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Use of Protease Produced by *Bacillus* sp. SJ-121 for Improvement of Dyeing Quality in Wool and Silk

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Abstract In this study, a microorganism-produced protease was used to improve the quality of fabrics. First, the protease-producing bacteria were isolated from soils, and one of them was selected and identified as *Bacillus* sp. SJ-121. The optimal medium composition for its growth and protease production was determined to be as follows: glucose 1 g/L, soybean meal 0.5 g/L, soy peptone 0.5, K_2HPO_4 0.2, $MgSO_4 \cdot 7H_2O$ 0.002, NaCl 0.002, and Na_2CO_3 g/L. Also, the optimal temperature for the production of the protease by *Bacillus* sp. SJ-121 was about 40°C at pH 7. The wool and silk were treated with the protease from *Bacillus* sp. SJ-121. Following the protease treatment, changes in the surface of a single yarn of the fabrics were observed by both an optical microscope and a scanning electron microscope (SEM). Changes in the K/S value of the wool and silk were measured by spectrophotometric analysis, in order to determine the amount of dye uptake in the fabrics. We also performed a tensile strength examination in order to determine the degree and nature of mechanical changes in single yarns of the wool and silk fabrics. By increasing the protease treatment time to 48 h, the dyeing characteristics of the fabrics were enhanced, and the surfaces of the single yarns of the fabrics became smoother, due to the removal of soil and scale in them. However, no mechanical changes were detected in the fabrics. Therefore, we suggest that proper treatment of the protease produced by *Bacillus* sp. can improve the quality of silk and wool.

Keywords: *Bacillus* sp., dyeing property, mechanical property, protease

INTRODUCTION

Bacillus subtilis produces seven kinds of proteases as a reaction to various substrates, some alkaline, and some neutral. One of these alkaline proteases (subtilisin) strongly degrades fibrin [1]. A previous study reported that the alkaline serine proteases produced by *Bacillus* sp. in fermented soybeans exhibiting fibrinolytic activity [2].

Wool fiber is, largely, chemically composed of keratin proteins, and it generates a bad smell when burned because it also contains sulfuric components. Its structure is characterized by two distinct components: (i) the cuticle, which is involved in the protection, luster, and elasticity of wool fibers, and it increases spinning ability due to its efficient ability to combine fibers; and the (ii) cortex, which comprises about 90% of wool fiber, and is involved in the intensity and elasticity of the wool fiber [3].

The highest grade of fabric, silk fabric, processes a fine luster, tactile sensation, and draft property, and therefore, its dyeing characteristics are distinct from those of other fabrics. Silk is composed of two major proteins (fibroin and sericin), and it also contains trace elements, such as wax and salts [4]. The sericin of the silk fabric exhibits hydrophilic characteristics, as glycine and serine, which contain polarity groups such as -OH, -COOH, and NH_2, comprising 50% of the fabric. It has been demonstrated that a technique involving acidic and alkali solutions to dye the silk fabric produces softer silk fabric, *via* the removal of fibroin [5]. However, this method has been insufficient in terms of producing quality silk fabric, because the technique does not produce uniform quality silk fibers [6].

To date, although there have been many studies regarding the improvement of the quality and manufacturing of silk and wool fabrics [7-12], similar studies involving a protease treatment have been sparse at best. For example, in the case of enzyme treatments on fabrics, most studies have focused on a cellulose treatment for the improvement of the quality of cotton and hemp fabrics [7,8], and a mixture treatment with protease and lipase, as produced by *B. licheniformis*, for determining the effects on the detergency of fabrics, according to KS (Korean Standards) K0905 [9,11].

In this study, we first isolated a bacterial strain produc-

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ing a highly active protease, and then used this protease to improve the tactile sensations and dyeing properties of commercial silk and wool fabrics for development of an effective dyeing method of them without damage of them. Following this protease treatment, we analyzed variations in surface properties, dyeing properties, and tensile strength in the silk and wool fabrics, *via* microscopic examination, K/S value tests, and tensile strength tests, in order to determine whether the protease treatment improved the quality of the fabrics.

MATERIALS AND METHODS

Isolation of Bacterial Strain

In order to isolate a bacterial strain producing a highly active protease, soil samples were diluted with a 5% skim milk solution, and spread onto plates containing 5% skim milk. After the plates were incubated for 24 h, individual colonies were isolated and transferred to selection plates (glucose 1 g/L, soybean meal 0.5 g/L, soytone peptone 0.5 g/L, K_2HPO_4 0.02 g/L, $MgSO_4 \cdot 7H_2O$ 0.02 g/L, NaCl 0.002 g/L, and Na_2CO_3 0.003 g/L, at pH 7.0), then incubated for 24 h. Subsequently, colonies exhibiting wide clear zones were inoculated into selection culture media, and incubated at 37°C with shaking (130 rpm) for 72 h, in order to determine protease activity [13,14].

Determination of Protease Activity

Protease activity was determined by measuring the degree of casein degradation. In brief, the protease activity in a broth culture was determined spectrophotometrically by optical density (O.D., λ = 590 nm), and total protein content was determined by a Bradford assay, after the removal of cells *via* centrifugation [15]. One milliliter of a substrate solution (0.6% casein in 50 mM Na_2HPO_4-NaOH buffer, at pH 10) was mixed with 0.2 mL of the culture supernatant, which had been diluted to appropriate levels with a coenzyme solution (Na_2CO_3-Na_2HPO_4, at pH 10), and the samples were incubated at 65°C for 30 min. A 15% TCA (trichloroacetic acid) solution was added to the samples to stop the reaction, and the samples were again incubated at 37°C for 30 min. The protease in the samples was precipitated by adding 2.5 mL of a Na_2CO_3 solution (0.55 M) with 0.5 mL of a phenol reagent, diluted three times with H_2O, in order to determine one unit of protease. One protease unit was determined by O.D. at 660 nm, and was defined as the amount of protease necessary to produce 1 μg of tyrosine from casein [16].

Protease Treatment of Silk and Wool Fabrics

The supernatant of the broth culture, which was diluted with the coenzyme solution, was prepared as a treatment for the silk and wool fabrics, in order to determine the effects of the protease produced by the bacteria. A fresh liquid medium and a heat-treated broth culture were also prepared, for use as a control. Forty milliliters of the prepared protease-containing broth samples, along with the control, was applied to both the silk and wool fabrics, whose sizes were 15 × 5 cm, in 50-mL conical tubes. They were then incubated at 37°C with shaking (130 rpm). Single yarns of both the silk and wool fabrics were then observed by optical microscopy at 3, 6, 9, 24, and 48 h. We used a 100× magnification for the wool and a 200× magnification for the silk. The silk and wool fabrics used in this study were certified by KS K0905 [17].

SEM (Scanning Electron Microscope) and Tensile Strength Test

Single yarns of the silk and wool fabrics were also observed by an SEM (Akasi Alpha 25A) at 1500× magnification. A tensile strength test of the single yarns was performed according to the KS K0520 protocol and with a tensile strength tester (Instron, model no.4468) [17]. Protease-treated single yarns, along with inactivated protease treated single yarn controls, were tested five times by the Ravelled strip method at 30 cm/min of tensile speed, and the means were generated statistically.

Dyeing Method

C. I. Reactive B-171 dye was used for the silk, and Syrozol N/B SHF-BP Blue 222 dye was used for the wool, and both dyes were applied at 2% (owf) in a dyebath. In order to analyze the dye ability of the fabrics, they were dyed at 60°C for 30 min, washed, and dried [18].

Determination of K/S Value

The K/S value, which indicates the degree of dye uptake in the fabrics, was analyzed spectrophotometrically (Color-Eye 3100, Macbeth Inc), and also using the Kubelka-Munk equation, which is as follows [16]:

$$K/S = (1-R)^2/2R$$

K: absorption coefficient of dyed goods
S: scattering coefficient of dyed goods
R: spectral reflectance

RESULTS AND DISCUSSION

Isolation of Bacterial Strain

A soil bacterial strain exhibiting a highly active protease was isolated and characterized. It was determined to be a Gram-positive, rod-shape, motile, spore-forming bacterium [19]. The growth of the strain was found to be optimal at conditions of 37°C, pH 7, and with 7% NaCl. The bacterial strain degraded starch and casein, but it was not able to produce urease. Based on these findings, it was presumptively identified as *Bacillus* sp. SJ-121.

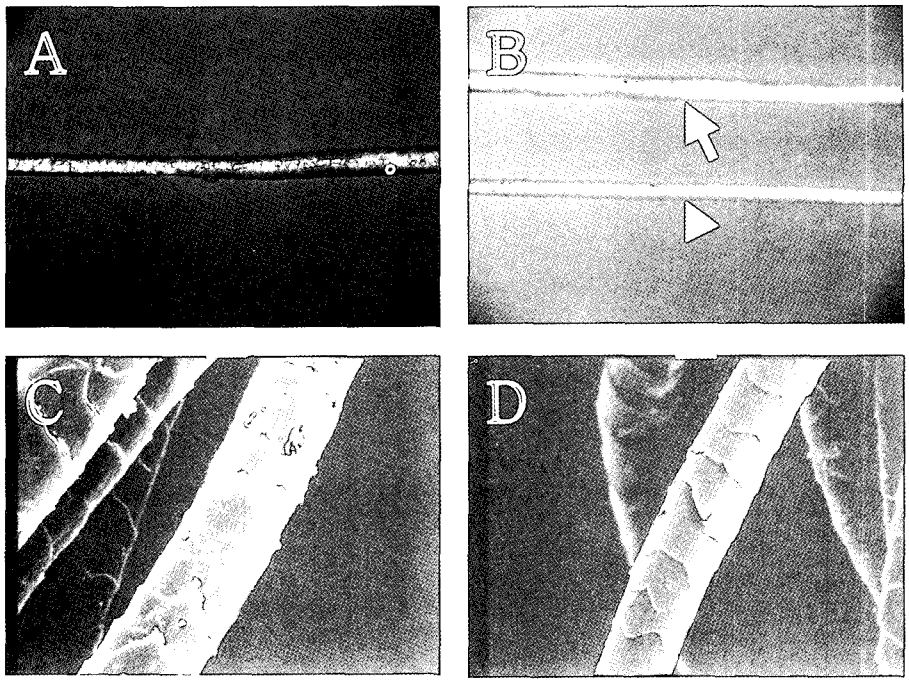

Fig. 1. Microscopic examination of single yarns of the wool fabrics. (A, B) The pictures were taken with an optical microscope. (C, D) The pictures were taken with an SEM. A, raw wool B, the protease treated sample after 48 h: arrow, the wool treated with the inactivate protease; arrowhead, the wool treated with the protease. C, The sample treated with the inactivated protease. D, The sample treated with the protease. Magnification was 100× for the microscope and 1,500× for the SEM.

Optimization of Protease Production

In previous reports, *Bacillus* sp. was found to strongly produce a protease in a medium containing glucose and soluble starch as carbon sources [20,21]. In order to assess the effects of carbon sources on protease production by *Bacillus* sp. SJ-121, 1% of glucose and soluble starch in the liquid medium were used as carbon sources, and the culture was incubated at 37°C for 72 h. *Bacillus* sp. SJ-121 produced the protease in a medium containing only glucose. Therefore, the glucose concentration was optimized in the range of 0.2 to 1.5% in the medium, and approximately 0.8% of glucose was found to be the most suitable concentration. In the optimization of pH, temperature, and time for the production of the protease by *Bacillus* sp. SJ-121, the protease was most efficiently produced at 35°C and pH 7, for 72 h, as has been described in previous reports [22].

Microscopic Examination of the Silk and Wool Fabrics

The silk and wool fabrics were treated with the protease produced by *Bacillus* sp. SJ-121, and were observed by an optical microscope and an SEM. When the protease was added to the wool for 48 h, each tough and thick single strand of yarn (Fig. 1A) was considerably altered, assuming a smooth, thin form, as is shown in Figs. 1B and 1D. The fabric was also treated with an inactivated protease as a control. The single yarn stands of silk exhibited no change and appeared to be identical to non-treated samples (Figs. 1B and 1C). In the case of the silk fabrics, an identical pattern was observed. The raw silk displayed in Fig. 2A was altered, assuming a smooth, thin form (Figs. 2B and 2D), and the protease-treated sample was much cleaner than both the non-treated sample and the control (Fig. 2C). These results can be explained; the protease degraded the tough and hard sericin of the raw silk, and it also removed the scale and possibly the soil from the wool.

Tensile Strength Analysis

In order to determine the effects of the protease treatment on the physical properties of the fabric, we determined its tensile strength. α-keratin, comprising the main protein of the wool, forms α-helices, which can be reversed in form to β-keratin by hot water, steam, or alkalis, due to the cleavage of α-helices via the weakening of hydrogen bonds among its amino acids. Also, when the tensile strength of β-keratin is obviated, it contracts and can reverse in form, assuming the shape of an α-helix [23]. In other words, treatment with hot water, steam, or alkali can increase the tensile strength of wool. As shown in Table 1, the results of the tensile strength analysis did not demonstrate considerable differences, after 48 h of the protease treatment, between the treated wool samples and the control samples treated with the heat-inactivated protease. Therefore, it appears that the protease produced by *Bacillus* sp. SJ-121 was able to remove the scales of the wool fabrics, but it did not change the actual structure of the wool.

It has been established that the fibroin comprising the

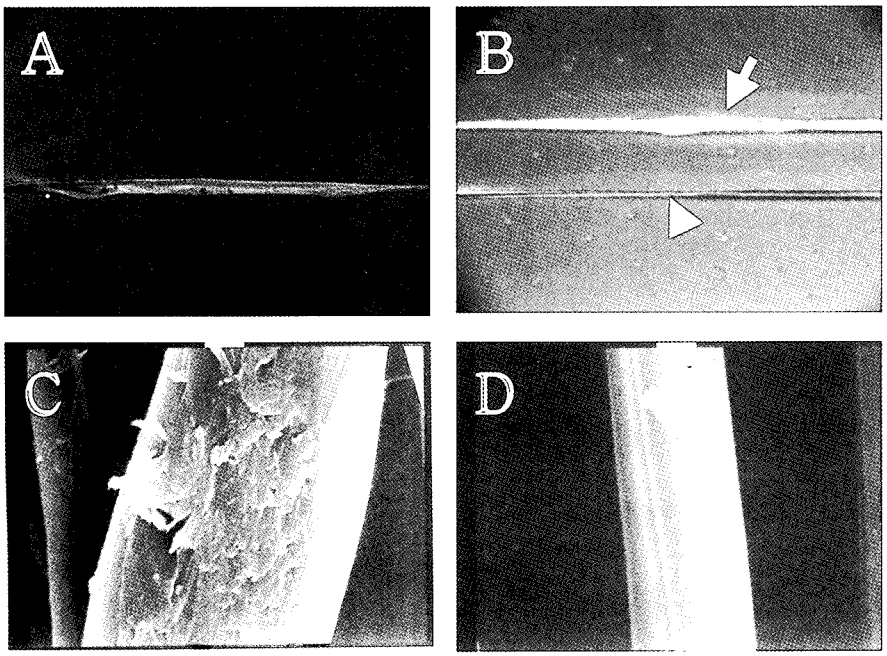

Fig. 2. Microscopic examination of single yarns of the silk fabrics. (A, B) The pictures were taken by an optical microscope. (C, D) The pictures were taken by an SEM. A, raw silk B, the protease-treated sample after 48 h: arrow, the silk treated with the inactivate protease; arrowhead, the silk treated with the protease. C, The sample treated with the inactivated protease. D, The sample treated with the protease. Magnification was 200× for the microscope, and 1,500× for the SEM.

Table 1. Modulus of wool treated with the protease and inactivate protease as a control Kgf/cm²

Reaction time (h)	Protease	Inactivated protease
3	2.94331	2.9133
6	3.0681	2.7649
9	2.9154	2.6288
24	2.7069	2.6349
48	3.01681	2.6726

Table 2. Modulus of silk treated with the protease and inactivate protease as a control Kgf/cm²

Reaction time (h)	Protease	Inactivated protease
3	2.1326	1.8359
6	1.9474	2.1392
9	2.1209	1.9179
24	2.2903	2.2263
48	1.9563	1.9413

majority of silk yarn is composed of polypeptide chains, which themselves are composed of 17 different amino acids [24]. The majority of these amino acids, however, are glycine and alanine, which possess shorter side chains than others and, therefore, hydrogen bonds can be more easily formed between their polypeptide chains. However, >C=O and –NH- in the polypeptide chains of silk fabrics contribute to the structural stability of silk against various stresses [24]. As shown in Table 2, our results indicated similar data patterns of the wool. Consequently, it was demonstrated that the protease removed scales in both the silk and wool fabrics, but it did not alter their tensile characteristics.

Change of K/S Value According to Protease Treatment Time in Silk and Wool

In order to characterize any changes of the K/S value, which indicates the amount of dye uptake in fabrics, the fabrics were treated with the protease at intervals of 3, 6, 9, 24, and 48 h, and the fabrics were dyed, washed, and dried. The K/S values were then determined. As shown in Fig. 3, when the wool fabrics were treated with the protease, the K/S values gradually increased. This indicates that the increases in the dye uptake profiles of the protease-treated wool fabrics were due to the protease-induced degradation of the scales, especially the serine residues, in the wool. These results, in which the protease contributed to the degradation of the wool scale, are consistent with data described in previous reports [23,25]. In a previous report, it was shown that a molecule of dye can diffuse into the CMS (cell membrane complex) of wool [26]. However, in the current study, the dye uptake values exhibited by the wool over 48 h were enhanced to 200% of the values obtained before testing. Therefore, it seemed that the protease did not degrade the serine resi-

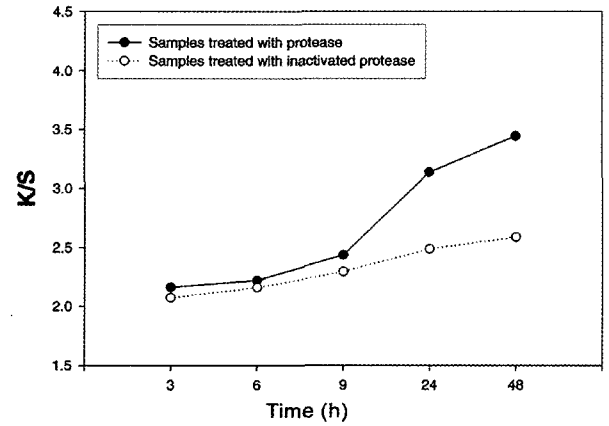

Fig. 3. Change in K/S values according to the protease treatment time in wool.

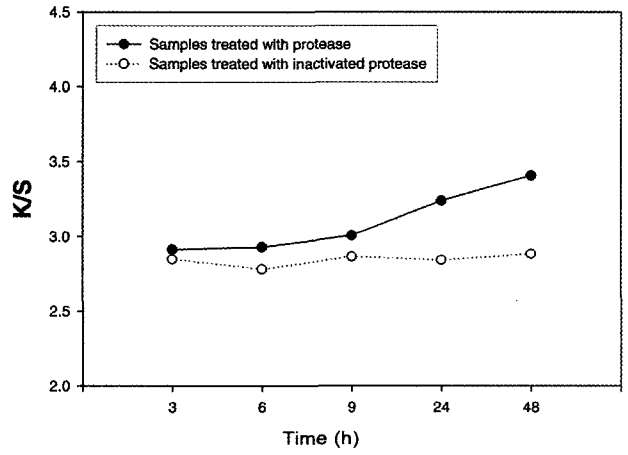

Fig. 4. Change in K/S values according to the protease treatment time in silk.

dues in the wool fabrics enough to increase its tensile strength, although the protease did enhance the wool's dye uptake characteristics.

As shown in Fig. 4, the dye uptake characteristics of the silk fabrics were consistently higher than those of the wool, and the dye uptake of the silk fabrics was enhanced during the 48 h, but the control fabrics exhibited no change in dye uptake. In a previous study, it was reported that an amino residue contributes to the enhancement of the dye uptake in silk [27]. Therefore, it appears that this enhancement was, in part, due to the amine residues in the silk. Although the surface of the silk yarn became smoother upon the protease treatment when compared with the wool fabrics as shown in Fig. 2, the ratio of enhancement in silk dye uptake was somewhat lower than expected. This was because the amine residues of the silk fabrics were excessively degraded by the protease, thereby reducing the interaction between the dye molecules and the amine residues in the silk.

According to the above data, we suggest that the protease produced by *Bacillus* sp. SJ-121 enhances the dyeing properties of wool and silk fabrics, but does not positively affect their tensile strength. Therefore, a microorganism-produced protease might have commercial applications in the fabric industry for improvement of the dyeing method.

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