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ORIGINAL PAPER

A green approach for modification and functionalization of wool fabric using bio- and nano-technologies

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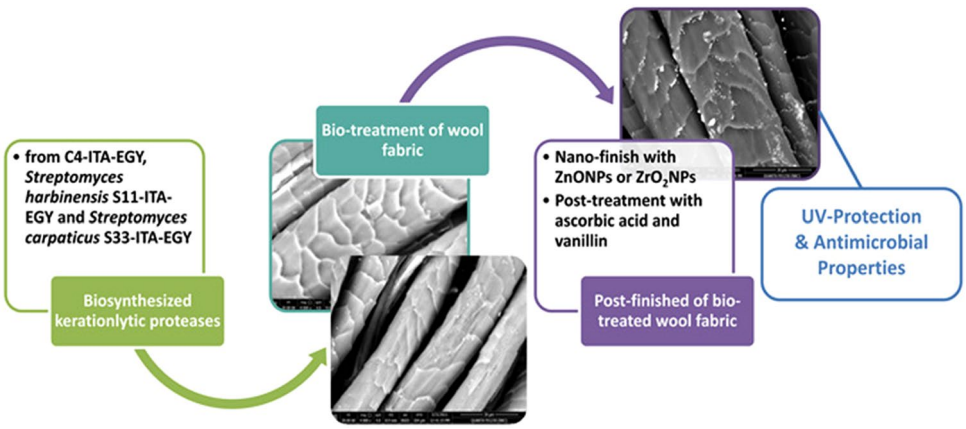
Abstract

In the present work, we propose a green and sustainable strategy for eco-friendly surface modification of wool structure using biosynthesized kerationlytic proteases, from C4-ITA-EGY, *Streptomyces harbinensis* S11-ITA-EGY and *Streptomyces carpaticus* S33-ITA-EGY, followed by subsequent environmentally sound functionalization of the bio-treated substrates using ZnONPs, ZrO_2NPs, ascorbic acid and vanillin, individually, to provide durable antibacterial as well as UV-protection properties. Both surface modification changes and the extent of functionalization of the final products were characterized by SEM, EDX, antibacterial efficacy, UV-blocking ability, loss in weight, nitrogen content and durability to washing analysis. The obtained data reveal that the developed green wool fabrics exhibit outstanding durable antibacterial activity and UV-blocking ability for fabricating multi-functional textile products that can be utilized in a wide range of sustainable protective textiles, irrespective of the used post-finishing formulation ingredients. The results also show that both modification and functionalization processes are governed by the type of enzyme and kind of active material respectively. Moreover, the biosynthesized kerationlytic proteases could be accessibly used to remove protein-based stains like blood and egg.

Graphical abstract



Keywords Wool · Enzyme and nano-technologies · Bio-active ingredients · Modification and functionalization · Sustainable textiles · Stain removal

Introduction

Microbial proteases are a commercially important group of industrial enzymes that have a variety of applications in textile, detergent, leather, pharmaceuticals, food and biotechnology industries (dos Santos Aguilar and Sato 2018; Mishra

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2016; Razzaq et al. 2019; Singh et al. 2016). A protease is a hydrolase enzyme that catalyzes the hydrolysis of the peptide bonds of protein molecules to liberate small peptides and amino acids (Dash and Sahoo 2021; Kumar and Sharma 2017). Among proteases, keratinases (E.C. 3.4.99.11) are important proteases for hydrolyzing feathers, hair, wool, horns and hooves and have several important biotechnological applications in textiles, leather, poultry processing industry, food industry, wastewater treatment, medicine and cosmetics (Bhange et al. 2016; Hamza 2017; Vidmar and Vodovnik 2018). Several microorganisms including bacteria (Bhange et al. 2016; Gong et al. 2015; Reddy et al. 2017), actinomycetes (Demir et al. 2015; Espersen et al. 2021) and fungi (Bohacz and Korniłłowicz-Kowalska 2019; Kumar and Mahal 2021) have been reported to produce keratinases. These keratinases are predominantly extracellular serine proteases or Metallo-endoproteases, and have a protease and disulphide reductase dual nature (Reddy et al. 2017).

The poultry and wool industries produced a large amount of keratin wastes in the form of feathers and wool. This kind of wastes accumulation took up a lot of space and could cause environmental issues. As a result, biotransformation of these wastes is thought to be of significant interest because of its roles in environmental management and high-value products production (Gong et al. 2015). Therefore, the development of biotechnological tools useful to fully exploit this bio-resource is a topic of major interest. Hydrolysis of keratinous wastes by keratinases is an attractive alternative method to the highly-energy requiring steam pressure cooking method (SPCM), which leads to the production of feather meal. Another potential application of keratinases is the possibility to use them for surface modification of protein-based fibers (Fu et al. 2015; Ibrahim et al. 2012). In addition, their use in the detergent industry to remove proteinaceous stains has gotten a lot of publicity (Habbeche et al. 2014; Paul et al. 2014).

Recently, the adoption and implementation of enzyme technology, nanotechnology and/or application of bioactive ingredients in conventional wet chemical processes to develop a green, sustainable and high-performance multifunctional products for diverse applications taken into account economy, ecology and social concerns have drawn great attention (Ibrahim et al. 2004; Madhu and Chakraborty 2017). However, to date, there is no integrated study disclosed in the literature about an eco-friendly sustainable route for integrated surface modification and multifunctionalization of a wool substrate using emerging technologies namely enzyme technology, nanotechnology and green bioactive agents to meet the ever-growing textile customer demands and environmental issues.

In the present article, we develop a novel, eco-friendly and integrated approach for surface modification and multifunctionalization of wool substrate using bio- and nanotechnologies, taking in consideration product, environment quality as well as consumer demands and awareness.

In this contribution, crude alkaline kertinases has been prepared by selected microbial strains having proteolytic activity using a cost effective method, characterized and utilized as a green bio catalyst for surface modification of grey wool substrate as well as in the removal of some protein-containing stains. Moreover, post-functionalization of bio-treated wool using ZnONPs, ZrO_2NPs ascorbic acid, or vanillin to develop an eco-friendly, durable, antibacterial, anti-UV and fashionable wool products with high value-added has been investigated.

Experimental part

Materials

Feathers and wool were collected from the local poultry industry and wool manufacturers. All the other buffer salts used for preparation were obtained from Sigma-Aldrich. As a starting fabric, a grey wool fabric (100%, 250 g/m^2, Misr Co. El-Mahalla El-Kobra, Egypt) was used.

A powder form of ZnO (10–30 nm), and ZrO (0.3–0.5 nm) nanoparticles were delivered from Sky Spring nanomaterials Inc. (USA). Ascorbic acid was purchased from Sigma. Vanillin R ($C_8H_8O_3$, molecular weight: 152.15) were purchased from SDFCL® India.

Methods

Preparation of keratinaceous materials

Native keratinaceous materials, chicken feathers and sheep wool, were prepared using the method of Friedrich et al. Native chicken feathers and sheep wool had been cut into small pieces, rinsed thoroughly with water, and defatted in chloroform–methanol (1:1 v/v) for four days. Once a day, the solvent was changed. Then, these materials were thoroughly washed, dried for 3 d at 50 °C and ground. Before being used as a source for powdered keratin, the powder was sifted throughout a 0.5 mm screen. Preparation of soluble keratin had been achieved by heating feathers in dimethyl sulphoxide (DMSO) at a temperature of about 100 ~ C for 2 h (Badrulzaman et al. 2021). In this manner, a 1.2 percent keratin protein solution in DMSO was ready. Acetone was used to precipitate the protein from the solution. For 1 volume of protein solution, 2 volumes of acetone were used. Phosphate buffer (0.1 M, pH 8) was used to re-suspend the keratin protein precipitate.

Isolation of actinobacteria

Homogenized soft corals, and sea sediments were subjected to serial dilution technique (10–1 to 10-6dilutions). Sterilized agar plates (15 cm) each having 50 ml of solidified isolation medium (containing g/l: yeast extract 5, glucose 10, peptone 5, KH_2PO_4 5, $MgSO_4$. 7, H_2O 1, Na_2CO_3 10 and agar 20). Na_2CO_3 was sterilized separately and added to the medium just before solidification giving final pH of 9.0 were prepared. Actinobacterial strains were isolated by inoculating the prepared agar plates with 200 µl from each dilution. The appeared actinobacterial colonies were picked and maintained on starch-nitrate slants after incubation at 30 °C for 7–14 days and then kept at 4 °C till the next use.

Screening and selection of the strains

For the 11 actinomycetes, primary screening experiments were conducted using the agar plates technique. The isolates' protease activity was assessed in this stage through their inoculation onto agar plates with the following ingredients (g/l): yeast extract 4, skim milk 10, starch 10, peptone 2 and agar 15 adjusted at pH 10 (Demir et al. 2015). Overflowing the Coomassie brilliant blue (CBB) R-250 solution, which was being destained with glacial acetic acid (10%) and methanol (40%), revealed activity as a clear zone all around the colony (Anbu et al. 2008). Good potential protease producers were chosen based on the clear zone diameter to colony diameter ratio.

Molecular identification of actinobacterial isolates

Actinobacterial isolates C4, S33 and S11 (high protease/keratinase producers) had been identified using 16S rDNA sequence analysis, which includes the following steps: extraction of genomic DNA, polymerase chain reaction (PCR) and 16S genes sequencing for amplification of genomic DNA. For genomic DNA extraction from 18 h old actinobacterial culture, DNA purification kit from GeneJET Genomic (Thermo Fisher Scientific, USA) was used. Biometra Thermal Cycler (Labrepco, Germany)was used for the amplification of 16S rDNA using two 16S rDNA universal primers 27F (AGAGTT TGA TCM TGG CTC Ag) and 1492R (TAC GGY TAC CTT GTTACG ACT T). PCR products were purified using a Montage PCR Clean-up kit (Millipore). The Big Dye terminator cycle sequencing kit (Applied Biosystems, USA) was used to complete the sequencing. The automated DNA sequencing system (Applied BioSystems, model 3730XL, USA) was used to resolve the sequencing products. The NCBI website's basic local alignment search tool (BLAST) was used to deposit the nucleotide sequence (1420 bp) into Genbank and compare to nucleotide sequence database. Alignment of 16S rDNA sequences was performed and phylogenetic trees were constructed. The taxonomic positions of strains based on 16S rRNA gene sequences of C4, S33 and S11 isolates are observed in the phylogenetic tree. This was determined using a workflow that began with a multiple sequence alignment using maximum parsimony (MP) trees and MUSCLE. The alignment with RAxML and TNT yielded maximum likelihood (ML).

For ML, rapid bootstrapping was used in connection with the auto-MRE bootstrapping characteristic, followed by a search for the best tree;1400 bootstrap replicates were used for MP, along with tree-bisection-and-reconnection branch swapping. To verify the sequences for compositional bias, the X2 test, as applied in PAUP, was used (Abdel-Aziz et al. 2019).

Enzyme production and culture conditions

Alkaline protease was produced by cultivating the selected strains (with a CFU value of about 2.1×10^8 cells/ml) on a production medium (g/l, w/v): 10 glucose, 5 casein, 5 yeast extract, 2 KH_2PO_4, 2 K_2HPO_4 and 1 $MgSO_4.7H_2O$ (Sedrah et al. 2021). The medium pH was adapted to 9.0–9.5 using Na_2CO_3. The culture was grown for 5 and 7 days in an incubating shaker incubator (150 rpm) at 30 °C after inoculation with 10% of cells/ml. At the end of the fermentation period, the produced culture was centrifuged at 8,000 rpm and 4 °C for 20 min. The collected supernatant was used as the crude enzyme.

In addition, using the chicken feathers as the sole carbon and nitrogen source, chosen strains were tested for feather degradation capacity. These strains were grown in mineral salt medium (50 ml/ 250 ml Erlenmeyer flask) supplemented with 1% chicken feather as a keratin source. The mineral salt medium composed of (g/l, w/v): 1.5 K_2HPO_4, 0.05 $MgSO_4.7H_2O$, 0.025 $CaCl_2$, 0.015 $FeSO_4.7H_2O$, 0.005 $ZnSO_4.7H_2O$, pH 7.5 (Anbu et al. 2008). Fermentation broth was centrifuged at 5000 rpm for 3 min to remove the produced biomass and leftover solid substrate. Extracellular keratinase and protease activities were assayed in the cell-free fermentation broth.

Keratinase activity measurement

Following Peng et al. (Peng et al. 2019) with a minor modification, 0.1 ml of culture supernatant was mixed with 0.4 ml of 1% (w/v) keratin (or other previously mentioned keratinaceous materials) in 50 mM Glycine/NaOH buffer (pH 10), and it was incubated at 40 °C for 1 h. The reaction was halted by the addition of 0.5 ml trichloroacetic acid (TCA, 10%). After 30 min at room temperature, the non-digested proteins (precipitated) were separated by centrifugation for 20 min at 10,000 × g. For Folin–Ciocalteu method, 200 µl Folin–Ciocalteu reagent and 1 ml Na_2CO_3 (4%) was added to 200 µl supernatant and incubated at 20 °C for 1 h. Its absorbance

was measured at 660 nm. TCA solution was added before the enzyme reaction as a control. Under specific conditions, one unit of keratinase activity was expressed as the enzyme proportion requisite to release 1 g of tyrosine/min. Keratinase activity is the average of at least two determinations where the difference in values is less than 5%. Using a Shimadzu spectrophotometer (UV-1601) the amount was calculated from standard tyrosine solution with a concentration range of 50–500 µgm/ml.

Protease activity measurement

The alkaline protease activity was determined in the cell-free supernatant using the previously described keratinase activity assay method, but with 1% (w/v) casein as a substrate in Glycine–NaOH buffer (50 mM; pH 10). Under standard conditions, one unit of protease activity was expressed as the enzyme proportion requisite to liberate 1 µg of tyrosine/min.

Hydrolysis of chicken feathers, wool or keratin

Except when differently specified, a sample containing 1% wool powder, chicken feather or keratin in 50 mM Gly/NaOH buffer (pH 10.0) was mixed with an appropriate dilution of enzyme. After 24 h at 40 °C, the reaction was terminated with the addition of 4% TCA. The hydrolysis yield was monitored as tyrosine by the Folin-Ciocalteu reagent. To test the hydrolysis in the presence of the reducing agent, the substrates were suspended in 50 mM Gly/NaOH buffer (pH 10.0) containing 0, 0.5 or 1% Na_2SO_3. The hydrolysis was done at 40 °C and the hydrolysis yield was evaluated by monitoring the increase in absorbance at 280 nm at a scheduled time (3, 6, 24, 48 and 72 h).

Bio-preparation of wool fabric using produced alkaline keratinolytic protease enzymes

Initially, a sample of a grey wool fabric was washed (W) using Na_2CO_3: $NaHCO_3$ (0.1 M: 0.1 M) buffers (pH 9), along with nonionic wetting agent (2 g /l), in aqueous solution with liquor to material (20: 1) at (50 °C), for (30 min). The treatment bath was carried out in IR–machine for dyeing followed by rinsing in tap water followed by distilled water.

Combined washing and bleaching

Grey fabric samples were washed and H_2O_2 -bleached in one step (W/B) using H_2O_2 (4% owf), along with nonionic wetting agent (2 g/L), at pH (9), and material to liquor ratio (1/20), for one hour at 50 °C, using IR-dyeing machine, followed by neutralization and washing for three times using distilled water. After washing, the treated fabric samples were oven dried to a constant weight.

Post-enzymatic treatment

Portions of grey (G), washed (W), and washed-bleached (W/B) wool samples were bio-treated with the produced alkaline keratinolytic protease enzymes using (5 ml/g fabric) enzyme dose, (2 g/l) sodium silicate, (2 g/l) nonionic wetting agent, and L/R (1/20), at pH (8) and temperature (50 °C) for time (60 min) in IR-dyeing machine. The enzyme-treated fabric samples were neutralized, using acetic acid to terminate the activity of the used enzyme, followed by thoroughly washing and drying.

Post-finished of bio-treated wool fabric

Portions of enzymatically treated wool fabric samples were post finishing using ZnONPs (5% owf), ZrO_2NPs (5% owf), ascorbic acid (10% owf), or vanillin (10% owf) at 60 °C for 30 min in a sonicator bath with 55 kHz frequency, followed by padding twice to 80% wet pick-up and microwave fixation at 400 watts for 6 min.

Stain removal

Cotton and cotton/polyester (50/50) were used in this study. Specimens of the used fabric were stained with blood and egg stains. The samples were washed with alkaline keratinolytic protease enzyme S11-E (1 g/l) in presence of a nonionic wetting agent (1 g/l). The washing process was carried out in an ultrasonic bath using 47 kHz at 50 °C for 30 min. After washing the washed specimens were thoroughly rinsed with cold running water to remove the removed stains and used chemicals.

Tests and analysis

- The weight loss of enzyme treated samples (WL %) was expressed as the percentage weight loss to the initial weight of untreated sample using the following equation: $WL\ (\%) = [(W_a - W_b)/W_a] \times 100$ (W_a and W_b are the weight of untreated and treated fabric samples respectively).
- The Kjeldahl method was used to determine the nitrogen content
- UV-protection properties of the treated samples were expressed as UPF value and determined using the Australian/New Zealand standard (AS/NZS 4366−1996).
- The antibacterial activity against pathogenic Gram-negative (G-ve, *E. coli*) bacteria, expressed bacteriostatic reduction rate quantitatively (BR %) was evaluated qualitatively using the AATCC test method (100–2019).

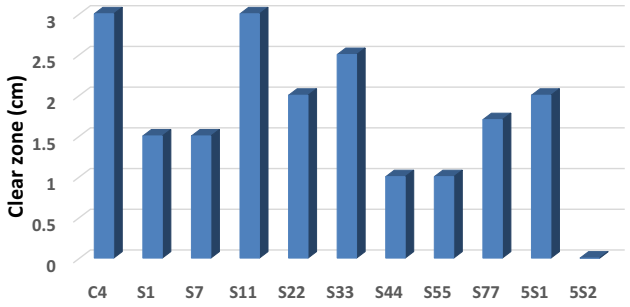


Fig.1 Preliminary screening of protease activity of different isolates using skim milk agar medium

- Washability was assessed using AATCC Test Method 61(2A-1996)
- Images from the scanning electron microscope (SEM) were obtained for selected samples using electron probe micro-analyzer (JEOL JXL-840A) equipped with energy dispersive X-ray spectroscopy (EDX) for elemental analysis.
- The extent of stain removal was assessed by measuring the reflectance of the stains before and washing by reflectance spectrophotometry.

Results and discussion

Screening for proteolytic activity

This test has been developed by cultivating the actinobacterial isolates on a medium containing skim milk and stained with Coomassie stain. As shown in Fig. 1, all of the tested isolates were found to have protease activities of varying abilities. The clear zone around colonies of isolates no. S11, S33 and C4 indicated their high protease activities (Fig. 2).

Table 1 Alkaline protease activity of selected actinobacterial isolates after 5 and 7 days incubation

Actinobacterial isolates	Protease activity (U/ml)	
	5d	7d
C4	366.84	367.35
S11	335.47	106.91
S22	49.68	46.48
S33	362.48	161.08
5S1	252.24	276.95

Table 2 Alkaline keratinase activity of selected actinobacterial isolates after 3, 6 and 9 days of incubation

Actinobacterial isolates	Keratinase activity (U/ml)		
	3d	6d	9d
C4	18.58	26.29	18.18
S11	19.16	17.71	15.940
S22	9.919	6.44	11.95
S33	14.49	21.91	16.59
5S1	6.75	13.48	26.40

Production of proteases by actinomycetes has been reported by many authors. (Leena and Yugandhar 2019; Rathore and Singh 2021).

Results in Tables 1 and 2 summarize the actual production of alkaline protease and keratinase activities, respectively, by the selected actinobacterial isolates. It has been noticed that no appreciable increase in protease activity has been found after the 5th-day incubation. Isolate no.C4 showed the highest enzyme activity of 366.84 U/ml followed by S11 (362.48 U/ml) and S33 (335.47 U/ml). Concerning keratinase activity of the selected isolates, it has been found that isolates C4, S11 and S33 exhibited relatively high keratinase activities with values of 26.29, 21.91 and 17.49 U/ml, respectively,

Fig. 2 Clear zone around colonies of actinobacterial isolates indicates protease activity

after 6 days incubation, while isolate 5S1 should the highest keratinase activity of 26.40 U/ml after 9 days incubation period (Table 2). Several actinomycetes like *Streptomyces* sp. 2M21 (Demir et al. 2015), *Amycolatopsis keratiniphila* $D2^T$ (Espersen et al. 2021) and *Actinomadura keratinilytica* strain Cpt29 (Habbeche et al. 2014) have been reported to produce keratinases. As shown in Tables 1 and 2, the protease and keratinase activities produced by each isolate are correlated. Consequently, isolates C4, S11 and S33 were selected for further investigation based on the produced protease and keratinase activities.

Molecular Identification of C4, S11 and S33 isolates

Sequencing of the 16S rRNA gene of C4, S11 and S33 isolates showed an ancestral association with other actinobacterial strains and their phylogenetic trees were also constructed (Fig. 3). C4 isolate was identified as *Streptomyces coelicolor* C4-ITA-EGY with Genbank accession number MW662631.1, S11 was identified as *Streptomyces harbinensis* strain S11-ITA-EGY with an accession number of MW662612.1, whereas S33 isolate was identified as *Streptomyces carpaticus* strain S33-ITA-EGYwith accession number of MW662616.1. The insight nucleotide matrix included 1569 characters (from which 283 were variable and 121 were parsimony-informative) and 37 operational taxonomic units. The check of base-frequency revealed no evidence of compositional bias ($p = 1.00$, $\alpha = 0.05$). The GTR + GAMMA model of ML analysis showed the maximum log—likelihood of—4972.25, with alpha parameter of 0.21. Because the ML bootstrapping did not combine, 1400 replicates were performed, with an average support of 58.29. The best score of MP analysis was 485 (retention index 0.84, consistency index 0.75) and the best trees were 70. The average support for MP bootstrapping was 53.29%. As shown in Fig. 3, the tree is drawn to expand, with branch lengths in just the same units as the evolutionary distances used to deduce the phylogenetic tree. All positions with gaps and missing data were removed. Type strains are indicated with a superscript T.

Preparation of alkaline keratinolytic proteases and testing their hydrolysis of different keratinaceous substrates

According to the recorded enzyme activities in the previous experiment, three actinobacterial strains namely were selected. Three crude alkaline keratinolytic proteases (C4-E, S11-E and S33-E) were prepared from *S. coelicolor* C4-ITA-EGY, *S. harbinensis* S11-ITA-EGY and *S. carpaticus* strain S33-ITA-EGY, respectively, by the cultivation of selected strains using chicken feathers as a sole carbon source. Chicken feathers in the enzyme production medium can act as a source of both carbon and nitrogen and also a keratinase inducer (Anbu et al. 2008). These preparations showed both protease

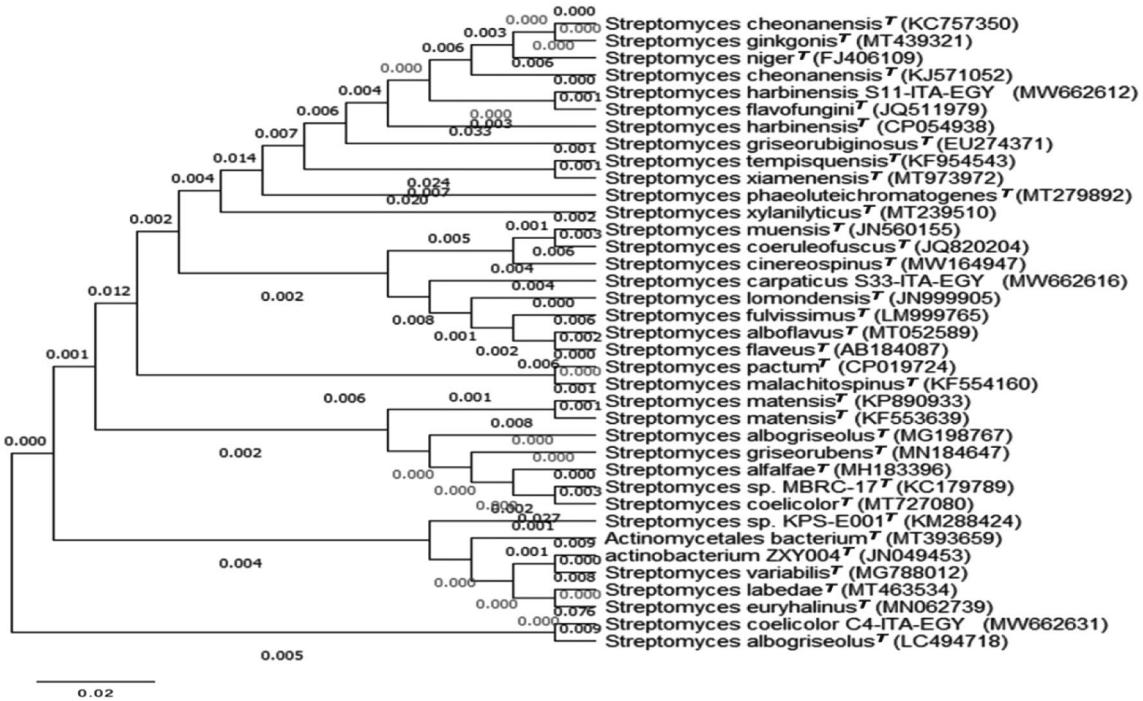


Fig. 3 The phylogenetic tree depicting the strains taxonomic positions relying on 16S rRNA gene sequences of the *Streptomyces coelicolor* strain C4-ITA-EGY, *Streptomyces harbinensis* strain S11-ITA-EGY and *Streptomyces carpaticus* strain S33-ITA-EGY. The NCBI accession number or gene ID number is in brackets. Bar, sequence divergence. Reference Type strains are indicated with a superscript T

and keratinase activities (data not shown). Keratinase was thought to be another possible enzyme for wool modification because it may minimize degree of cross-linking in keratinous proteins, making successive proteolysis with protease from the wool surface easier (Wang et al. 2011).

Different keratinaceous substrates (keratin, wool and chicken feather) were subjected to hydrolysis by different prepared keratinolytic proteases (Fig. 4). A common feature of all the tested substrates is that they were not soluble. In fact, the fibrous structure of keratins requires appropriate ionic strength and oxidative or reductive conditions to break the S–S bonds to be solubilized (Sinkiewicz et al. 2018). The tested substrates were chicken feathers, keratin and wool. The yield of hydrolysis originates from the fraction of peptides containing aromatic amino acids passing into the solution after and because of the hydrolysis. As shown in Fig. 4, generally hydrolysis of both wool and chicken feathers was harder than that of extracted keratin as they have a more complex structure and S–S bonds needed to be solubilized. C4-E keratinolytic protease exhibited better degradative activity against keratin whereas S11-E exhibited better degradative activity with the wool substrate. Regarding these results, C4-E and S11-E keratinolytic proteases produced by a cost-effective method were chosen to be applied for surface modification of protein-based fibers and removing proteinic stains.

Although the enzymatic hydrolysis of keratinaceous substrates is preferred because it operates under mild reaction conditions and consumes little energy, the soluble keratin yield of is insufficient to support the industrial enzymatic applications (Sinkiewicz et al. 2017). As a result, enzymatic hydrolysis should be aided by chemical reducing agents that degrade the disulphide bonds in keratin (Sinkiewicz et al. 2018). As an example of using a reducing agent, hydrolysis of keratinaceous substrates by C4-E alkaline keratinolytic protease (Fig. 5a, b and c) was investigated in the presence and absence of sodium sulphite agent. As shown in Fig. 5a, b and c, the presence of 0.5–1% sodium sulphite in the enzyme reaction markedly increases the hydrolysis yield whatever the used substrate is. Generally, the best used Na_2SO_3 concentration was 0.5%, except in the case of wool hydrolysis it was 1%. The yield of hydrolysis was in the following order CF<W<K according to the used substrate. Consequently, wool fabrics were treated with H_2O_2 before their application by C4-E and S11-E alkaline keratinolytic protease from *S. coelicolor* C4-ITA-EGY, *S. harbinensis* S11-ITA-EGY strains, respectively.

Some potential applications in textile chemical processing

Wool surface modification and functionalization

Applications of locally produced alkaline keratinolytic proteases, under appropriate conditions in surface modification of wool substrates followed by subsequent functional finishing using eco-friendly active ingredients namely ZnONPs, ZrO_2NPs, ascorbic acid and vanillin individually to impart highly demanded antibacterial, and UV-shielding functionalities to the biotreated substrates have been investigated.

The data in Table 3 revealed that pre-washing and pre-washing/H_2O_2 bleaching of grey wool samples result in increment in weight loss, decrement in nitrogen content, a slight improvement in antibacterial activity against the tested pathogenic *E. coli* bacterium along with a remarkable decrease in UPF values. The variation in the tested properties reflects the differences between the tested pretreatment steps in the extent of hydrophobic contaminants removal, e.g. fatty acids, grease, wax, natural colorants, etc., along with attacked, ruptured and removal of wool scales and the amino acids in the keratins due to oxidative effect of H_2O_2, which in turn resulted in increased weight loss and decreased nitrogen content of pretreated substrates (Ibrahim et al. 2012; Madhu and Chakraborty 2017; Radhakrishnan 2014; Wang et al. 2012).

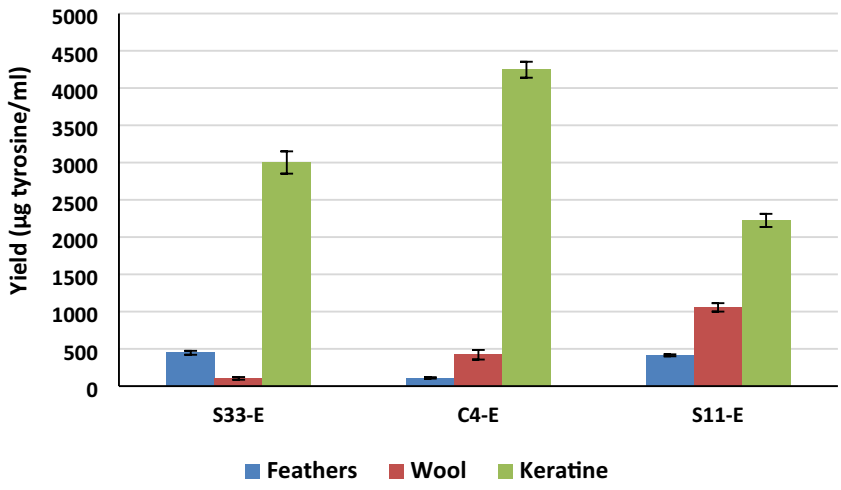


Fig. 4 The hydrolysis yield of prepared enzymes against different keratinaceous substrates. The hydrolysis was done in glycine buffer pH 10 for 24 h at 40 °C and the hydrolysis yield was monitored as tyrosine by Folin–Ciocalteu reagent

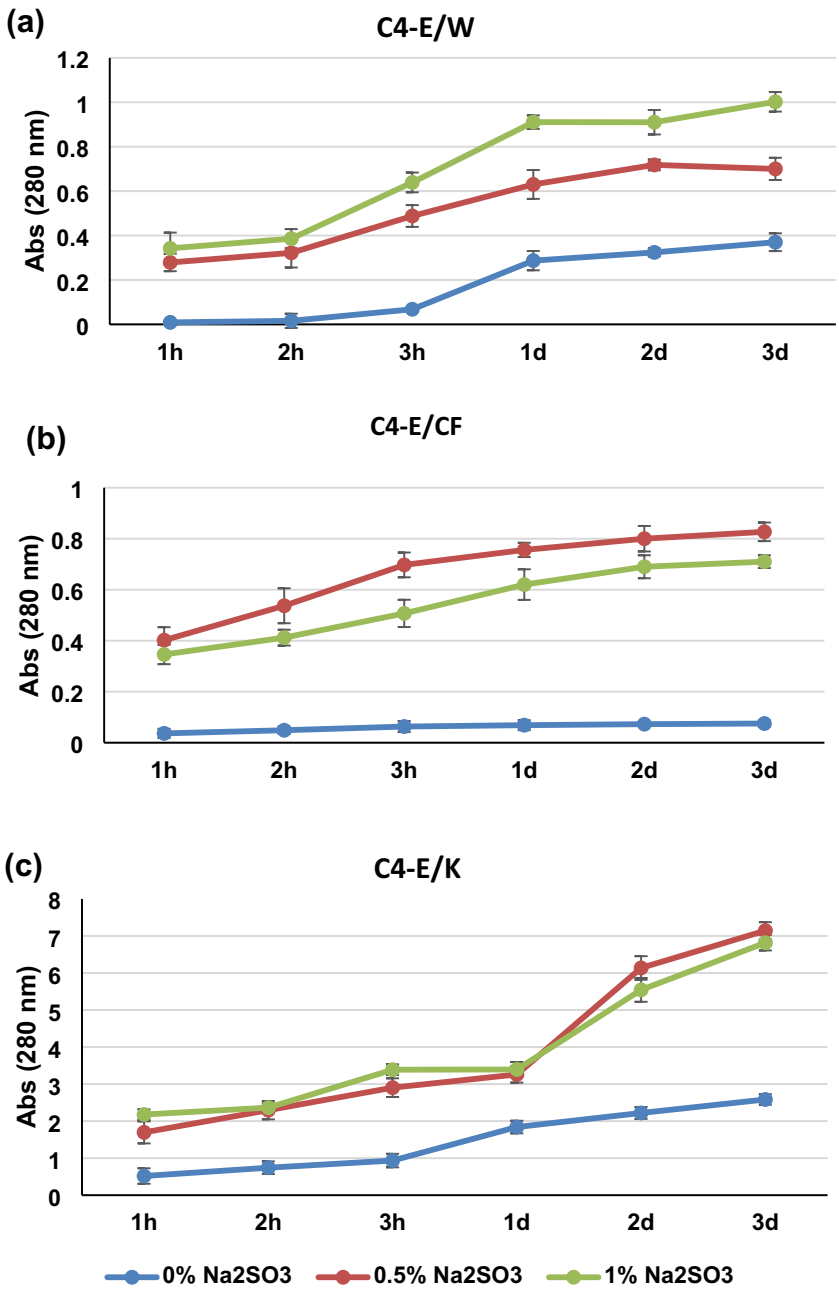


Fig. 5 Hydrolysis of **a** wool (W), **b** chicken feathers (CF), and **c** keratin (K) by C4-E alkaline keratinolytic protease. The hydrolysis was done in glycine buffer pH 10 at 40 °C and the hydrolysis yield was evaluated by monitoring the increase in absorbance at 280 nm

It is also observed that addition of H_2O_2-bleaching agent to the prewashing formulation improves both hydrophilicity and wettability along with partial oxidation of natural colorants, i.e. improved whiteness, which in turn resulted in minimizing the UV-protection functionality, expressed in UPF value (Shen 2019).

On the other side, the positive role of H_2O_2 species like per hydroxyl species in attacking the keratins-amino acids and rupturing of the disulfide linkages of cysteine and hence increases the number of available active sites, e.g. −COOH, $-NH_2$, −SH, etc. consequently results in inhibiting the growth of pathogenic bacterium via altering the microbial cell membrane and thus damage the cell (Ibrahim et al. 2019).

Also, the data represented in Table 3 clearly show that post-enzymatic treatment of the pre-washed fabric samples using the produced proteolytic enzymes C4-E and S11-E results in a reasonable loss in weight, a slight decrease in nitrogen content, an increase in antibacterial activity and a significant reduction in UV-shielding functionality of bio-treated fabric samples. The degree to which the tested physicochemical and functional properties decrease or increase is governed by kind of substrate, G, W, or W/B and type of enzyme, i.e.

Table 3 Effect of post-enzymatic treatment on some properties of pretreated wool fabric samples

Fabric sample	Fabric properties								
	Pretreated				Pretreated and post-treated with enzyme				
	WL (%)	N (%)	BR (%)	UPF	Enzyme	WL (%)	N (%)	BR (%)	UPF
Grey (G)	–	19.74	–	128	C4-E	1.42	19.35	15.92	93
					S11-E	1.67	19.56	21.44	106
Washed (W)	0.54	18.85	5.67	115	C4-E	1.80	18.28	25.67	85
					S11-E	2.37	18.60	27.49	97
Washed/ H_2O_2bleached (W/B)	1.07	18.15	11.48	92	C4-E	2.23	17.40	29.82	80
					S11-E	2.53	17.62	31.30	85

WL: weight loss; N: nitrogen content, R: reduction of *E. coli* bacteria; UPF: UV-protection factor. Treatment cycle: Washing in absence (W) and presence of H_2O_2 (W/B) followed by subsequent enzymatic treatment using alkaline keratinolytic proteases C4-E from *Streptomyces coelicolor* C4-ITA-EGY, and S11-E from *Streptomyces harbinensis* S11-ITA-EGY

Table 4 Effect of alkaline keratinolytic protease S11-E on the extent of stain removal

Fabric	Stain type	Reflectance (R)	
		Unwashed	Washed with alkaline Keratinolytic protease S11-E
Cotton	Blood	3.63	17.62
	Egg	8.71	16.78
C/PET	Blood	5.14	16.27
	Egg	9.66	15.34

its source, enzymatic activity and stability, active sites, biocatalytic efficiency, molecular size and hence the extent of diffusion and penetration within the wool structure (Madhu and Chakraborty 2017).

As far as change in WL (%), N (%), BR (%) and UPF values of bio-treated substrates vary according to enzyme type. Table 4 data show that the decrease in weight, nitrogen content and UV-shielding ability follows the decreasing order: S11-E > C4-E, keeping other parameters constant. The experimental results demonstrate the variation in surface modification and functionalization of bio-treated substrates, the extent of degradation of cuticle scales and removal of hydrophobic matters, subsequent improvement in hydrophilicity and wettability, along with the creation of more accessible cationic active sites like amine terminal groups as a direct consequence of the enzymatic attack (Ibrahim et al. 2012).

The enhancement in antibacterial functionality with the combination of pre-and bio-treatments reflects the positive role of enzymatic post-treatment on enhancing the cationic sites on/within the wool structure thereby enabling the electrostatic interaction with the negatively charged bacterial cell membrane and consequently leading to leakage on intercellular components of the bacterial cell (Sheikh and Bramhecha 2018). Also, partial immobilization of the used proteolytic enzyme onto/within the bio-treated wool structure via adsorption, entrapment and/or chemical bonding makes the pathogenic bacterial protein more susceptible to subsequent attack, and hence the imparted antibacterial functionality is improved (Shen 2019).

EDX and SEM analysis

Figure 6 depicts the EDX spectra and SEM images of untreated (a, b), prewashed then bio-treated using S11-E enzyme (c&d), and prewashed/H_2O_2 bleached then bio-treated (e, f) selected wool fabric samples. As seen incorporation of H_2O_2 bleaching along with post-enzymatic treatment with S11-E enzyme are accompanied by a noticeable modification and improvement of the surface of wool scales as a direct consequence of removal and/or modification of these scales via an oxidative treatment using H_2O_2 followed by proteolysis (e, f). The extent of descaling and surface modification follows the decreasing order: W/B → enzymatic treatment (Fig. 6e) > W → enzymatic treatment (Fig. 6c) > > untreated (Fig. 6a) (Ibrahim et al. 2012; Jajpura 2018).

On the other hand, EDX elemental analysis (Fig. 6b,d,e) demonstrates the decrease in the S- element of the treated substrates as a direct consequence of pre−H_2O_2 bleaching and subsequent enzymatic treatment which in turn resulted in the rupture of some cysteine disulfide bonds (Wang et al. 2012). The extent of decrease in S-content follows the above-mentioned order.

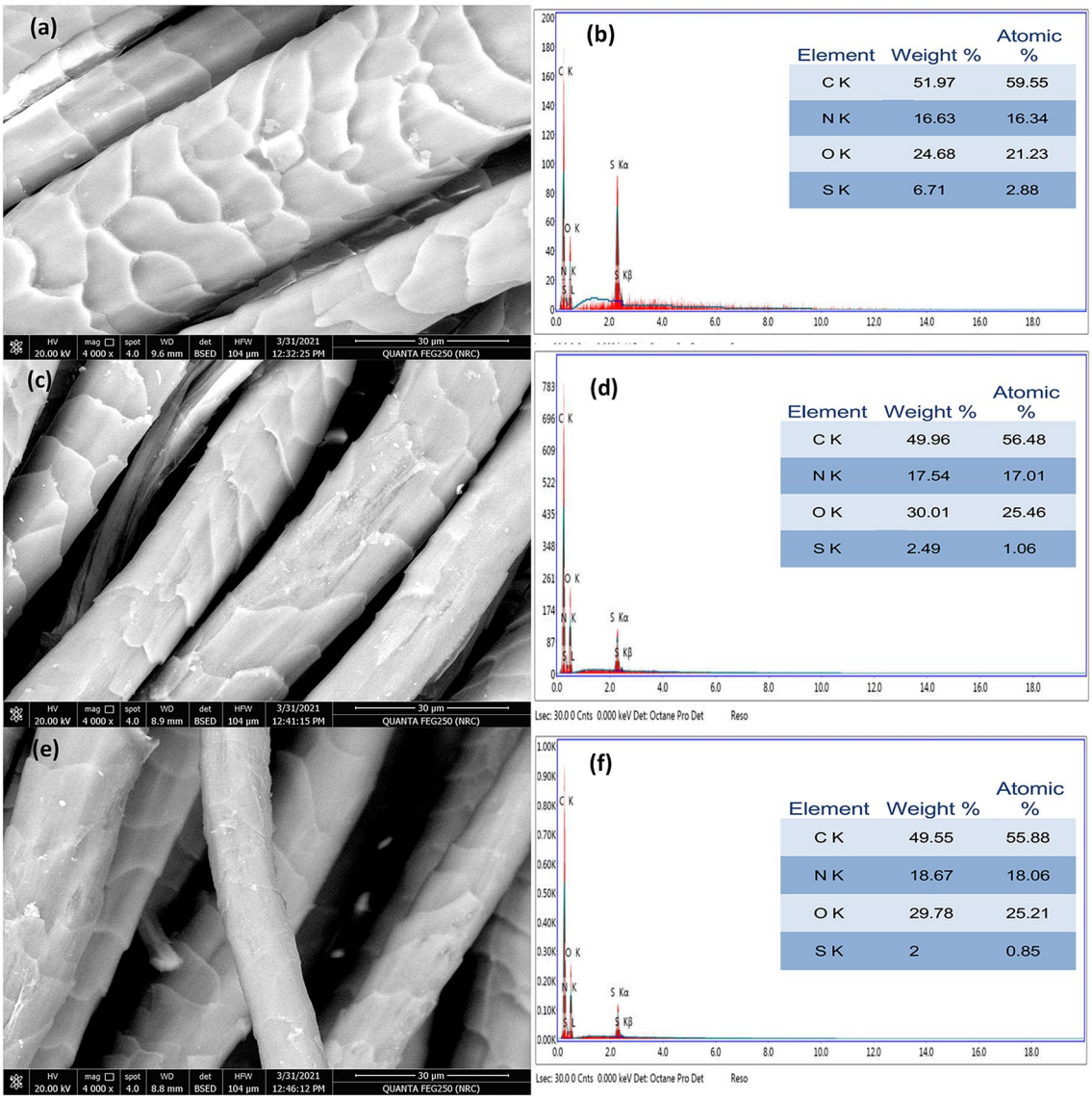


Fig.6 SEM images and EDX spectra of untreated wool fabric (**a** and **b**), wool fabric sample washed followed by treatment with alkaline keratinolytic protease enzyme S11-E (**c** and **d**), wool fabric sample treated with H_2O_2 followed by alkaline keratinolytic protease enzyme S11-E **e** and **f**

Nano-finish of bio-treated wool samples

As shown in Fig. 7a post-treatment of bio-treated wool fabric samples with ZnONPs or ZrO_2NPs leads to a reasonable increase in the antibacterial functionality against the tested *E. coli* bacterium, which is one of the most common pathogenic negative bacteria, regardless of the used alkaline keratinolytic protease enzyme and type of nanometal oxide (NMO). The type of alkaline keratinolytic protease determines the extent of antibacterial functionality enhancement that follows the reducing order: S11-E > C4-E, which reflects their differences in the extent of modification of wool structure, generating of accessible active sites e.g. $-NH_2$, $-COOH$, $-SH$ etc. and consequently uptake, fixation and immobilization of NMO onto/ within the wool structure. Figure 7a also demonstrates that the imparted antibacterial activity to the wool substrate follows the descending order: ZnONPs > ZrO_2NPs > > None, keeping other parameters constant.

The imparted antibacterial functionality could be discussed in terms of the photocatalytic activity of loaded NMO under UV/Vis irradiation and subsequent generation of highly reactive species, e.g. H_2O_2, $^{\cdot}OH$, $^{\cdot}O_2^-$, single oxygen (El-Nahhal et al. 2020; Ibrahim 2015; Ibrahim et al. 2018a, 2018b) as follow:

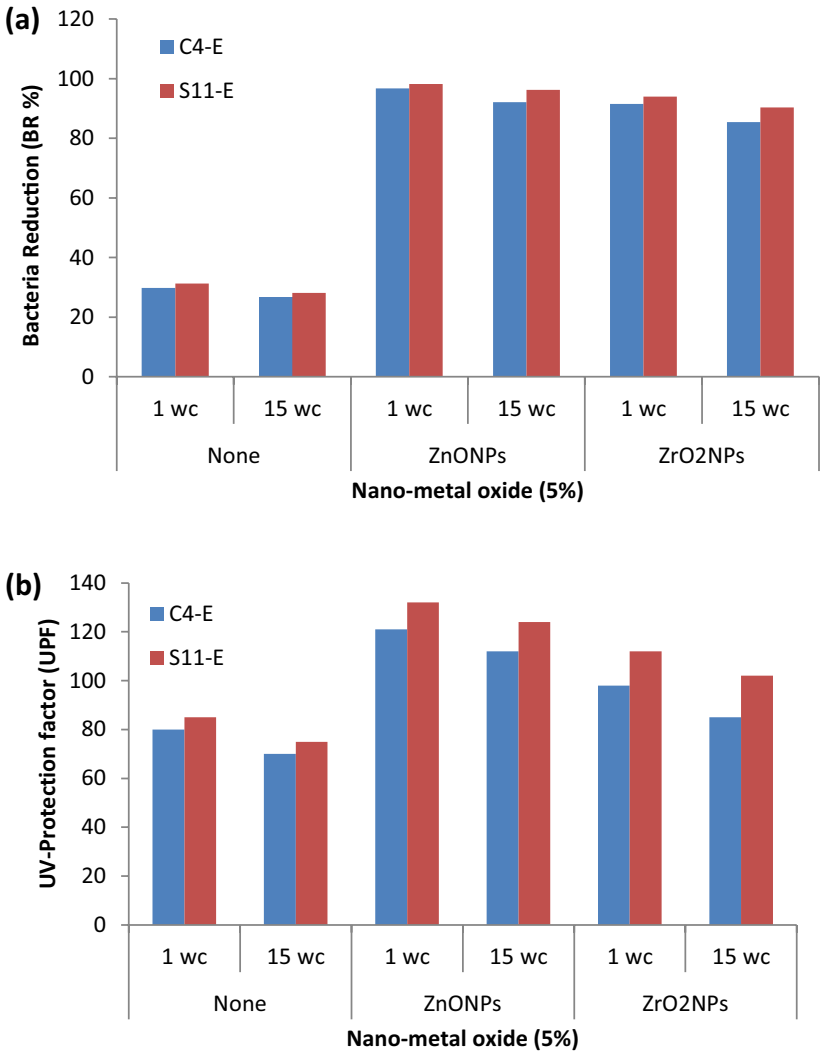


Fig. 7 Effect of ZnONPs or ZrO_2NPs post-treatment on: **a** antibacterial activity and **b** UV-protection properties of bio-treated wool samples

$$NMO + h\nu \xrightarrow{UV/V\text{ is irradiation}} NMO + h^+ + e^- \quad (1)$$

$$h^+ + H_2O_2 \rightarrow^{\cdot} OH + H^+ \quad (2)$$

$$e^- + O_2 \rightarrow^{\cdot} O_2^- \quad (3)$$

$$^{\cdot}O_2^- + H^+ \rightarrow HO_2^{\cdot} \quad (4)$$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad (5)$$

which have the ability to inhibit the normal metabolism of the pathogenic bacteria, and oxidize the organic components in the bacterial cell thereby contributing to the imparted antibacterial efficacy. Additionally cell wall damage and change in the cell wall permeability due to the abrasion of the loaded NMO particles along with releasing of metal cations leading to the death of pathogenic *E.coli* bacterium (El-Nahhal et al. 2020; Prasad et al. 2016).

It is quite clear from Fig. 7b that loading of ZnONPs or ZrO_2NPs onto the bio-treated wool fabric samples results in a significant increase in UV-protection functionality, demonstrated as UPF value, irrespective of the used alkaline keratinolytic protease and follows the decreasing order: ZnONPs > ZrO_2NPs > > None. On the other hand, bio-treated fabric samples using S11-E, in the absence and presence of NMO particles, demonstrate better UPF in comparison with C4-E bio-treated ones, which could be discussed in terms of the degree of the nano-finished materials distribution onto/within the nano-finished fabric structure. The substantial improvement in the UV-shielding capacity of the bio-treated then nano-finished fabric samples, (UPF > 50^+, excellent), reflects the capability of the loaded ZnONPs and ZrO_2NPs in shielding and blocking

the harmful UV radiation most probably as a result of their greater surface area and intense absorption in the UV-range (Harifi and Montazer 2017; Ibrahim et al. 2018d).

On the other hand, Fig. 7a and b demonstrate that even after 15 consecutive washing cycles, the imparted functional properties remain > 90% and > 50^{+} for antibacterial and UV-protection functional respectively, which reflects the high extent of fixation and immobilization of the used ZnO and ZrO_2 NPs onto/within the bio-treated then nano-finished wool fabric samples.

Once again, the so obtained results ultimately indicate that enzymatic treatment of wool fabric with the prepared enzymes results in surface modification, degradation of cuticle scales, removal of hydrophobic contaminants, enhancing fabric hydrophilicity and wettability along with creation of more accessible active sites, e.g. $–NH_2$, –COOH, –NH, thereby improving the extent of loading/fixation and immobilization of ZnONPs and ZrO_2NPs onto/within the wool structure and hence upgrading its antibacterial and anti-UV functionalities compared with the untreated ones.

SEM images and EDX spectra

Figure 8 demonstrates the surface morphology and EDX spectra of selected wool fabric samples bio-treated and then nano-finished with ZnONPs and ZrO_2NPs respectively, as shown in Fig. 8a, b, c and d). SEM images Fig. 8a and c confirmed the deposition of ZnONPs and ZrO_2NPs onto the surface of nano-finished wool fabric samples. Additionally, EDX spectra of nano-finished bio-treated wool fabric samples Fig. 8b and e showed peaks of Zn and Zr-elements in their pattern confirming the immobilization and loading of both ZnONPs and ZrO_2NPs onto the bio-treated then the nano-finished wool fabric samples in comparison with the biotreated ones Fig. 6e and f.

Post-treatment with ascorbic acid and vanillin

Changes in the antibacterial properties along with UV-shielding capacity of bio-treated wool fabric samples as a function of the type of environmentally sound active ingredient, the data in Fig. 9a and b demonstrate that individual incorporation of ascorbic acid and vanillin in the finishing bath as active phenolic compounds leads to a substantial enhancement in both antibacterial activity against *E. coli* (pathogenic Gram-negative bacteria), and UV-protection functionality. The extent of improvement in the aforementioned functionality demonstrates the following order: S11-E > C4-E and ii) vanillin > ascorbic acid > > None, keeping other parameters constant.

The imparted antibacterial activity to the bio-treated fabric samples loaded with ascorbic acid and vanillin most probably is attributed to the negative impacts of the loaded phenolic bioactive compounds on DNA and disrupting effect on the bacterial cell which in turn adversely affected its growth and survival (Gegel et al. 2018; Hong 2014, 2015; Ibrahim et al. 2019, 2018c; Kumaraswamy et al. 2019).

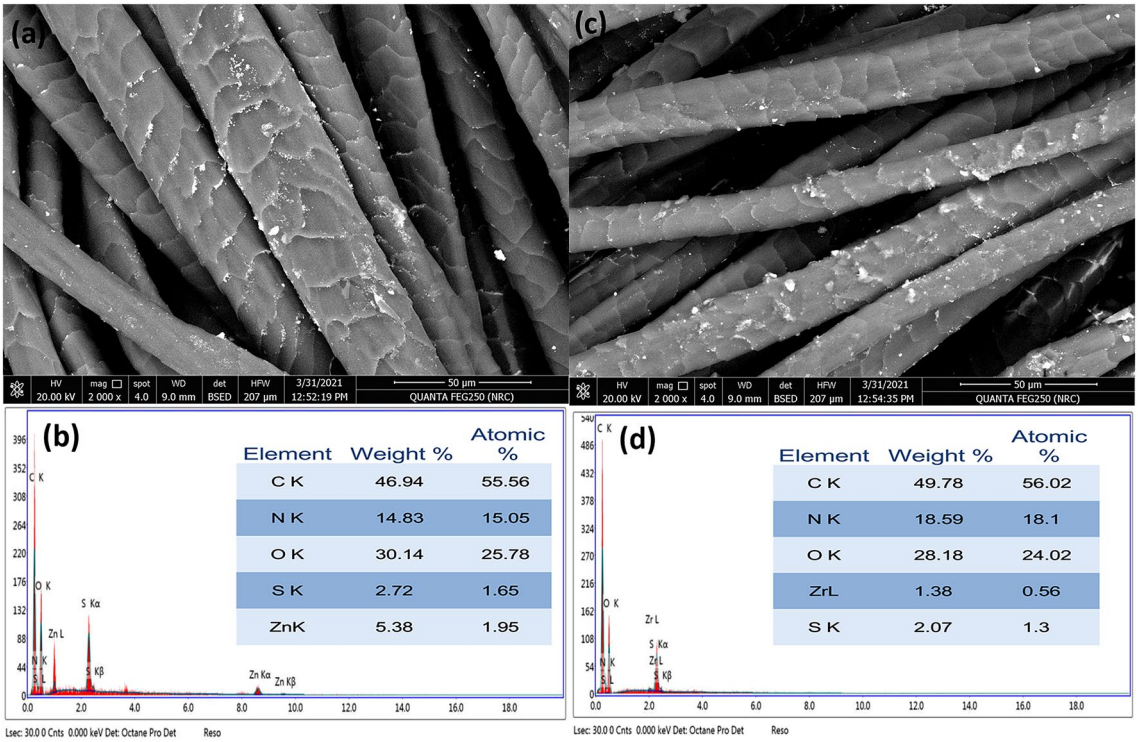


Fig. 8 SEM images and EDX analysis of enzymatically treated wool fabric followed by ZnONPs (**a** and **b**), ZrO_2NPs (**c** and **d**)

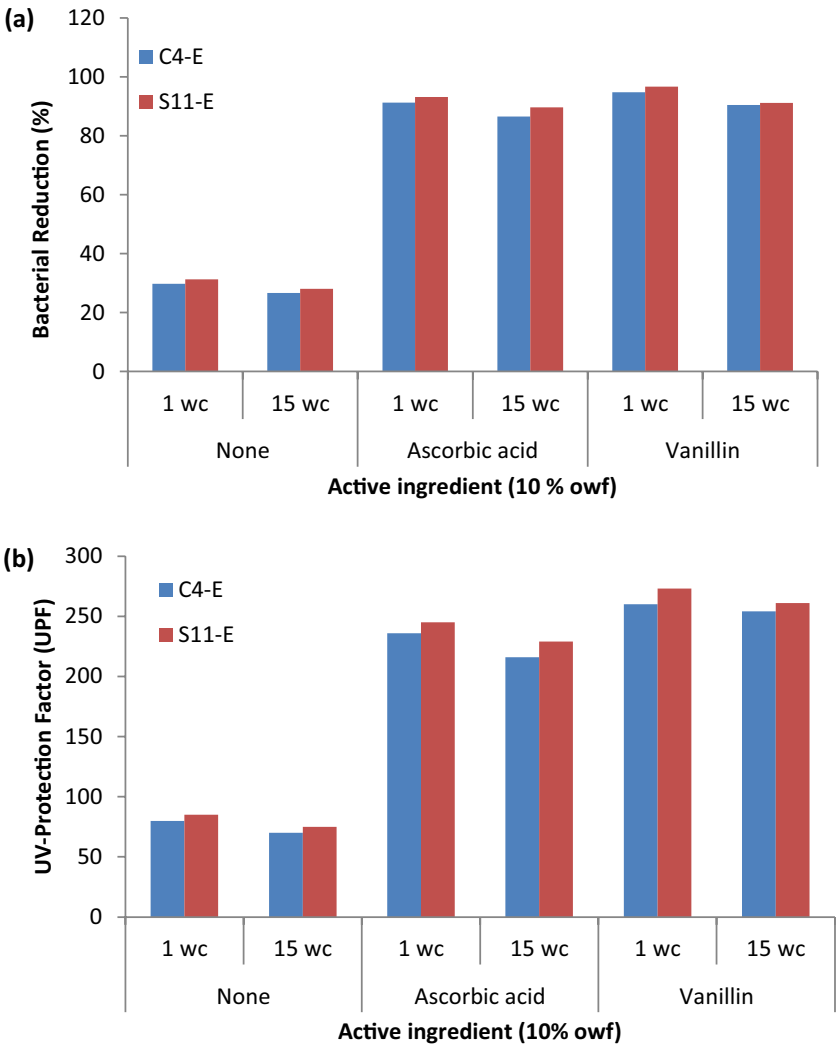
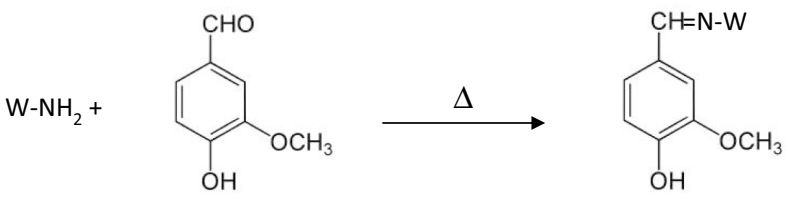


Fig. 9 Effect of post-treatment with ascorbic acid or vanillin on: **a** antibacterial activity and **b** UV-protection properties of bio-treated wool samples

On the other hand, the inclusion of any antibacterial ingredients, ascorbic acid and vanillin phenolic compounds, in the post-functional finishing of bio-treated wool samples is accompanied by a remarkable enhancement in their UV-protection capacity, expressed as UPF value, reflecting the positive role of bonded phenolic compounds in blocking the fabric porosity, in shielding and/or absorbing the extra UV-harmful radiation (Hong 2015; Ibrahim et al. 2019; Kim 2015). Additionally, loading of vanillin fragrant onto/within the bio-treated wool structure adds the fragrance functional property to the finished substrate.

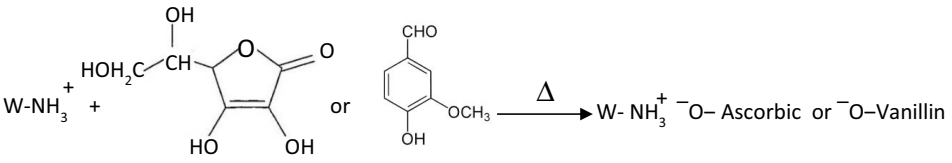
Moreover, Fig. 9a and b further demonstrate that, all post-finished fabric samples, after 15 wash cycles, exhibited a small reduction in both BR (< 15%) and UPF (< 10%), irrespective of the loaded phenolic compound, due to the release of unfixed, physically bonded and/ or entrapped active ingredients by increasing the wash cycles. The degree of fixation and immobilization of the nominated phenolic compounds is governed by chemical structure, molecular size, functional groups as well as a mode of interaction and chemical fixation onto/within the bio-treated wool accessible active sites as follows:



(6)

Wool vanillin

$$W\text{–}NH_2 + H^+ \longrightarrow W\text{–}NH_3^+ \quad (7)$$



wool ascorbic acid vanillin Ascorbic or vanillin- loaded wool fabric (8)

which in turn enhances the degree of fixation and immobilization of the used active ingredients, i.e. high durability to wash.

Removal of some protein-containing stains

As far as the potential application of the produced *S. harbinensis* alkaline keratinolytic protease, S11-E, in the removal of some protein-containing stains namely blood and egg stains, the data in Table 4 disclosed that the inclusion of the alkaline keratinolytic protease enzyme (1 g/l) along with the nonionic wetting agent (1 g/l) in the washing bath results in a remarkable improves in the extent of stain removal, expressed as R-value, as a direct consequence of its proteolytic ability to disrupt and degrade these proteinaceous stains into more easily removed fragments thereby facilitating their removal by the used nonionic surfactant. Additionally, the degree of stain removal is affected by the stains type and chemical composition, location and distribution onto/within the stained substrate (Madhu and Chakraborty 2017; Matkawala et al. 2019; Niyonzima and More 2015), availability and accessibility to enzyme attack as well as the hydrophobic nature of the stained substrate along with the synergistic action of alkaline keratinolytic protease and the used nonionic wetting agent under the given washing conditions.

Conclusion

Green surface modification of wool structure using the produced alkaline keratinolytic proteases under appropriate conditions followed by subsequent nano-finishing using ZnONPs and ZrO_2 NPs or post-treatment with environmentally sound bioactive agents namely ascorbic acid and vanillin to enhance and develop the targeted antibacterial and UV-blocking functionalities are investigated.

The extent of wool modification and post-functionalization is determined by bio-treatment conditions and proteolytic activity of the used enzyme as well as post-finishing formulations. For a given set of bio-treatment conditions, the extent of wool modification follows the decreasing order: *S. harbinensis* alkaline keratinolytic protease (S11-E) > *S. coelicolor* alkaline keratinolytic protease (C4-E), and (W/B) → enzymatic treatment > W → enzymatic treatment > > Untreated.

The enhancement in antibacterial activity against the tested Gram-negative bacteria (*E. coli*) and the improvement in UV-blocking efficacy of the bio-treated –nano-finished wool substrates follow the descending order: ZnONPs > ZrO_2 NPs > > None, keeping other parameters constant.

Also, the increase in antibacterial functionality and UV-blocking capacity using the ascorbic acid and vanillin bioactive agents can be ranked as follows: vanillin > ascorbic acid > > None.

Moreover, SEM and EDX analysis for selected samples confirm both surface modifications by enzymatic treatment and surface deposition and immobilization of nanometal oxides used on the biotreated-finished fabric surfaces.

The results obtained further signify that all bio-treated finished fabric samples exhibit pronounced antibacterial activity against the tested pathogenic bacterium and excellent UV-protection functionality ($> 50^+$) even after 15 consecutive piles of washing.

The developed durable eco-friendly, multifunctional wool products can have a wide range of potential applications like outdoor textiles, medical textiles, sportswear, etc.

Additionally, it was observed that potential utilization of the *Streptomyces harbinensis* S11-ITA-EGY alkaline keratinolytic protease (S11-E) in textile aftercare is accompanied by a remarkable improvement in the extent of removal of some common protein-based stains like blood and egg.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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