R_{22}-C(O)-NH-R_{23}-N(-CH_3)_2-O$, wherein R_{22} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, R_{23} is an alkyl group with a hydrocarbon chain length of 1 to 4 carbon atoms;

An alkylbetaine with the general formula: $R_{24}-N(-CH_3)_2-CH_2-CO_2$, wherein R_{24} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms;

An alkylhydroxysultaine with the general formula: $R_{25}-N(-CH_3)_2-CH_2-CH(-OH)-CH_2-SO_3$, wherein R_{25} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms;

An alkylsultaine with the general formula: $R_{26}-N(-CH_3)_2-CH_2-CH_2-CH_2-SO_3$, wherein R_{26} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms;

An alkylamine oxide with the general formula: $R_{27}-N(-CH_3)_2-O$, wherein R_{27} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms.

Nonionic surfactants:

[0052]

An alkyl glucoside with the general formula: $R_{28}-O-[G]p_3$, wherein R_{28} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 4 to 22 carbon atoms, G is a saccharide fragment containing 5 or 6 carbon atoms, p_3 can take values from 1 to 4;

An alkylpolyethylene glycol with the general formula: $R_{29}-O(-CH_2-CH_2-O-)_{n_3}H$, wherein n_3 can take values from 2 to 20, and denotes the number of polyethylene glycol groups, R_{29} being an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms;

An alkylpolyethylene/propylene glycol with the general formula: $R_{30}-O(-CH_2-CH_2-O-)_{n_4}(-CH(-CH_3)-CH_2-O-)_{n_5}H$, wherein n_4 can take values from 2 to 20 and denotes the number of polyethylene glycol groups, n_5 can take values from 2 to 20 and denotes the number of polypropylene glycol groups, R_{30} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms;

A dialkyl polyethylene glycol with the general formula: $R_{31}-O(-CH_2-CH_2-O-)_{n_6}R_{32}$, wherein n_6 can take values from 2 to 20, and denotes the number of polyethylene glycol groups, R_{31} being an alkyl and/or alkenyl group with

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a hydrocarbon chain length of 6 to 22 carbon atoms, R32 being an alkyl and/or alkenyl group with a hydrocarbon chain length of 1 to 12 carbon atoms;

A dialkylpolyethylene/propylene glycol with the general formula: $R_{33}-O(-CH_2-CH_2-O-)_{n7}(-CH(-CH_3)-CH_2-O-)_{n8}-R_{34}$, wherein $n7$ can take values from 2 to 20, and denotes the number of polyethylene glycol groups, $n8$ can take values from 2 to 20, and denotes the number of polypropylene glycol groups, R33 is an alkyl and/or alkenyl group with a hydrocarbon chain length from 6 to 22 carbon atoms, R34 is an alkyl and/or alkenyl group with a hydrocarbon chain length from 1 to 12 carbon atoms.

Dispersing medium for the polysaccharide/solvent:

[0053]

An organic alcohol with the general formula: $R_{35}(-OH)_{s1}$, wherein R35 is an alkyl group with a hydrocarbon chain length from 3 to 12 carbon atoms, $s1$ can take values from 1 to 12, and denotes the number of hydroxyl groups arranged randomly in a hydrocarbon radical relative to each other;

An alkylpolypropylene glycol with the general formula: $H(-CH(-CH_3)-CH_2-O-)_{n9}R_{36}$, wherein $n9$ can take values from 2 to 10 and denotes the number of polypropylene glycol groups, R36 is an alkyl group with a hydrocarbon chain length from 1 to 10 carbon atoms.

pH regulators:

[0054]

Organic acids with the general formula: $R_{37}(-OH)_{s2}(-COOH)_{m1}$, wherein R37 is an alkyl group with a hydrocarbon chain length of 1 to 12 carbon atoms, $s2$ can take values from 1 to 12 and denotes the number of hydroxyl groups arranged in the hydrocarbon radical in random order with respect to each other, $m1$ can take values from 1 to 4 and denotes the number of carboxyl groups arranged in the hydrocarbon radical in random order with respect to each other;

Solutions of alkali or alkaline earth metal hydroxides, ammonia, primary and tertiary alkylamines, primary and tertiary alkanolamines, primary and tertiary glucamines, basic amino acids, disodium salt of citric acid, trisodium salt of citric acid.

Chelator:

[0055]

Trisodium salt of methylglycidylacetic acid, tetrasodium salt of glutamylacetic acid, trisodium salt of ethylenediamine-(N,N)-disuccinate;

Phosphonic acid esters with the general formula $RP(O)(OR'_1)_n(OH)_{2-n}$, wherein R, R' are organic radicals, in particular the alkyl, alkenyl or aryl radical, and can be primary ($n=1$, acidic phosphonates) and secondary ($n=2$, full phosphonates) depending on the hydroxyl groups;

Organic acids, as well as salts of alkali metals, ammonium, alkylammonium, alkanolammonium, glucoammonium, corresponding to these acids: citric acid, malic acid, tartaric acid, glutaric acid, adipic acid, glucuronic acid, galacturonic acid, galactaric acid, gluconic acid, phytic acid, polytaconic acid, polyacrylic acid, polymethacrylic acid, copolymer of acrylic acid and maleic acid as well as organic acids with the general formula $R_{38}(-OH)_{s3}(-COOH)_{m2}$, wherein R38 is an alkyl group with a hydrocarbon chain length from 1 to 12 carbon atoms, $s3$ can take values from 1 to 12 and denotes the number of hydroxyl groups arranged in a hydrocarbon radical in random order with respect to each other, $m2$ can take values from 1 to 4 and denotes the number of carboxyl groups arranged in a hydrocarbon radical in random order with respect to each other.

Inhibitors of the reverse sedimentation of contamination:

[0056]

Polysaccharide derivatives: sodium salt of carboxymethyl polysaccharide, hydroxyalkyl polysaccharide, alkyl polysaccharide and cellulose gum;

Polyvinylpyrrolidone and its derivatives, copolymers of polyvinylpyrrolidone and vinylimidazole;

Water soluble salts of polyacrylic acid, polymethacrylic acid, copolymers of acrylic/methacrylic and maleic acid.

Defoamers:

[0057]

Higher carboxylic acids with the general formula: R39-CO₂H, wherein R39 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms;
Higher carboxylic alcohols with the general formula: R40-COH, wherein R40 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms;
Esters of higher carboxylic alcohols with the general formula: R41-O-R42, wherein R41, R42 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 4 to 22 carbon atoms;
Bisamides of alkyl diamines and higher carboxylic acids with the general formula: R43-C(O)-NH-R44-NH-C(O)-R45, wherein R43, R45 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms and R44 an alkyl radical with a hydrocarbon chain length of 1 to 12 carbon atoms;
Builders and fillers: sodium silicate, sodium sulfate, sodium chloride, sodium carbonate and other commercially available components;

Preservatives:

[0058]

Organic acids and salts of alkali and alkaline earth metals, ammonium, alkylammonium, alkanolammonium, glucoammonium corresponding to these acids: benzoic acid, sorbic acid, 4-methoxybenzoic acid, salicylic acid, undecylenic acid;
Organic alcohols and phenols: phenoxyethanol, benzyl alcohol, caprylyl glycol, ethylhexyl glycerol, phenethyl alcohol, 3-methyl-4-isopropyl phenol, 2,4-dichlorobenzyl alcohol;
Broad spectrum biocides: benzisothiazolinone, dodecylpropylene triamine, methylisothiazolinone, mixture of these, sodium pyrithione, laurylamine dipropylenediamine and other commercially available components;
Fungicides: sodium pyrithione, climbazole and other commercially available components.
Enzymes: protease (ficin, bacillolysine, subtilisin), alpha-amylase, pectate lyase, mannanase, mannosidase, cellulase, amino oxidase, feruloyl esterase, beta-glucanase, tannase, alpha-glucosidase, beta-glucosidase, alpha-galactosidase, beta-galactosidase, laccase, manganese peroxidase, licheninase, xylanase and other commercially available enzymes used in laundry, dishwashing, floor, glass and all-purpose cleaners.
Bleaching agents based on oxygen compounds: hydrogen peroxide, calcium peroxide, carbamide peroxide, ε-phthalimidoperoxycaproic acid, sodium carbonate peroxide, TAED and other commercially available components.
Essential oil fragrances or essential oils in pure form or as blends in various proportions: orange, bergamot, lemon, lime, mandarin, grapefruit, neroli, rosewood, yuzu, lemongrass, lavender, sage, thyme, melissa, mint, tea tree, eucalyptus, cedar, sandalwood, black pepper, pink pepper, cinnamon, cardamom, coriander, jasmine, rose, peony, blue camomile.

[0059] In another aspect, the invention relates to the use of the composition according to the invention for enzymatic cleavage of biostains, regulation of nucleic acid enriched sweat crystallisation and neutralisation of unpleasant odours on various fabrics, maintaining a long lasting purity and pleasant fragrance. The use may be characterized in that said fabric is selected from cotton, synthetic, blended, delicate (wool, silk, cashmere, merino, down, feather and their mixtures), membrane.

EXPERIMENTAL SECTION

[0060] The examples included in the present description are not intended to limit the claimed invention and are merely intended to illustrate and confirm the achievement of the expected technical results. These examples are among the many experimental data obtained by the authors of the invention which confirm the effectiveness of the means within the scope of the invention. With regard to the examples of the invention given below, it should be noted that a bioprotease having the CAS number 9014-01-1 and a bionuclease having the CAS number 9003-98-9 were used.

[0061] Before proceeding to a detailed examination of the examples illustrating the invention according to the claims, it should be recalled that the present invention was made on the basis of the surprising discovery that component C) appears to be a "game-changer" for combining components A) and B) to obtain beneficial and synergistic technical effects of the claimed composition, as described in paragraph [0007] above. When these three components A, B and C are used together as defined in claim 1, a high cleaning performance of the composition was observed. It is important to note that the applicants' own studies and experiments also showed that the addition of EDTA or replacement of

component C) of claim 1 with EDTA had no effect (no experimental data are provided herewith), which appears to be due to digestion and/or inhibition of the nuclease by the proteinase, as indicated with reference to prior art studies mentioned above. In addition, on the other hand, additional data of the applicants (not presented in the present application) show that the advantageous technical and synergistic effect provided by the combination of the claimed protease and nuclease can be further enhanced by adding glycerol to the said composition as an additional component.

Example 1

[0062] The components for inclusion in the composition according to the invention were investigated in various laundry detergents. A liquid detergent (formula No. 1), in particular a universal laundry detergent for white and coloured laundry, was prepared within the scope of the invention (Table 1).

Table 1. Formulation of the liquid laundry detergent with the claimed composition

No.	Component	Content, wt. %
1	purified water	up to 100.00
2	alkyl polyethylene glycol sulfate with the general formula $R^1-O(-CH_2-CH_2-O)n^1(SO_3)n^2X^1$, wherein n^1 takes a value from 0 to 10 and denotes the number of polyethylene groups, R^1 represents an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 22 carbon atoms, n^2 takes a value from 0 to 1 and denotes number of sulfate groups, X^1 represents alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid	2.5-10.00
3	alkyl glucoside with the general formula: $R^{28}-O-[G]p^3$, wherein R^{28} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 4 to 22 carbon atoms, G is a saccharide fragment containing 5 or 6 carbon atoms, p^3 can take values from 1 to 4, in particular, C10-16 alkyl polyglucoside and C8-10 alkyl polyglucoside	1.5-10.00
4	acylamidoalkylbetaine with the general formula: $R^{18}-C(O)-NH-R^{19}-N(-CH_3)_2-CH_2-CO_2$, wherein R^{18} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms, R^{19} is an alkyl group with a hydrocarbon chain length of 1 to 4 carbon atoms	0.5-5.00
5	Mixture of ethoxylated alcohols and fatty acids with pH of 4.5 (EN 1262 method), cloud point 44-53 °C (EN 1890 method), density 0.95 g/ml (DIN 51757 method) and viscosity 70 mPa*s (EM 12092 method, 23 °C, Brookfield, 60 rpm)	1.5-7.50
6	bionuclease	0.001-0.10
7	bioprotease	0.001-0.10
8	complexing agent MGDA	0.1-1.00
9	complexing agent, corrosion inhibitor based on an inulin derivative	0.1-1.00
10	glycerol of natural origin	1.0-5.00
11	trinatrium citrate dihydrate	0.5-3.00
12	sodium chloride	1.0-5.00
13	cotton seed extract <i>Cotton seed extract</i>	0.005-0.50
14	preservative	0.005-0.75
15	sodium hydroxide or potassium hydroxide	0.005-0.50
16	excipients if necessary	2.0-25.00

[0063] Prepared liquid detergent for laundry washing and stain removal provides high efficiency of nucleic acids breakdown in the biostains and reduction of crystallisation of sweat enriched with nucleic acids from various fabrics, in particular cotton, synthetic, membrane, linen, at any hardness of tap water of 0-15 °dH and at any washing temperature from +15 °C to +60 °C. It does not cause colour change and dye washout, retains the product's original appearance,

leaves no streaks, is completely rinsed from the surface of fabric, suitable for washing children's laundry and people with sensitive hands, stable in storage for 24 months (observation time).

Example 2

[0064] The components for inclusion in the composition according to the invention were investigated in various detergent formulations. A dry detergent (formula No. 2), in particular a powdered detergent for washing coloured laundry and stain removal, was prepared within the scope of the invention (Table 2).

Table 2. Formulation of the powdered detergent for washing coloured laundry with the claimed composition

No.	Component	Content, wt. %
1	purified water	2.5-7.5
2	alkyl sulphate with the general formula $R^3\text{-OSO}_3 X^3$, wherein R^3 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms and X^3 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium	2.5-10.0
3	alkylpolyethylene glycol ether with the general formula: $R^{29}\text{-O}(-\text{CH}_2\text{-CH}_2\text{-O})_{n^3}\text{H}$, wherein n^3 can take values from 2 to 20 and denotes the number of polyethylene glycol groups, R^{29} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms	2.5-10.0
4	alkyl carboxylate with the general formula $R^1\text{-O}(-\text{CH}_2\text{-CH}_2\text{-O})_{n^1}(\text{SO}_3)_{n^2} X^1$, wherein n^1 takes a value from 0 to 10 and denotes the number of polyethylene groups, R^1 represents an alkyl and/or alkenyl group with a hydrocarbon chain length from 5 to 22 carbon atoms, n^2 is 0 to 1 and denotes the number of sulfate groups, X^1 is	1.0-10.0
	an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid	
5	sodium carbonate	10.0-40.0
6	sodium sulphate	10.0-50.0
7	sodium silicate	0.5-10.0
8	sodium aluminosilicate in the form of zeolites	5.0-20.0
9	amorphous sodium aluminosilicate	1.0-10.0
10	TAED	0.1-1.5
11	bionuclease	0.001-0.25
12	bioprotease in the form of subtilisin	0.001-0.25
13	lipase, mannanase, cellulase, amylase	0.001-0.25
14	carboxymethylcellulose sodium salt	0.15-2.50
15	carboxymethyl cellulose	0.5-2.00
16	cotton seed extract <i>Cotton seed extract</i>	0.005-0.50
17	excipients if necessary	2.0-25.0

[0065] Prepared powdered detergent for washing coloured linen and stain removal ensures high efficiency of breaking down nucleic acids in the composition of biostains and reduction of crystallisation of sweat enriched with nucleic acids from various fabrics, in particular cotton, synthetic, membrane, linen, at any hardness of tap water 0-5 mg-eq/l and at any washing temperature from +15°C to +60°C. It does not cause colour change and dye washout, retains the product's original appearance, does not leave streaks, rinses off the surface of fabric, suitable for washing children's linen and people with sensitive hands, stable when stored for 36 months (observation time).

Example 3

[0066] The components for inclusion in the composition according to the invention were investigated in various detergent formulations. A dry detergent (formula No. 3), in particular a powdered detergent for washing white laundry and stain removal, was prepared within the scope of the invention (Table 3).

Table 3. Formulation of the powdered detergent for washing white laundry with the claimed composition

No.	Component	Content, wt. %
1	purified water	2.0-7.5
2	alkyl sulphate with the general formula $R^3\text{-OSO}_3 X^3$, wherein R^3 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms and X^3 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium	2.5-10.0
3	alkylpolyethylene glycol ether with the general formula: $R^{29}\text{-O}(-\text{CH}_2\text{-CH}_2\text{-O})_{n^3}\text{H}$, wherein n^3 can take values from 2 to 20 and denotes the number of polyethylene glycol groups, R^{29} is an alkyl and/or alkenyl group with a hydrocarbon chain length of 6 to 22 carbon atoms	1.0-10.0
4	alkyl carboxylate with the general formula $R^1\text{-O}(-\text{CH}_2\text{-CH}_2\text{-O})_{n^1}(\text{SO}_3)_{n^2} X^1$, wherein n^1 takes a value from 0 to 10 and denotes the number of polyethylene groups, R^1 represents an alkyl and/or alkenyl group with a hydrocarbon chain length from 5 to 22 carbon atoms, n^2 is 0 to 1 and denotes the number of sulfate groups, X^1 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid	1.0-10.0
5	sodium carbonate	10.0-40.0
	sodium carbonate peroxide	5.0-20.0
6	sodium sulphate	4.0-30.0
7	sodium silicate	0.5-10.0
8	sodium aluminosilicate in the form of zeolites	5.0-20.0
9	amorphous sodium aluminosilicate	1.0-10.0
10	TAED	0.5-5.0
11	bionuclease	0.001-0.25
12	bioprotease in the form of subtilisin	0.001-0.25
13	lipase, mannanase, cellulase, amylase	0.001-0.25
14	carboxymethylcellulose sodium salt	0.15-2.50
15	carboxymethyl cellulose	0.5-2.00
16	cotton seed extract <i>Cotton seed extract</i>	0.005-0.50
17	excipients if necessary	2.0-25.0

[0067] Prepared powdered detergent for washing white laundry and stain removal provides high efficiency of breaking down nucleic acids in the composition of biostains and reducing the crystallisation of sweat enriched with nucleic acids from various fabrics, in particular cotton, synthetic, membrane, linen, at any hardness of tap water of 0-15 °dH and at any washing temperature from +15°C to +60°C. It does not cause destruction of fabric structure and excessive washout of dye, retains the same appearance of the product, does not leave streaks, rinses off the surface of fabric, suitable for washing baby or children laundry and people with sensitive skin of hands, stable in storage for 36 months (observation time).

Example 4

[0068] A laboratory study of the detergent effectiveness of the composition as part of a universal liquid laundry detergent was carried out. As a base for introducing the components, a liquid universal laundry detergent for white and coloured laundry was used, as defined in Table 1.

[0069] The test methodology is based on the generally recognised recommendations of the European Association A.I.S.E. [A.I.S.E. Laundry Detergent Testing Guidelines Minimum requirements for comparative detergents testing, see https://www.aise.eu/documents/document/20180625164030-laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf,

to determine the detergent efficacy with regard to stain removal index (SRI) and the values L,a,b. The SRI is calculated using the formula from the ASTM D 4265 Standard guide for evaluating stain removal performance in home laundering [DOI: 10.1520/D4265-14]. This guide was prepared by the American Society for Testing and Materials (ASTM) Committee D-12 on soaps and other detergents and was published in 2014. This test evaluates the effectiveness of the composition in removing fresh and stale stains of various nature from cotton and synthetic materials simulating actual washing conditions. Washing was carried out in a Bosch WAB 24272 CE washing machine. Common recommendations in the European Union and Russian Federation were chosen as test conditions, in particular a temperature of 40 °C, a water hardness of 10.9 °dH and a standard wash mode (mixed fabrics). The concentration of the universal liquid detergent for white and coloured laundry was 4.5 g/l per standard load of a washing machine being 2-4 kg. Three repetitions (n=3) were carried out, and the deviation was no more than 0.18% for each sample.

[0070] Hard-to-remove biostains of different nature were used for the test (Table 4). The selected stubborn biostains allowed the overall detergent efficacy of the formulation to be assessed, as well as the efficacy against individual contaminants due to the presence of the composition of the invention.

Table 4. Biostains and biocontaminants tested from A.I.S.E.

Designation	Composition	Type
EMPA 106	mineral oil/soot	pigment-oil-based, dispersible
EMPA 118	skin grease/pigment	pigment-oil-based, dispersible
EMPA 116	blood/milk/ink	mixed: protein + pigment
EMPA 111	blood	enzymatic protein
CFT CS-27	starch/dye	enzymatic amylase
CFT CS-44	chocolate drink	mixed: protein + bleach
EMPA 164	grass	mixed: protein + bleach
C-P244PDE	human sebum with DNA	mixed: enzymatic + pigment

[0071] SRI is a quantitative measure of the removal of dirt and stains in units. The values are based on whiteness L and chromaticity a,b, calculated mathematically using a special formula from ASTM D 4265-14. The method has numerous advantages, in particular, high accuracy, reproducibility, low error rate (less than 5%), takes into account colours of laundry and their change in the process of washing, different types of fabric and is the closest to the real visual perception and balances out the effect of optical dyes, common in detergents for laundry washing. A difference of 2 or more SRI units is effective and statistically significant. In practical experience, increased 1 unit of SRI corresponds to 5% increased detergent efficacy and gives a significant contribution to the formulation.

Results

[0072] According to the results of evaluation of effectiveness and removal rate of stubborn biostains of different nature, it was established that the studied composition in the liquid universal laundry detergent for white and colored laundry has a strong stain-removing effect in respect of complex soiling of protein, pigment and oil, mixed nature based on low-fusible and refractory saturated and/or unsaturated acids and their monoacyl, diacyl and triacylglycerides, nucleic acids, coloured pigments and organic biopolymers, compared to the comparison means, not containing the composition according to the invention.

[0073] At the end of the study, marked changes were observed in the assessed index of removal efficiency of stubborn biostains. According to the dynamics of the index, the addition of high performance bionuclease in combination with bioprotease together with selected components of the composition increased the degree of removal of complex pigment-

oil, food, biological (blood, sebum, sweat) stains based on saturated and/or unsaturated acids and acylglycerides and nucleic acid derivatives. The addition of 0.006% bioprotease increased the SRI index by 54.2 units for protein, food enzymatic and stained contaminants saturated with biological substances based on peptides and proteins. When the components were combined, an increase in SRI was observed. The composition with 0.006% bioprotease and 0.005% bionuclease increased SRI by 77.9 units for all stains and in particular for sweat, skin sebum, grease and blood based on nucleic acids, showing synergism between the components and statistically significant result in removing biostains during washing ($p < 0.05$). The base sample without the composition of the invention did not show a very high removal of the selected biostains, indicating a lack of efficacy of the surfactants in removing the soiling during laundry (Table 5).

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Table 5. Assessment of the effectiveness of biocontamination removal

Test specimen	Components of the composition	SRI score								SRI amount
		EMPA 106	EMPA 118	EMPA 116	EMPA 111	EMPA 164	CFT CS-27	CFT CS-44	C-P244PDE	
sample No. 1 base	aerobically fast biodegradable surfactant SLES 5.25+0.25%	70.9	88.9	71.5	83.5	78.2	56.7	79.8	41.7	571.2
sample No. 2 base + bioprotease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.006%	71.2	88.9	79.5	88.9	81.0	79.1	85.3	51.5	625.4
sample No. 3 base + bioprotease + bionuclease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.006% bionuclease 0.005%	72.3	90.3	80.5	90.6	81.2	79.5	87.5	70.2	649.1

*Mean reliable difference, statistically significant (p<0.05), 10-20% increase in standard washing efficiency

**Best reliable difference, statistically significant (p<0.05), more than 20% improvement in standard washing efficiency

[0074] Complex biological contamination based on saturated and/or unsaturated acids and their acylglycerides, nucleic acids and protein residues, are difficult to remove in laundry washing due to fixation in hard to reach places and fibres of different fabrics, the formation of "biofilms" of skin sebum, oxidation of unsaturated fatty acids and insufficient action of surfactants. High levels of surfactants increase the residue on the fibres of the fabric, create large amounts of foam and drastically reduce the durability of the fabric, which can negatively affect the environment, increase water consumption, reduce washing efficiency and significantly increase fabric wear and colour fade.

[0075] The composition of a universal liquid detergent for washing coloured and white laundry based on natural aerobically biodegradable surfactants, bioprotease and bionuclease allows effective removal of soiling from surface and hard-to-reach areas of various fabrics, thus reducing the total amount of surfactants in household detergents, maintaining dermatological safety for humans and achieving almost complete removal of biological soiling from the laundry. Removal of fatty grease stains, sweat and sebum crystals, epidermal cell proteins and exfoliated particles provides a hygienic function, prevents excessive sorption of contaminants on fabric surfaces, rapid accumulation of bad smells on garments and reduces the number of washings in the long term. Increased SRI demonstrates high light-reflectivity of the fabric, the appearance of whiteness and a reduction of jaundice on the surface of the garment, indicating crystal-clear laundry without optical brighteners. The addition of bionuclease improves the effectiveness of removing food stains (cocoa, grass, fruit) and blood stains without damaging the fabric, and allows to regulate the content of bioprotease in the product, without exceeding the dermatological tolerance of protease in laundry detergents from HERA and ECHA. The reduced content of enzymes, in particular proteases, and surfactants allows for low toxicity, low skin irritation potential on the hands and the possibility of allergic reactions in humans when wearing laundry washed with the product.

Example 5

[0076] A laboratory study of the detergent efficacy of the composition in a powdered detergent for washing white laundry with the claimed composition was carried out. A powdered laundry detergent for coloured laundry was used as a base for introducing components, as shown in Table 2.

[0077] The test methodology is based on the generally recognised recommendations of the European Association A.I.S.E. [A.I.S.E. Laundry Detergent Testing Guidelines Minimum requirements for comparative detergents testing, see [https://www.aise.eu/documents/document/20180625164030-](https://www.aise.eu/documents/document/20180625164030-laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf)

[laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf](https://www.aise.eu/documents/document/20180625164030-laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf), to determine the detergent efficacy with regard to stain removal index (SRI) and the values L,a,b. The SRI is calculated using the formula from ASTM D 4265 Standard guide for evaluating stain removal performance in home laundering [DOI: 10.1520/D4265-14]. This guide was prepared by the American Society for Testing and Materials (ASTM) Committee D-12 on soaps and other detergents and was published in 2014. This test evaluates the effectiveness of the composition in removing fresh and stale stains of various nature from cotton and synthetic materials, simulating actual washing conditions. Washing was carried out in a Linetest B laboratory washing machine. Common recommendations within the European Union and Russian Federation were used as test conditions, in particular a temperature of 40°C, a water hardness of 10.9 °dH and a standard wash cycle (mixed fabrics). The concentration of powder detergent for washing white laundry was 7.0 g/l per standard washing machine load of 2-4 kg. Three repetitions (n=3) were carried out, and the deviation was no more than 0.20% for each sample.

[0078] Hard-to-remove biostains of different nature were used for the test (Table 6). The selected stubborn biostains allowed the overall detergent efficacy of the formulation to be assessed, as well as the efficacy against individual contaminants due to the presence of the composition of the invention.

Table 6. Biostains and biocontaminants tested from A.I.S.E.

Designation	Composition	Type
C-P244PDE	human sebum with DNA	mixed: enzymatic + pigmental

[0079] SRI is a quantitative measure of the removal of dirt and stains in units. The values are based on whiteness L and chromaticity a,b, calculated mathematically using a special formula from ASTM D 4265-14. The method has many advantages, in particular, high accuracy, reproducibility, low error (less than 5%), takes into account colours of laundry and their change in the process of washing, different types of fabric and is the closest to the real visual perception and balances out the effect of optical dyes, common in detergent composition for laundry washing. A difference of 2 or more SRI units is effective and statistically significant. In practical experience, increased 1 unit of SRI corresponds to 5% increased detergent efficacy and gives a significant contribution to the formulation.

Results

[0080] According to the results of evaluation of effectiveness and removal rate of stubborn biostains of different nature, it was found that the studied composition in the powdered detergent for washing white laundry has a strong stain-removing effect against complex biological stains based on low-fusible and refractory saturated and/or unsaturated acids and their monoacyl, diacyl and triacylglycerides, nucleic acids, coloured pigments compared to the comparison means, not containing the composition according to the invention.

[0081] At the end of the study, marked changes were observed in the assessed index of removal efficiency of stubborn biostains. According to the dynamics of the index, the addition of high performance bionuclease in combination with bioprotease together with selected components of the composition increased the degree of removal of complex pigment-oil, food, biological (blood, sebum, sweat) stains based on saturated and/or unsaturated acids and acylglycerides and nucleic acid derivatives. The addition of 0.05% bioprotease did not significantly increase the SRI with regard to the base product for biological contamination based on sebum, sweat crystals, bacterial biofilms and nucleic acid-rich skin epidermis. When the components were combined, an increase in SRI was observed. The composition with 0.05% bioprotease and 0.005% bionuclease increased the SRI by 4.7 units for the sweat and skin sebum biocontamination based on nucleic acids, which is a manifestation of synergy between the components and statistically significant result in biostain removal during washing ($p < 0.05$). The base sample without the composition of the invention showed a low removal of the selected biostain, indicating a lack of efficacy of the surfactants for the removal of contaminants during laundering (Table 7).

Table 7: Assessment of the effectiveness of C-P244PDE specific contaminant removal

Test specimen	Components of the composition	SRI score
		C-P244PDE (in. contamination 32.2)
sample No. 1 base	aerobically fast biodegradable surfactant SCS 4.50+0.25%	36.7
sample No. 2 Base + bioprotease	aerobically fast biodegradable surfactant SCS 4.50+0.25% bioprotease 0.06%	36.8
sample No. 3 Base + bionuclease	aerobically fast biodegradable surfactant SCS 4.50+0.25% bionuclease 0.005%	38.8*
sample No. 3 base + bioprotease + bionuclease	aerobically fast biodegradable surfactant SCS 4.50+0.25% bioprotease 0.06% bionuclease 0.005%	41.4**
*Mean reliable difference, statistically significant ($p < 0.05$), 10-20% increase in standard washing efficiency		
**Best reliable difference, statistically significant ($p < 0.05$), more than 20% improvement in standard washing efficiency		

[0082] Complex biological contaminants based on saturated and/or unsaturated acids and their acylglycerides, nucleic acids and protein residues, are difficult to remove in laundry washing due to fixation in hard to reach places and fibres of different fabrics, the formation of "biofilms" of skin sebum, oxidation of unsaturated fatty acids and lack of sufficient action of surfactants. High levels of surfactants increase the residue on the fibres of the fabric, create large amounts of foam and drastically reduce the durability of the fabric, which can negatively affect the environment, increase water consumption, reduce washing efficiency and significantly increase fabric wear and colour fade.

[0083] The composition of a powdered detergent for washing white laundry based on natural aerobically biodegradable surfactants, bioprotease and bionuclease enables effective removal of soiling from surface and hard-to-reach areas of various fabrics, thus reducing the total amount of surfactants in household detergents, maintaining dermatological safety for humans and achieving almost complete removal of biological soiling from laundry. Removal of fatty grease stains, sweat and sebum crystals, epidermal cell proteins and exfoliated particles provides a hygienic function, prevents excessive sorption of contaminants on fabric surfaces, rapid accumulation of bad smells on garments and reduces the number of washings in the long term. Increased SRI demonstrates high light-reflectivity of the fabric, the appearance of whiteness and a reduction of jaundice on the surface of the garment, indicating crystal-clear laundry without optical brighteners. The addition of a bionuclease enhances the removal of stains from human excreta without damaging the fabric, and controls the bioprotease content of the product, without exceeding the dermatological tolerance of the protease in HERA and ECHA laundry detergents. The reduced content of enzymes, in particular proteases, and surfactants allows for low toxicity, low skin irritation potential on the hands and the possibility of allergic reactions in humans when wearing laundry washed with the detergent.

Example 6

[0084] A laboratory study of the detergent effectiveness of the composition as part of a universal liquid laundry detergent was carried out. As a base for introducing the components, a liquid universal laundry detergent for white and coloured laundry was used, as defined in Table 1.

[0085] The test methodology is based on the generally recognised recommendations of the European Association A.I.S.E. [A.I.S.E. Laundry Detergent Testing Guidelines Minimum requirements for comparative detergents testing, see [https://www.aise.eu/documents/document/20180625164030-](https://www.aise.eu/documents/document/20180625164030-laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf)

[laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf](https://www.aise.eu/documents/document/20180625164030-laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf),

to determine the detergent efficacy with regard to stain removal index (SRI) and the values L,a,b. The SRI is calculated using the formula from ASTM D 4265 Standard guide for evaluating stain removal performance in home laundering [DOI: 10.1520/D4265-14]. This guide was prepared by the American Society for Testing Materials (ASTM) Committee D-12 on soaps and other detergents and was published in 2014. This test evaluates the effectiveness of the composition in removing fresh and stale stains of various nature from cotton and synthetic materials simulating actual washing conditions. Washing was carried out in a Linetest B laboratory washing machine. Common recommendations in the European Union and Russian Federation were chosen as the test conditions, in particular a temperature of 40 °C, a water hardness of 10.9 °dH and standard wash mode (mixed fabrics). The concentration of the universal liquid detergent for white and coloured laundry was 5.0 g/l per standard washing machine load of 2-4 kg. Three repetitions (n=3) were carried out, and the deviation was no more than 0.20% for each sample.

[0086] Hard-to-remove biostains of different nature were used for the test (Table 8). The selected stubborn biostains allowed the overall detergent efficacy of the formulation to be assessed, as well as the efficacy against individual contaminants due to the presence of the composition of the invention.

Table 8. Biostains and biocontaminants tested from A.I.S.E.

Designation	Composition	Type
EMPA 116	blood/milk/ink	mixed: protein + pigmented

[0087] SRI is a quantitative measure of the removal of dirt and stains in units. The values are based on whiteness L and chromaticity a,b, calculated mathematically using a special formula from ASTM D 4265-14. The method has numerous advantages, in particular, high accuracy, reproducibility, low error (less than 5%), takes into account colours of laundry and their change in the process of washing, different types of fabric and is the closest to the real visual perception and balances out the effect of optical dyes, common in detergents for laundry washing. A difference of 2 or more SRI units is effective and statistically significant. In practical experience, 1 unit of SRI corresponds to 5% detergent efficacy and gives a significant contribution to the formulation.

Results

[0088] According to the results of evaluation of effectiveness and removal rate of stubborn biostains of different nature, it was found that the studied composition in the liquid universal laundry detergent for white and colored laundry has a strong stain-removing effect against complex stains of protein, pigment and oil, mixed nature based on nucleic acids, pigments and protein compounds, compared to the comparison means, not containing the composition according to the invention.

[0089] At the end of the study, marked changes were observed in the assessed index of removal efficiency of stubborn biostains. According to the dynamics of the index, the addition of high performance bionuclease in combination with bioprotease together with selected components of the composition increased the degree of removal of complex protein contamination based on proteins, nucleic acids and ink. The addition of 0.006% bioprotease increased the SRI index by 2 units with respect to blood stains, which would be discernible by the human eye in 1 shade. When the components were combined, an increase in SRI was observed. The composition with 0.006% bioprotease and 0.005% bionuclease increased the SRI index by 4.8 units with regard to protein and nucleic acid based contamination, which is a manifestation of synergy between the components and statistically significant result in the removal of biostains during washing ($p < 0.05$). The base sample without the composition of the invention did not show high removal of the selected biostains, indicating insufficient efficacy of the surfactants for the removal of soiling during laundering (Table 9).

Table 9. Evaluation of the effectiveness of EMPA 116 biocontamination removal

Test specimen	Components of the composition	SRI score
		EMPA 116
sample No. 1 base	aerobically fast biodegradable surfactant SLES 5.25+0.25%	57.7
sample No. 2 base + bioprotease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.006%	59.7
sample No. 3 base + bionuclease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bionuclease 0.005%	58.4
sample No. 3 base + bioprotease + bionuclease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.006% bionuclease 0.005%	62.5**
*Mean reliable difference, statistically significant ($p < 0.05$), 10-20% increase in standard washing efficiency		
**Best reliable difference, statistically significant ($p < 0.05$), more than 20% improvement in standard washing efficiency		

[0090] Complex biocontaminants based on nucleic acids and protein residues, are difficult to remove in laundry washing due to their fixation in inaccessible areas and fibres of different fabrics, the formation of "biofilms" of skin sebum, oxidation of unsaturated fatty acids and the insufficient action of surface-active agents. High levels of surfactants increase the residue on the fibres of the fabric, create large amounts of foam and drastically reduce the durability of the fabric, which can negatively affect the environment, increase water consumption, reduce washing efficiency and significantly increase fabric wear and colour fade.

[0091] The composition of the universal liquid laundry detergent for coloured and white laundry based on natural aerobically biodegradable surfactants, bioprotease and bionuclease allows effective removal of soiling from surface and hard-to-reach areas of various fabrics, thus reducing the total amount of surfactants in household detergents, maintaining dermatological safety for humans and achieving almost complete removal of biological soiling from the laundry. Removal of sweat crystals and sebum, epidermal cell proteins and exfoliated particles provides a hygienic function, prevents excessive sorption of contaminants on fabric surfaces, rapid accumulation of unpleasant odours on garments and reduces the number of washings in the long term. Increased SRI demonstrates high light-reflectivity of the fabric, the appearance of whiteness and a reduction of jaundice on the surface of the garment, indicating crystal-clear laundry without optical brighteners. The addition of a bionuclease enhances the removal of blood stains without damaging the fabric, and controls the bioprotease content of the product, without exceeding the dermatological tolerance of the protease in HERA and ECHA laundry detergents. The reduced content of enzymes, in particular proteases, and surfactants allows for low toxicity, low skin irritation potential on the hands and the possibility of allergic reactions in humans when wearing laundry washed with the product.

Example 7

[0092] A laboratory study of the detergent efficacy of the composition in a universal liquid laundry detergent was carried out. A universal liquid laundry detergent for white and coloured laundry listed in Table 1, containing no bionucleases and bioproteases, was used as the basis for introducing the components.

[0093] The test methodology is based on the generally recognised recommendations of the European Association A.I.S.E. [A.I.S.E. Laundry Detergent Testing Guidelines Minimum requirements for comparative detergents testing, see https://www.aise.eu/documents/document/20180625164030-laundry_detergent_testing_guidelines_v_5_2_june_2018_.pdf, to determine the detergent efficacy with regard to the stain removal index (SRI) and the values L,a,b. The SRI is calculated using the formula from ASTM D 4265 Standard guide for evaluating stain removal performance in home laundering [DOI: 10.1520/D4265-14]. This guide was prepared by the American Society for Testing and Materials (ASTM) Committee D-12 on soaps and other detergents and was published in 2014. This test evaluates the effectiveness of the composition in removing fresh and stale stains of various nature from cotton and synthetic materials, simulating actual washing

conditions. Washing was carried out in a Miele washing machine. Common recommendations in the European Union and Russian Federation were chosen as test conditions, in particular a low temperature mode of 30°C, water hardness of 15°dH, accelerated washing mode (cotton), ballast 4 kg. The concentration of the liquid all-purpose detergent for white and coloured laundry was 6.0 g/l per standard washing machine load of 2-4 kg. Three repetitions (n=2) were carried out, and the deviation was no more than 0.10 % for each sample.

[0094] Hard-to-remove biostains of different nature were used for the test (Table 10). The selected stubborn biostains allowed the overall detergent efficacy of the formulation to be assessed, as well as the efficacy against individual contaminants due to the presence of the composition of the invention.

Table 10. Biostains and biocontaminants tested from A.I.S.E.

Designation	Composition	Type
EMPA 116	blood/milk/ink	mixed: protein + pigmented
EMPA 117	blood/milk/ink	mixed: protein + pigmented
WE5DASBWK	blood	enzymatic protein
CFT CS-44	chocolate drink	enzymatic protein
CFT CS-07	grass	enzymatic protein
WFK 20PF	vegetable oil	enzymatic oil
CFT CS-32	skin sebum	enzymatic oil
CFT CS-406	balsamic sauce	enzymatic mannanase
EMPA 165	chocolate pudding	enzymatic mannanase
WE5TPWK	tomato puree	enzymatic pectate
C-H147	fruit jam	enzymatic pectate
CFT CS-28	rice starch coloured	enzymatic amylase
CFT CS-29	tapioca starch coloured	enzymatic amylase
KC-H097	porridge	enzymatic cellulase
C-01	mineral oil	pigmento-oil
WE5FSMWK	mustard	pigmento-oil
WE5FM2WK	decorative cosmetics	pigmento-oil

[0095] Nucleic acid (DNA) based skin sebum stain removal was also evaluated: NZ-117 DNA and PC-H244 DNA (Table 11). The selected hard to remove nucleic acid based biostains allowed both the overall detergent efficacy of the formulation and the efficacy against individual contaminants to be evaluated due to the presence of a bionuclease and bioprotease according to the invention. The test was carried out in a TOM laboratory washing machine at 30° C, a washing time of 20 minutes, spin speed of 120 PRM, a water hardness of 15° dH, and a dosage of 3.33 g/l. The test was carried out twice (n=2). SRI was measured at Rem 460 nm.

Table 11. Biostains and biocontaminants tested from A.I.S.E.

Designation	Composition	Type
C-P244 DNA	human sebum with DNA	mixed: enzymatic + pigmental
NZ-117 DNA	human sebum with DNA	mixed: enzymatic + pigmental

[0096] SRI is a quantitative measure of the removal of dirt and stains in units. The values are based on whiteness L and chromaticity a,b, calculated mathematically using a special formula from ASTM D 4265-14. The method has numerous advantages, in particular, high accuracy, reproducibility, low error (less than 5%), takes into account colours of laundry and their change in the process of washing, different types of fabric and is the closest to the real visual perception and levels the effect of optical dyes, common in detergents for laundry washing. A difference of 2 or more SRI units is effective and statistically significant. In practical experience, increased 1 unit of SRI corresponds to 5% increased detergent

efficacy and gives a significant contribution to the formulation.

Results

[0097] According to the results of evaluation of efficiency and speed of removal of stubborn biostains of different nature, it was established that the studied composition in the liquid universal laundry detergent for white and colored laundry has a strong stain-removing effect in respect of complex soiling of protein, pigment-oil, polysaccharide, lipid, bleach and mixed nature based on low-fusible and refractory saturated and/or unsaturated acids and their mono-, diacyl- and triacylglycerides, nucleic acids, stained pigments and organic biopolymers.

[0098] At the end of the study, marked changes were observed in the assessed SRI removal efficiency of difficult to remove biostains. According to the dynamics of the index, the addition of 0.0025% high-performance bionuclease in combination with 0.0035% bioprotease together with selected components of the composition increased the removal of complex pigmented oil, food, biological, stained and mixed soiling. When the components were combined, an increase was observed in SRI for all stains, and in particular for sweat, sebum, grease, nucleic acid based blood, which is a manifestation of synergism between the components and a statistically significant result in the removal of biostains during washing ($p < 0.05$). The base product sample without bionuclease according to the invention did not show very high removal of selected biostains, indicating insufficient effectiveness of surfactants and bioprotease for the removal of soiling during laundry (Table 12).

Table 12. Assessment of the effectiveness of biocontamination removal

Test specimen	Main components	SRI amount for 11 spots	SRI score					
			protein stains (5 spots)	starchy stains (2 spots)	grease stains (2 stains)	mannose- based stains (2 spots)	pectin- based stains (2 spots)	cellulose- based stains (1 spot)
sample No. 1 base + bioprotease 60 g	aerobically fast biodegradable surfactant SLES 5.25±0.25% bioprotease 0.006%	768	215	99	93	89	109	65
sample No. 2 base + bioprotease + bionuclease 60 g	aerobically fast biodegradable surfactant SLES 5.25±0.25% bioprotease 0.006% bionuclease 0.005%	930**	277**	126*	108*	117**	126*	79*
sample No. 3 commercially available sample of laundry gel with bioprotease 65 g	aerobically fast biodegradable surfactant SLES non-biodegradable LAS aerobically fast degradable surfactant SCS (total of 5-15% anionic surfactants) bioprotease 0.0005-0.1%	916**	255*	128*	119**	113*	114	66

*Mean reliable difference, statistically significant (p<0.05), 10-20% increase in standard washing efficiency

**Best reliable difference, statistically significant (p<0.05), more than 20% improvement in standard washing efficiency

[0099] According to the index dynamics, the addition of a high-performance bionuclease in combination with a bioprotease together with the selected components of the composition increased the degree of removal of complex nucleic acid (DNA)-based sebum contaminants. The addition of 0.005% bionuclease significantly increased the SRI relative to the base product with respect to biological contamination based on sebum, sweat crystals, bacterial biofilms and nucleic acid-rich skin epidermis. The composition with 0.0035% bioprotease and 0.005% bionuclease increased the SRI score by 4.7 units for nucleic acid-based sweat and sebum biocontamination, which is a manifestation of synergism between the components and statistically significant result in biostain removal during washing ($p < 0.05$). The base sample without the composition of the invention showed a low removal of selected biostains, indicating the insufficient effectiveness of surfactants in removing these contaminants during laundering (Table 13).

Table 13. Assessment of the effectiveness of nucleic acid based stain removal

Test specimen	Components of the composition	SRI score		
		C-P244 DNA	NZ-117 DNA	total SRI for 2 spots
sample No. 1 base	aerobically fast biodegradable surfactant SLES 5.25+0.25%	28.4	20.4	48.8
sample No. 2 base + bioprotease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.0035%	29.6	20.9	50.5
sample No. 3 base + bioprotease + bionuclease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.0035% bionuclease 0.0025%	41.4**	29.7*	71.1**
* Mean reliable difference, statistically significant ($p < 0.05$), 10-20% increase in standard washing efficiency				
** Best reliable difference, statistically significant ($p < 0.05$), more than 20% improvement in standard washing efficiency				

[0100] The composition of the universal liquid detergent for washing coloured and white laundry based on the natural aerobically biodegradable surfactants, bioprotease and bionuclease allows effective removal of soiling from surface and hard-to-reach areas of various fabrics, thus reducing the total amount of surfactants in household detergents, maintaining dermatological safety for humans and achieving almost complete removal of all types of biological soiling from laundry. The combination of bionuclease and bioprotease significantly increases the overall washing efficiency against all types of stains, improving the removal of protein, mannan, pectin and cellulose based stains, indicating the role of nucleic acids in fixing contaminants. Consequently, the addition of a bionuclease improves the removal efficiency of food stains (cocoa, grass, fruit), blood stains and DNA-rich sebum stains without damaging the fabric and allows the bioprotease content of the product to be adjusted without exceeding the dermatological tolerance of the protease in HERA and ECHA laundry detergents.

Example 8

[0101] A laboratory study was carried out on the effectiveness of neutralising unpleasant food odours during washing with a universal liquid laundry detergent containing the composition according to the invention. The liquid universal laundry detergent for white and coloured laundry listed in Table 1, containing no bionucleases or bioproteases, was used as the basis for introducing the components.

[0102] The test methodology was based on the scientific publication "Deodorizing Ability of Houttuynia cordata Thunb. (Dokudami) for Masking Garlic Odor - Hiromi Ikeura" to determine neutralization of unpleasant odours by the organoleptic method. The sources of unpleasant odours were strong food odours in an alcoholic solution: garlic extract, atlantic herring extract and oil acid extract, which is part of the fatty acids of sweat, sebum. This test evaluates the effectiveness

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of neutralising unpleasant odours using a composite of cotton and synthetic materials, simulating real washing conditions.

[0103] The test was carried out on 4.5 cm × 9.0 cm pieces of cotton fabric which were pre-sprayed with these extracts by 3-fold spraying at a distance of 10-15 cm from the contaminated fabric sample. The tissue samples were then air dried in a horizontal position for 45-60 min. One sample obtained in this way was used as a control initial smell, and the other 4 samples after 20 min soaking in 1 l solution of laundry gel (4.5 g/l) was washed in a Linitest laboratory washing machine by 2 pieces in 100 g of this solution in 2 separate cups in the appropriate washing programme for 20 min at 40 °C with 2 subsequent rinses for 2 min. The samples were air dried horizontally for 2 hours at room temperature after washing.

[0104] Comparison of odours was carried out organoleptically by comparing the initial smell of the fabric (unwashed) with the smell of fabric samples after washing by placing a fabric sample in the bottom of a 1 litre plastic cup. The odour of the original fabric was evaluated by intensity:

- 5 - there is no odour;
- 4 - a faint odour;
- 3 - noticeable odour;
- 2 - strong odour;
- 1 - very strong odour.

[0105] The washing results were evaluated on an odour scale of 5 compared to the baseline:

- 5 - there is no odour;
- 4 - a faint odour;
- 3 - noticeable odour;
- 2 - the odour is slightly less than the original odour;
- 1 - there is almost no difference in odour.

Results

[0106] According to the results of evaluation of the effectiveness of neutralization of unpleasant odours, it was found that the studied composition in the liquid universal laundry detergent for white and colored laundry has a pronounced deodorizing effect against specific sources of odours based on sulfur compounds, amines and fatty acids of sebum. A single wash of contaminated fabric samples for 40 minutes eliminated unpleasant odours by more than 90%, indicating a pronounced effect of the product (Table 14). A single wash of contaminated fabrics with a base product without bioprotease and bionuclease did not achieve the same efficacy and did not eliminate odour, indicating a synergistic effect of the composition against unpleasant odours, including sweat and sebum.

Table 14. Assessment of the effectiveness of nucleic acid based stain removal

Test specimen	Components of the composition	Odour intensity					
		garlic		herring		oleic acid	
		prior	after	prior	after	prior	after
sample No. 1 base	aerobically fast biodegradable surfactant SLES 5.25+0.25%	3.5	4	4	4	1	2.5
sample No. 2 Base + bioprotease + bionuclease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.0035% bionuclease 0.0025%	3.5	5*	4	5*	1	4.5**
*statistically significant result (p<0.05)							
**statistically significant result (p<0.01)							

[0107] The composition of a universal liquid detergent for washing coloured and white laundry based on natural

aerobically biodegradable surfactants, bioprotease and bionuclease allows effective removal of unpleasant odours from surfaces and hard-to-reach areas of various fabrics, thereby improving the organoleptic profile of the laundry. The combination of bionuclease and bioprotease significantly increases the overall deodorising effectiveness against common unpleasant odours, including sweat, nucleic acid-rich sebum.

Example 9

[0108] The washing effectiveness of the composition in a universal liquid laundry detergent was investigated in relation to sweat crystals enriched with nucleic acids and mineral deposits of sweat glands, and hard-to-remove fixable skin sebum. A liquid all-purpose laundry detergent for white and coloured laundry, as listed in Table 1, was used as the base for introducing the components.

[0109] The test methodology is based on the study of cotton pillowcase samples before and after washing to determine the removal efficiency of hard-to-remove fixable skin sebum and to assess the kinetics of nucleic acid-rich sweat crystal formation. A group of 10 healthy volunteers took part in the study. At the beginning of the consumer study, participants were given clean white cotton pillowcases to use during the 14 days of the study. At the end of the study, participants returned the contaminated pillowcases, evaluated the aroma intensity using the methodology in Example 8, and the appearance of the pillowcases.

[0110] Next, fabric samples (pillowcases) with contamination causing a specific odour and colour change with plaque formation were sent to the laboratory to study the surface and structure under an electron microscope in order to visualise the contamination before and after washing. A 2×2 cm² piece was cut from a whole pillowcase sample for analysis, placed on a $76 \times 26 \times 1$ mm slide and fixed with adhesive tape around the edges of the sample. A preliminary search for the most contaminated areas was carried out for subsequent high-resolution SEM imaging (FEI Teneo scanning electron microscope). Tissue surface imaging was performed using a FEI CorrSight confocal fluorescence microscope with localization of the most contaminated areas. To quantify the area and degree of contamination present, the images were analyzed to obtain data on the fraction of area from which the fluorescence signal from contamination arrives and the signal intensity. Based on the results, the proportion of contaminated area and the number and size of contaminant particles, in particular sweat crystals and dermal sebum, were estimated using ImageScope software.

[0111] The samples were washed once in a Miele washing machine. Common recommendations in the European Union and Russian Federation were chosen as test conditions, in particular a temperature of 40 °C, a water hardness of 10.9 °dH and a standard wash mode (mixed fabrics). The concentration of the universal liquid detergent for white and coloured laundry was 5.0 g/l per standard washing machine load of a 2-4 kg. Three repetitions (n=3) were carried out and the measurement error was 0.20% or less for each sample.

Results

[0112] According to the results of evaluation of effectiveness of removal of hard-to-remove fixed skin sebum, it was found that the studied composition in the liquid universal laundry agent for white and coloured laundry has a pronounced washing effect against with regard to nucleic acid-based soiling compared to the means of comparison, not containing the composition according to the invention, and the initial condition of pillowcases. A statistically significant reduction of 83.07% in contaminated fabric surface area in 1 wash was observed, indicating high removal efficiency of these contaminants and deep hygienic cleaning in the laundry process (Table 15). Average size of particles of dirt and sweat crystals statistically significantly decreased by 34.27% in comparison with initial value and control (washes base), that allows to state about regulation of kinetics of sweat crystallization on fabrics from various material (Table 15).

Table 15. Assessment of contaminant removal efficiency and reduction of nucleic acid-enriched sweat crystallisation.

Test specimen	Components of the composition	Contaminated surface area, %		Average size of sweat crystals, µm	
		prior	after 5 washings	prior	after 5 washings
sample No. 1 base	aerobically fast biodegradable surfactant SCS 5.00±0.25%	14.65±5.73	0.53±0.13*	2.15±0.78	2.15±0.78

(continued)

Test specimen	Components of the composition	Contaminated surface area, %		Average size of sweat crystals, μm	
		prior	after 5 washings	prior	after 5 washings
sample No. 2 base + bioprotease + bionuclease	aerobically fast biodegradable surfactant SCS 5.00+0.25% bioprotease 0.006% bionuclease 0.006%	14.65+5.73	0.11+0.04**	2.48+1.26	1.63+0.43*
*statistically significant result ($p<0.05$) **statistically significant result ($p<0.01$)					

[0113] Visually, there is a reduction in the area of sweat, sebum, invisible to the eye due to imaging with a Laser light source (488 nm) and overlaying a mask of indexed contaminants, as shown in Fig. 1.

[0114] At the end of the consumer and laboratory trials, marked changes were observed in the assessed rate of removal of fixable sebum and reduction of nucleic acid-enriched sweat crystals. According to the dynamics of the indicator, the addition of a high-performance bionuclease in combination with a bioprotease together with selected components of the composition increased the removal of fixable nucleic acid-based skin sebum. The composition with 0.006% bioprotease and 0.005% bionuclease reduced invisible soiling by 83.07% after one wash, and reduced sweat crystal size by 34.27% after one wash, showing synergies between the components and a statistically significant result in hygienic cleaning of laundry after washing ($p<0.05$). Base sample without the composition according to the invention showed removal of selected biocontaminants due to surfactants, but poorly regulated the crystal formation of sweat enriched with nucleic acids, indicating insufficient effectiveness of surfactants for deep hygienic cleaning in the laundry, the subsequent preservation of bad odours and creating a favourable environment for the growth of microflora on the laundry.

Example 10

[0115] A laboratory study was carried out on the stability of the enzymes of the composition according to the invention, in particular bioproteases and bionucleases, in the universal liquid laundry detergent indicated in Table 1.

[0116] The test was carried out in the Novozymes A/S laboratory using the company's own methodology to determine enzyme activity after 4 weeks in a chamber at 30 °C. This test evaluates the preservation of enzyme activity in the base of the finished product using an accelerated temperature stability technique to identify the residual activity over the shelf life and expiry live, and thus the efficacy of the product. Samples of the universal liquid laundry detergent indicated in Table 1 were prepared and bioprotease and bionuclease were added separately in one test specimen and together in another test specimen. The samples were incubated in a heat chamber for 4 weeks, then the enzyme activity was assessed according to Novozymes A/S developed methods for each enzyme. This assay detects any change in the activity of sensitive enzymes from the protease and nuclease groups. The measurement error for residual activity estimation is +3%. Tests were performed once ($n=1$).

Results

[0117] According to the results of evaluation of residual activity of enzymes it was found that the studied bioprotease, bionuclease and combination of enzymes retain their activity in conditions of said accelerated stability method (Table 16). The investigated composition based on bioprotease and bionuclease in the composition of liquid universal laundry agent for white and coloured laundry retains its high activity after 2 and 4 weeks of accelerated storage, which indicates the possibility of combined presence of two enzymes in household detergent formulations and preservation of high values of efficiency demonstrated in Examples 4-9.

Table 16. Residual enzyme activity of the composition after accelerated storage

Test specimen	Components of the composition	Residual enzyme activity, %		
		0 week	2 weeks	4 weeks
sample No. 1 base + bioprotease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.01%	100%	102%	103%
sample No. 2 base + bionuclease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bionuclease 0.025%	100%	97%	97%
sample No. 2 Base + bioprotease + bionuclease	aerobically fast biodegradable surfactant SLES 5.25+0.25% bioprotease 0.01% bionuclease 0.025%	100%	96%	95%

[0118] Composition of universal liquid detergent for washing coloured and white laundry based on natural aerobically biodegradable surfactants, bioprotease and bionuclease retains its activity during product shelf life to achieve high removal of a wide range of human biostains and biocontaminants, regulation of crystal formation kinetics, neutralization of unpleasant odours from surface and hard to reach areas of different fabrics, thereby improving organoleptic profile of the laundry. The bionuclease is stable in the presence of bioprotease due to the selected combination of formulations given in Examples 1-3 and the use of aerobically biodegradable surfactant as a mycelling and stabilising agent. As the nuclease is not stable in the presence of the protease in most means, the results presented above show a new technical result achieved by the characteristics of the raw materials and the possibility of combined use to achieve the claimed effects of the composition according to the invention.

Example 11

[0119] The effects of the composition according to the invention, in particular bioproteases and bionucleases, on the destruction of biofilms formed by bacteria on the various house surfaces were investigated in the laboratory. The universal liquid household detergent (universal detergent) indicated in Table 17 was used as the base for incorporating the components of the composition. Samples of the liquid detergent with a pH of 7.5-8.0 were prepared under laboratory conditions.

Table 17. Formulation of the universal liquid detergent with the claimed composition

No.	Component	Content, wt. %
1	purified water	up to 100
2	alkyl carboxylate with the general formula: $R^1-CO_2X^1$, wherein R^1 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 21 carbon atoms and X^1 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid	1-15
3	alkyl glucoside with the general formula: $R^{28}-O-[G]p^3$, where R^{28} is an alkyl and/or alkenyl	2-15
	group with a hydrocarbon chain length from 4 to 22 carbon atoms, G is a saccharide fragment containing 5 or 6 carbon atoms, p^3 can take values from 1 to 4	
4	bionuclease	0.001-0.25
5	bioprotease in the form of subtilisin	0.001-0.25
6	complexifier	0.1-0.8
7	cotton extract	0.05-0.15
8	citric acid monohydrate	0.01-0.3
9	preservative	0.2-0.8

[0120] Microbial strains widely represented on various surfaces in the home were selected [Lax S., Smith D.P., Hampton-Marcell J. et al Longitudinal analysis of microbial interaction between humans and the indoor environment // Science, 2014, 345(6200): 1048-1052]: conditionally pathogenic and pathogenic microorganisms *Staphylococcus aureus* ATCC® 29213, *Escherichia coli* ATCC® 25922, *Klebsiella pneumoniae* (Institute for Medical Microbiology, Giessen, Germany), *Pseudomonas aeruginosa* ATCC® 27853, and representatives of the normoflora *Staphylococcus epidermidis* ATCC® 14990 and *Micrococcus luteus* (clinical isolate). The components of the composition were diluted separately and together in a universal liquid detergent to a concentration of 4 wt.%. The universal detergent without the components of the composition was used as a control.

[0121] The method of analysis is based on the assessment of the antibiofilm activity of compounds and the biofilm-destroying activity of compounds. The first activity is related to the prevention of biofilm formation on surfaces, while the second activity is related to the destruction of already formed stable biofilm on surfaces for elimination and cleaning of surfaces. A special broth was used as a nutrient medium: tryptone 1.0%; yeast extract 0.5%; sodium chloride 0.5%; pH 8.0 [Kayumov et al., 2015], which was prepared from dry components. The medium was sterilized by autoclaving at 121°C for 20 minutes. Agarized medium contained additional 1.5% agar. Glucose was then added to 1% w/v.

[0122] For quantitative analysis of biofilm formation by bacteria, it was stained with crystal violet [Peeters et al., 2008]. Microbial biofilms were formed by growing bacteria for two days on BM broth medium containing glucose (1% w/v) in adhesive plates, the culture liquid was removed and test compounds at concentrations of 4, 1, 0.25, 0.06% in a universal liquid detergent were added. After incubation at room temperature for 15 min, the liquid was removed and the wells were washed with PBS, then dried overnight. The wells were then incubated with 100 µl of a 0.5% solution of crystal violet (Sigma) dissolved in 96% ethanol, followed by a 20 minute incubation period. The plate was then washed with PBS 3-4 times after removing the crystal violet solution from the wells. After the plates were dried, 1 ml of 96% ethanol was added to the wells to elute crystal violet bound to the biofilm, and the optical density was measured at 550 nm using an Infinite 200 Pro microplate spectrophotometer (Tecan). Studies were performed three times (n=3) for statistical evaluation of M±SD results.

[0123] The parameters for the overall evaluation of the effect of the components of the composition on microbial biofilms were the minimum biofilm suppressing compound concentration (%) and % of the residual microbial biofilm after 15 minutes of exposure to the compounds in the base of said universal liquid detergent, determined by microbiologists according to the results of a laboratory study. The indicator of the residual biofilm of microorganisms makes it possible to assess the effect on the house bacteria and to draw conclusions about the inherent effect of the components of the composition on the various groups of microflora in the house. The general positive tendency of application of components of the applied composition - absence of negative influence on normal microflora of the house and strong antibiofilm effect on conditionally pathogenic and pathogenic microflora, which will provide maintenance of natural nonpathogenic microflora on various surfaces of the house for health of house residents.

Results

[0124] Based on the evaluation of the effect on biofilms of representatives of normal and transient house microflora, it was found that the composition of the invention has a high antibiofilm activity against various bacteria. Bioprotease and bionuclease have different activity against biofilms of bacteria due to chemical composition of biofilms and predominance of one or another component. It is known that in biofilms of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* protein components dominate, while in biofilms of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* - DNA. The highest antibiofilm activity is achieved by combining a bioprotease and a bionuclease in the base of a universal detergent containing alkyl and/or alkyl polyethylene glycol sulphate with the general formula $R^1-O(-CH_2-CH_2-O)_n(SO_3)_nX^1$. Combining the components of the composition in a single agent makes it possible to achieve a high biofilm effect at a minimum antibiofilm concentration of both components (Table 18). It is worth noting that the components of the composition have low antibiofilm activity against *Staphylococcus epidermidis* and only at high concentrations, which makes it possible to target the microflora of house surfaces and retain favourable microorganisms for dermatological tolerance and the health of house residents.

Table 18. Estimates of the antibiofilm activity of compounds

Bacterial strain		Base + Bioprotease	Base + Bionuclease	Base + Bioprotease + Bionuclease (1:1)
Minimum antibiofilm concentration, wt. %	<i>Staphylococcus aureus</i> ATCC® 29213	0.05%	>0.4%	0.025%
	<i>Escherichia coli</i> ATCC® 25922	0.0125%	0.0125%	0.003%
	<i>Klebsiella pneumoniae</i>	>0.4%	0.025%	0.0125%
	<i>Pseudomonas aeruginosa</i> ATSS® 27853	>0.4%	0.0125%	0.006%
	<i>Staphylococcus epidermidis</i> ATCC® 14990	>0.4%	>0.4%	>0.4%

[0125] At the end of the study a significant reduction in the biofilm mass of *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* was observed after 15 minutes of exposure. Individually components are able to destroy biofilms of conditionally pathogenic microorganisms, but the combination of components of the composition allows to achieve a synergistic antibiofilm effect with preservation of growth of *S. epidermidis* normoflora (Table 19). Bioprotease allows to destroy biofilms saturated with protein components, while the bionuclease is effective against biofilms of *P. aeruginosa* and *K. pneumoniae*, saturated with DNA, which are reservoirs for the growth of microorganisms with resistance to antibacterial agents. The combination of bioprotease and bionuclease (1:1 ratio) can reduce the residual biofilm mass of microorganisms from 20% to 45% during 15 minutes of exposure.

Table 19. Residual biofilm (%) of bacteria after 15 minutes exposure

Bacterial strain		Base	Base + Bioprotease 0.0125%	Base + Bionuclease 0.0125%	Base + Bioprotease 0.0125% + Bionuclease 0.0125% (1:1)
% of residual biofilm	<i>Staphylococcus aureus</i> ATCC® 29213	100 $\pm$ 5	92 $\pm$ 2	105 $\pm$ 4	81 $\pm$ 10
	<i>Escherichia coli</i> ATCC® 25922	100 $\pm$ 5	65 $\pm$ 12	73 $\pm$ 9	55 $\pm$ 1
	<i>Klebsiella pneumoniae</i>	100 $\pm$ 5	104 $\pm$ 6	85 $\pm$ 12	78 $\pm$ 17
	<i>Pseudomonas aeruginosa</i> ATSS® 27853	100 $\pm$ 5	125 $\pm$ 21	82 $\pm$ 11	80 $\pm$ 13
	<i>Staphylococcus epidermidis</i> ATCC® 14990	100 $\pm$ 5	96 $\pm$ 12	98 $\pm$ 17	101 $\pm$ 13

[0126] The microflora of the home is a harmonious system, so it is important to maintain a balance of micro-organisms both on the skin and on surfaces. In particular, *Staphylococcus aureus*, together with other conditionally pathogenic microorganisms such as *Escherichia coli*, can form stable biofilms from the exopolysaccharide matrix and contribute to various dermatological diseases, including atopic dermatitis [E. Scott, S. Bloomfield. The survival and transfer of microbial contamination via cloths, hands and utensils. J. Appl. Bacteriol. 68 (1990) 271-278].

[0127] The components of the composition according to the invention specifically influence the formation of stable protective biofilms of microorganisms, to which resistance quickly develops, but do not have a negative effect on the normoflora, which allows to provide directed protection against the development of conditionally pathogenic representatives and to maintain a harmonious biodiversity of the house microflora. The reduction of biofilms of conditionally pathogenic and pathogenic strains will help to shift biodiversity towards beneficial resident skin microflora and improve the dermatological condition of the skin in general when in contact with various surfaces. Thus, combining the components into a single complex makes it possible to achieve a synergistic effect against conditionally pathogenic microorganisms (*S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*) and to preserve representatives of normal microflora (*S. epidermidis*). The claimed composition has a high antibiofilm effect against conditionally pathogenic transient strains that are a causal factor in the development of dermatological and systemic diseases and representatives of the transient microflora at home. Because it preserves the normal microflora of the surfaces of the home at the place of application, it is possible to use it as part of household cleaning products on a regular basis.

Claims

1. A composition comprising the following components:

- A) a serine protease;
- B) a nuclease;
- C) a surfactant, wherein said surfactant is selected from at least one of the following:

- i) an alkyl carboxylate;
- ii) an alkyl sulfate;
- iii) an alkyl polyethylene glycol sulfate with the general formula

$R^1-O(-CH_2-CH_2-O)_{n^1}(SO_3)_{n^2}X^1$, wherein

- n^1 is 0 to 10 and denotes the number of polyethylene groups,
- R^1 is an alkyl and/or alkenyl group with a hydrocarbon chain length of 5 to 22 carbon atoms,
- n^2 is 0 to 1 and denotes the number of sulfate groups,
- X^1 is an alkali and/or alkaline earth metal cation, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid,

and wherein the mass ratio of said components is the following:

A	:	B	:	C
0.00125-0.25		0.0005-0.25		0.50-25.00

2. The composition of claim 1, wherein said serin protease is subtilisin, preferably subtilisin produced from *Bacillus subtilis*, further preferably subtilisin having the CAS number 9014-01-1.
3. The composition of claim 1 or 2, wherein said nuclease is a deoxyribonuclease, further preferably phosphodiesterase, further preferably phosphodiesterase from fungi, further preferably phosphodiesterase from fungi having the CAS number 9003-98-9.
4. The composition of any of the preceding claims, wherein said surfactant is degradable, preferably aerobically degradable.
5. The composition of any of the preceding claims, wherein said composition further comprises glycerol and/or sorbitol.
6. A laundry washing detergent comprising the composition of any of the preceding claims.
7. The laundry washing detergent of claim 6, wherein said laundry washing detergent comprises 0.50-25.50 wt.% of said composition of claims 1-5.
8. The composition of claims 1-5 or the laundry washing detergent of claims 6-7 assembled as a single composition comprising all said components.
9. The composition of claims 1-5 or the laundry washing detergent of claims 6-7 assembled as a kit-of-parts, wherein said kit-of-parts preferably separates components A) and B).
10. The composition or laundry washing detergent of any of the preceding claims, further defined by at least one of the following:
 - i) said serine protease is obtained biotechnologically from microorganisms selected from the serine endopeptidase group and/or with an activity of at least 80 U/g, preferably of at least 100 U/g, a pH 1-10% solution 4.0-7.0 at 25 °C, and a viscosity of 1-10% solution 0-1000 sPas at 25 °C;
 - ii) said nuclease is obtained biotechnologically from fungi or yeast and/or with an activity of at least 100 U/g, a pH 1-10% solution 4.0-7.0 at 25 °C, and a viscosity of 0-1000 sPas at 25 °C;
 - iii) said composition or laundry washing detergent is in a powdered, granulated, in gel or liquid form;
 - iv) said laundry is a fabric, wherein said fabric is preferably selected from the group consisting of: cotton, synthetic, wool, silk, cashmere, merino, down, feather and mixtures thereof.
11. The composition or laundry washing detergent of any of the preceding claims, wherein said composition or laundry washing detergent does not comprise ethylenediaminetetraacetic acid and/or any non-aerobically rapidly biodegradable surfactant, preferably not comprising any surfactant different from those as defined in item C) of claim 1.
12. The composition or laundry washing detergent of any of the preceding claims, wherein said composition or laundry washing detergent comprises additional components, wherein said additional components are selected

i) from the group consisting of organic solvents, film-formers and alcohols, quaternary ammonium bases, sili-

cones, optical brighteners, chlorine, phosphorus derivatives and mixtures thereof; or

ii) from the group consisting of organic solvents, surfactants, biodegradable chelates, preservatives, botanical extracts, citrate silver, essential oils, organic acid, nonorganic bases, dispersants, builders.

13. Use of the composition or laundry washing detergent of any of the preceding claims, wherein said use is selected from at least one of the following:

- a) use as an antimicrobial, including as a bactericide and fungicide, including as a bactericide and fungicide against bacteria or fungi biofilms;
- b) use as a laundry washing detergent;
- c) use as a fabric whitener.

14. The use of claim 13, wherein said use is further for the treatment of fabrics and/or fabric care, wherein said fabrics are preferably fabrics comprising at least one of the following:

- skin sebum;
- sweat crystals and/or sweat crystal formation;
- protein,
- DNA;
- RNA;
- microorganism selected from the group consisting of bacteria and fungi;
- odorous and/or metabolic products of microorganism, including bacteria and fungi, wherein said products are preferably selected from the group consisting of aldehydes, ketones, terpenes, amines, indoles, sulphur-containing components, including mercaptans, organic acids and their esters, phenols and cresols.

15. A method for cleaning fabrics and/or for fabric care, said method comprising:

- a) solving the composition or laundry washing detergent of claims 6-12 in water for obtaining an aqueous solution;
- b) optionally, adjusting

- the pH of said aqueous solution to a pH of 5.5 - 11.0, preferably 5.5-10.0, further preferably 6.0 - 10.5, and
- a water hardness of 0-15 °dH; and

- c) applying said aqueous solution to said fabric, preferably in a temperature range of 15 °C to 60 °C.

Microscopic assessment of the tissue surface

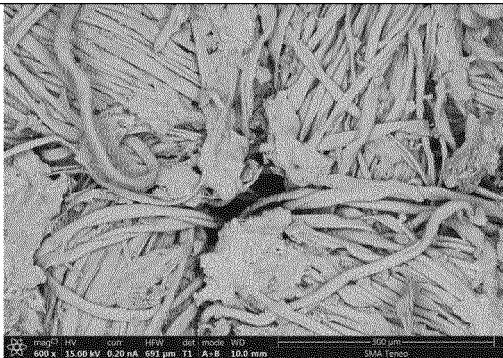
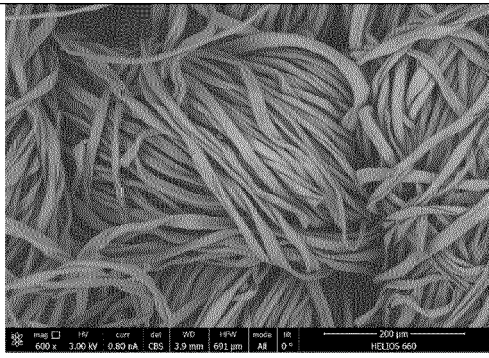
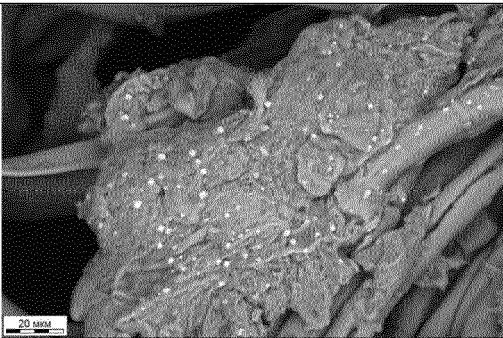
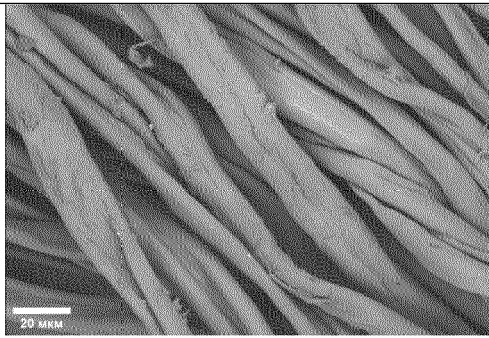
	Before washing with the composition	After 5 washings with the composition
Contaminated surface area of the fabric		
Visualisation of plaque particles on the fabric surface		

Fig. 1



EUROPEAN SEARCH REPORT

Application Number

EP 22 20 4178

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 2022/333039 A1 (LANT NEIL JOSEPH [GB] ET AL) 20 October 2022 (2022-10-20) * paragraphs [0060] - [62; 63; 69; 88; 289; 352; 304; 309; 357]; examples 13, 14, 17, 18 *	1-15	INV. C11D1/04 C11D1/14 C11D1/29 C11D3/386 C11D10/04 C11D11/00
X	US 2020/190436 A1 (GORI KLAUS [DK]) 18 June 2020 (2020-06-18) * 0005; 0009; 0016; 0017; 0019-0021; 0153; 0971; 10 34; 1072; 1073; 1081; 1083; 1190; 1191; claim 1; examples 1-3; tables 1-10 *	1-15	
			TECHNICAL FIELDS SEARCHED (IPC)
			C11D
The present search report has been drawn up for all claims			

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EPO FORM 1503 03.82 (P04C01)

Place of search	Date of completion of the search	Examiner
The Hague	21 March 2023	Douelle, Frédéric
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document		

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO.

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2022333039 A1	20-10-2022	CA 3160579 A1	01-07-2021
		CN 114761527 A	15-07-2022
		EP 4081626 A1	02-11-2022
		JP 2023507760 A	27-02-2023
		US 2022333039 A1	20-10-2022
		WO 2021133701 A1	01-07-2021

US 2020190436 A1	18-06-2020	BR 112019020960 A2	05-05-2020
		CA 3058519 A1	11-10-2018
		CN 110651030 A	03-01-2020
		EP 3607038 A1	12-02-2020
		JP 7032430 B2	08-03-2022
		JP 2020516707 A	11-06-2020
		PH 12019502279 A1	28-09-2020
		US 2020190436 A1	18-06-2020
		US 2022282185 A1	08-09-2022
		WO 2018184817 A1	11-10-2018

REFERENCES CITED IN THE DESCRIPTION

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Non-patent literature cited in the description

- **LAX S. ; SMITH D.P. ; HAMPTON-MARCELL J. et al.** Longitudinal analysis of microbial interaction between humans and the indoor environment. *Science*, 2014, vol. 345 (6200), 1048-1052 **[0120]**
- *J. Appl. Bacteriol.*, 1990, vol. 68, 271-278 **[0126]**