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Research Article

EVALUATION OF *BACILLUS SONORENSIS* ASKL-09 PROTEASE FOR BIOMEDICAL AND INDUSTRIAL APPLICATIONS

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Abstract- In the present study, protein digestion, blood stain removal and de-hairing ability of protease produced by *Bacillus sonorensis* ASKL-09 was evaluated. The protease produced by *Bacillus sonorensis* ASKL-09 showed excellent fibrinolytic action and hydrolyse egg albumin and blood clots. The protease produced by *Bacillus sonorensis* ASKL-09 removed bloodstains on the cloth within short time (25 min). The protease produced by *Bacillus sonorensis* ASKL-09 was better for de-hairing process which finds an excellent application in leather industry and could reduce environmental hazards that usually generated by conventional method of de-hairing at leather industries.

Keywords- *Bacillus sonorensis ASKL-09, Protease, Protein digestion, Blood stain removal, De-hairing*

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Introduction

Protease belongs to a group of proteolytic enzymes that hydrolyse peptide bonds of proteins [1]. It is widely distributed in nature and captures almost 60% of the total enzyme market [2]. Proteases were the first enzymes to be commercialized [1] and historically used in laundry detergents [3]. Other than the use in laundry detergents, they have become increasingly important in de-hairing animal skin and hides in the leather industry. The conventional de-hairing process is performed with a saturated solution of lime and sodium sulfide, as a consequence, the conventional process has been estimated to contribute 60–70% of the total pollution load associated with leather industrial processing. Furthermore, the extensive use of sulfide is harmful to the health of the workers [4]. Protease especially alkaline proteases are active from neutral to alkaline pH, which makes them highly desired in industries such as detergent manufacturing, food and feed production, peptide synthesis, leather processing, photography, silk degumming, and waste management [3-4]. Owing to the application of protease in different filed, the present study was carried out to evaluate the biomedical and industrial application of protease produced by *Bacillus sonorensis* ASKL-09.

Materials and Methods

Bacillus sonorensis ASKL-09 protease

Bacillus sonorensis ASKL-09 was isolated from garden soil (Gulbarga University) Karnataka, India and identified based on microscopic, biochemical and 16S rRNA gene sequencing [5]. The *Bacillus sonorensis* ASKL-09 produced protease of molecular mass 44 kDa and active at pH 10 and temperature 50°C [6].

Evaluation of protein digestion, blood stain removal and dehairing property

In this investigation, egg albumin and clotted blood were used to analyse protein digestion. The protein digestion was carried out as per the method of Najafi *et al*., [7] and incubated for 12h at 50ºC in pH system of 10. To analyse bloodstain removal efficacy, the cotton clothes of uniform size were stained with blood and allowed them for drying. The clothes were washed with enzyme solution in combination with or without detergent following our earlier protocol [8].

The washing performance was visualized at 10, 15, 20 and 25 min. Goatskin was used to analyse dehairing property and performed as per the method described by Vishalakshi *et al*., [8] Uniform sized two pieces of goatskin were taken and one piece was used as control and it was subjected for dehairing with conventional method wherein the skin piece was treated with 10% lime and 2% sulfide. Another piece of skin was incubated with purified alkaline protease (1%) at 50ºC. Later, the skin hair was removed with the help of a blunt knife. For the preparation of 1% alkaline protease solution was prepared in 0.2 mM phosphate buffer of pH 10 [8].

Result and Discussion

The protease produced by *Bacillus sonorensis* ASKL-09 was investigated to analyse protein digestion. The protease showed positive results for the hydrolysis of egg albumin and blood clot indicates the fibrinolytic action [Fig-1]. The mechanism involves the breakdown of cross-links between fibrin molecules with disruption of the structural integrity of blood clots due to proteolytic action of the enzyme [7]. The ability of alkaline protease in converting insoluble forms of blood clot to soluble form designates its clinical and medicinal applications in the thrombolytic drug. A similar type of results was reported for the alkaline protease from Pseudomonas for hydrolysis of blood clots [7].

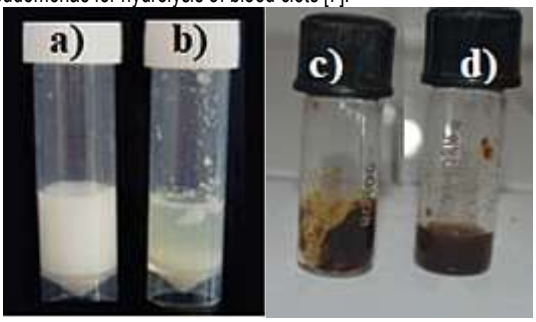

Fig-1 Evaluation of protein digestion: a) egg albumin without protease, b) egg albumin with protease, c) blood clot without protease d) blood clot with protease

In recent years, enzyme detergents (often referred to as green chemicals) were used which reduce environmental pollution and considered as eco-friendly [10]. Washing performance of protease produced by *Bacillus sonorensis* ASKL-09 was evaluated. The results revealed that the bloodstains on the cloth remained as it is even after 25 min of rinsing in the case of control whereas bloodstains were completely removed from the cloths after rinsing them with a combination of detergent and partially purified enzyme for a period of 25 min [Fig-2]. These results clearly indicate that the enzyme was stable in the presence of detergent. The result indicates that in future the protease from present work may be used as an excellent bio-cleaner. Similarly, Vishalakshi *et al.* [8] reported that the bloodstains were completely removed by protease with distilled water from cloth after 25 min. In a similar kind of investigation was reported where alkaline protease produced by soil bacterium was able to remove blood stains within one hour from cotton fabric [9].

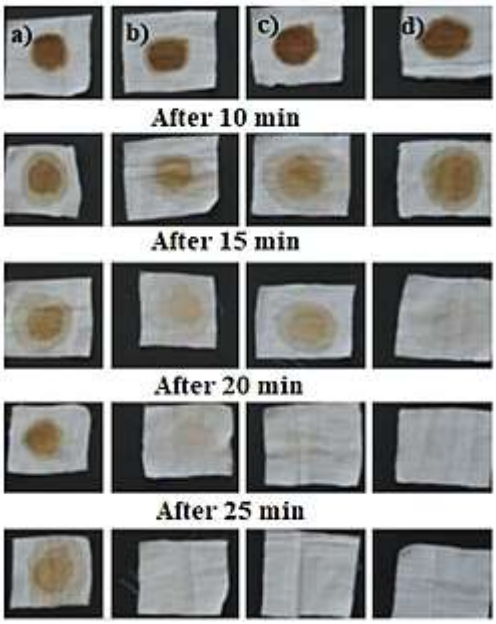

Fig-2 Removal of blood stains: a) water b) enzyme c) detergent d) enzyme and detergent

One of the important steps in leather industries includes de-hairing, which is performed by hazardous chemicals [11]. The use of proteases as alternatives to chemicals has proved successful in improving leather quality and in reducing environmental pollution [2]. In the present study, de-hairing efficiency of protease produced by *Bacillus sonorensis* ASKL-09 was evaluated visually with respect to the quality of the hair recovered. After 18 h of treatment with *Bacillus sonorensis* protease, intact hair was recovered by simple scraping (Figure 3).

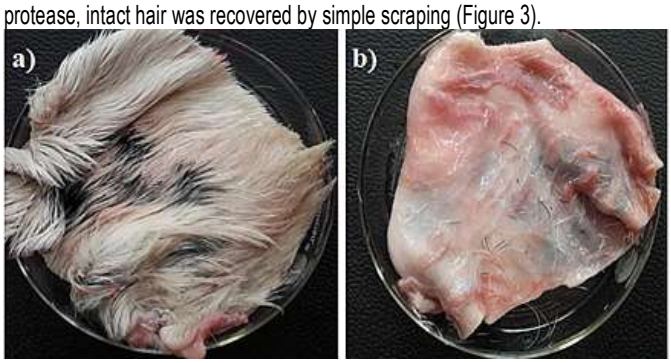

Fig-3 Dehairing by protease produced by *Bacillus sonorensis* ASKL-09, a) control, b) treated with protease.

Application of research: The protease produced by *Bacillus sonorensis* ASKL-09 finds an excellent application in the leather industry which could reduce environmental hazards that usually generated by conventional methods of de-hairing at leather industries.

Research Category: Biomedical and Industrial Application

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Study area / Sample Collection: Gulbarga University, Kalaburgi, 585106

Strain name: *Bacillus sonorensis* ASKL-09

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Department of Microbiology, Gulbarga University, Kalaburgi, 585106, Karnataka, India.
Ethical Committee Approval Number: Nil

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