



A novel detergent-stable protease from *Penicillium chrysogenum* X5 and its utility in textile fibres processing

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Received: / Accepted: / Published:

Abstract:

The consumption of energy and raw-materials, as well as increased awareness of environmental concerns related to the use and disposal of chemicals into landfills, water or release into the air during chemical processing of textiles are the principal reasons for the application of enzymes in finishing of textile materials. The aim of this study is to isolate a new fungi protease, with excellent proprieties in order to be used as a bio-additive in detergent formulation with any destructive effect on textile supports. SAPTEX is a new extracellular thermostable serine alkaline protease (designed as SAPTEX) was purified to homogeneity and biochemically characterized from *Penicillium chrysogenum* strain X5 as a monomer with 43 kDa. The experimental purification protocol comprises three steps: heat treatment optimized by experimental design followed by an ammonium sulfate precipitation, and a FPLC/UNO Q-12 anion exchange chromatography. The optimum pH and temperature values for protease activity were pH 10 and 80°C, respectively. According to morphological, physico-chemical, and metrological evaluation, SAPTEX has no destructive impact on fibers after the enzyme treatment and a very slight effect on textile support. Data suggested that SAPTEX may be considered a potential candidate as a protein stain removal product from textile supports.

Keywords: *Protease; Penicillium chrysogenum; Detergent; Textile.*

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1. Introduction

According to the quality of the workforce, political stability, economic, and geographical approximation with Europe, textile sector has a significant importance in the Tunisian economy. Today enzymes have become an integral part of the textile processing. At present, proteases are used in textile processing. However, due to the protease action, severe damage on natural protein fibers, results after washing with detergents containing proteases.

2. Results and Discussion

Purification procedure of SAPTEX

The purified enzyme preparation contained about 35% of the total activity of the crude and had a specific activity of 78,500 U/mg using casein as substrate.

Molecular weight determination of SAPTEX

To analyze the homogeneity and molecular weight of the purified SAPTEX, native-PAGE and SDS-PAGE were performed. The molecular mass of the purified enzyme was estimated to be approximately 43 kDa as assessed by native-PAGE.

Wash performance analysis of SAPTEX, Flavourzyme® 500L, and Alcalase Ultra 2.4L proteases

In order to evaluate the performed of SAPTEX in detergence area, some visual tests was done. This test was done compared to Flavourzyme® 500L or Alcalase Ultra 2.4L proteases. The proteases tasks were blood and egg stains on the cotton fabric. The washing is carried out at 40 °C for 30 min. It is noted that the addition of the SAPTEX protease to the detergent solution improves the performance of the Ariel detergent to ensure better discoloration of blood stains and egg stains.

Morphological evaluation of the performance of SAPTEX on textile supports

Comparing these images, no fissures or alterations in the surface after enzymatic treatment with SAPTEX are observed. The cotton fibers have the same appearance for both the control and the treated sample with SAPTEX. The state of the fibers has not changed indicating that the enzyme SAPTEX has not altered the surface condition of the cotton support.

Accordingly, we should evaluate the quality of stain removal and its impact on the shade of textile substrates and their mechanical properties. The aim is guaranteeing the whiteness of the textile support, softening hard water in order to optimize the conditions of action of the active ingredients, preserving the brightness of the colors and the general appearance of the textile support and fighting against the redeposition of dirt.

Spectrophotometry with dispersive energy (EDS)

The enzyme SAPTEX did not alter the structure or the chemical composition of the corresponding support (EDX) which both contained the same chemical elements (carbon, nitrogen, oxygen, and calcium).

FTIR

The FT-IR transmission can measure the bulk composition of fabrics [1]. It shows a comparison between different spectrograms (the red spectrum is the cotton treated with SAPTEX previously stained by egg; purple spectrum is the cotton treated with SAPTEX previously stained with a blood stain) above cotton fibers treated with the enzyme SAPTEX and control cotton (The blue spectrum).The treatments in textile substrates generally affect the cotton [2, 3], but the effect of this treatment remains negligible because the chemical structure doesn't changed.

Tear strength

The enzymatic treatment did not affect the warp yarns of the textile support unlike the weft threads, where the tearing force decreased. These threads are generally less resistant than weft threads and more sensitive to chemical and mechanical stresses. Enzymatic treatment slightly altered their tear resistance.

Dynamometry

The tensile strength of textile substrates is determined by the GrabTest2 method (ISO13934-2 standard). The obtained results indicated that there is a slight decrease in the strength and elongation at break of the treated cotton which remains insignificant, indicating that the enzyme treatment did not alter the cotton fibers

3. Materials and Methods

Experimental

Purification of the SAPTEX from the strain X5

A five hundred mL of a 72-h culture of *Penicillium chrysogenum* strain X5 were centrifuged for 20 min at $10,000 \times g$ to remove microbial cells. First, a heat treatment was done at 80 °C for 10 min. Second, the salting out with ammonium sulfate: the supernatant was precipitated between 30 and 50% ammonium sulfate saturation and dialyzed overnight against repeated changes of 50 mM Tris HCl pH 8.5. Insoluble material was removed by centrifugation at $9,000 \times g$ for 30 min. The supernatant obtained was deposited on a UNO Q-12 column (12 mm \times 53 mm) (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using Fast protein liquid chromatography (FPLC) system previously equilibrated with buffer B. The proteins were eluted with the same buffer containing an increasing concentration of NaCl of 0 to 500 mM at a rate of 30 mL/h.

Proteins measurement, electrophoresis, and mass spectrometry analysis

The subunit molecular weight of the purified SAPTEX was estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions as described by Laemmli [4], using 12% separating gel (pH 8.8) and 5% stacking gel (pH 6.8). The native-PAGE (non-reducing conditions) of the purified SAPTEX was performed using 10% resolving gel (stacking gel was omitted) in Tris-glycine buffer (pH 8.5) at 4 °C. The protein bands were visualized with Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) staining. Casein zymography staining was performed as described elsewhere [5].

Removal of blood and egg stains from cotton fabrics

New cotton cloth pieces (6 cm \times 6 cm) were stained with blood and egg stains and used to simulate the washing condition and determine the efficiency of SAPTEX as a bio-detergent additive compared to the commercially proteases Flavourzyme® 500 L and Alcalase® 2.4L. The

stained cloth pieces were shake-incubated (250 rpm) in different wash treatments at 40 °C for 1 h in 1-litre beakers containing a total volume of 100 mL of: tap water, Ariel detergent (7 mg/mL, in tap water), and detergent added with SAPTEX (500 U/mL) or commercial Flavourzyme® 500 L and Alcalase® 2.4L (500 U/mL). After treatment, the cloth pieces were taken out, rinsed with water, dried and submitted to visual observation to examine the stain removal effects of the enzymes. The untreated blood-stained piece of cloth was taken as a control.

Effect of SAPTEX on the fiber morphology using Scanning Electron Microscopy (SEM)

The study of SAPTEX's effect on the fiber morphology using SEM was done for both treated and untreated samples. It is generally used to study the 3D morphology of a surface or an object and also the chemical composition (Microanalysis X).

Determination of maximum force and elongation at maximum force by Grab Test2 (ISO13934-2 Standard)

This norm based to determine the maximum tensile strength of textile fabrics. A comparison between the cotton fibers before and after the SAPTEX treatment was done in warp and weft direction. A dynamometric study, performed under specific conditions, is essential to predict its behavior toward the constraints of use.

Determination of the force of the strength tear (Standards ISO NF G07 147 - NF EN ISO 13937-1)

In order to highlight the resistance of tissues to the tear the Elmendorf Tear Tester (Elmendorf method) was used. The influence of the SAPTEX treatment on the tensile strength of the weft and warp support was determinate.

Fourier transforms infrared spectral analysis (FTIR)

The structural changes of textile supports upon SAPTEX treatment were studied by FTIR. It is a measurement technique for the acquisition of infrared spectra. The vibration patterns that appear in the infrared spectra provide information about the chemical functional groups

of a sample, which leads to the characterization of the fibers or the identification of specific compounds.

Electronic differential lock

In order to determinate qualitatively the nature of the fibers tested by SAPTEX treatment, the SEM can be coupled to a dispersive energy spectrophotometer (EDS system). The SEM technique with Energy-Dispersive X-ray spectroscopy (EDX) analysis (FEI QUANTA 200) was used to analyze the surface fabrics.

4. Conclusions

The present investigation revealed fungus identified as *Penicellium chrysogenum* strain X5 and producing extracellular alkaline protease called SAPTEX with attractive biochemical characteristics as a good bio-additif with a wide

Acknowledgments

The authors would like to express their gratitude to Mr. K. Walha, Mrs. N. Kchaou, and Mrs N. Masmoudi (Analysis Unit-CBS) for their technical assistance with the HPLC and FPLC. We extend our thanks to Mr. M. Marzouki (INRAP, Technopole Sidi Thabet, Ariana, Tunisia) for SEM and EDS analyses and Mr. N. Baccar (LEBP, CBS) for FITR analysis.

Author Contributions

Conceived and designed the experiments: NK, BJ.

Performed the experiments: MOB, EM.

Analyzed the data: MOB, SB, RZ, AB, NK, JB.

Contributed reagents/materials/analysis tools: MOB, SM.

Wrote the text of the paper: MOB, BJ.

Critical revision of manuscript: RZ, AB, NK, BJ.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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range of commercialized laundry detergents for textile supports.

Due to the morphological, physico-chemical, and metrological evaluation, SAPTEX demonstrated no destructive impact on fibers after the enzymatic treatment and showed a very slight effect on textile support. Overall, the findings indicate that SAPTEX is bestowed with a number of promising properties that may be considered as potential candidate for protein stain removal from textile supports in the textile processing applications.