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APPLICATION OF PROTEASE FROM BACILLUS CEREUS MCM B-326 AS A BATING AGENT IN LEATHER PROCESSING

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ABSTRACT

Laboratory scale experiments were carried out to test the efficiency of the extracellular protease from Bacillus cereus MCM B-326; cattle dung and commercial bate powder (ComBate) as bating agents on delimed buffalo hide. Protease treated pelt was free from scud and pigments, clean and fine grain, white, smooth and silkier with loosen fat. Histological sections of bated pelts showed greater opening up of collagen fibers with Bacillus protease. The studies indicated potential importance of Bacillus protease as effective bating agent in leather processing.

Keywords: *Bacillus cereus; protease; bating agent; buffalo hide; leather processing*

[I] INTRODUCTION

Enzymes have considerable potential in the bioprocessing of skins and hides for leather production, offering effective biotreatments particularly for the dehairing and bating processes [1]. The six processing steps viz. curing, soaking, dehairing, bating, degreasing and tanning are clearly identified steps in the preparation of leather [2]. In soaking, dehairing, bating and degreasing steps enzymes play an important role. It is common knowledge that, of the various unit operations involved in making leather, it is only with bating that the enzymatic process can't be substituted by chemical method. Bating is the treatment of dehaired hides or skins with an aqueous solution of an enzyme to remove certain undesirable proteinaceous constituents [3]. The main function of bating was the removal of and hydrolysis of the keratose substance remaining on the surface of the delimed pelt, i.e. removal of scud. The scud consists of epidermis, hair roots, pigments, fats, sebaceous glands and sweat gland debris [4]. Bating following the dehairing process produces softer, stretcher, silkier leather. Bating process acts on elastin and increase suppleness, pliability, elasticity and feel of the leather [5]. Depending on its proposed use like cloths, car seats etc. different enzyme treatments may be necessary. Puvankrishnan & Dhar reported several *Bacillus* sp. as a source of bating enzyme [6]. Three types of enzymes used as bating agents are (i) pancreatic protease i.e. trypsin from bovine and swine pancreas glands, (ii) protease from moulds, fungal origin which works in the pH range of 3.5- 5.0 referred to as an acid bate (iii) bacterial protease which work in the neutral pH range 6.2- 7.0 [7].

The present work deals with the application of the extracellular protease from *Bacillus cereus* MCM B-326. The efficiency was compared with that of the cattle dung and ComBate.

[II] MATERIALS AND METHODS

2.1. Organism and chemicals

A strain of *Bacillus cereus* MCM B-326 used was a buffalo hide isolate which had the ability to secrete protease. All chemical and media were purchase from Hi- Media Laboratories, Mumbai.

2.2. Enzyme preparation

The strain was maintained as a glycerol stock at -20^{0}C. 2.8 x 10^8 cells of the organism *Bacillus cereus* MCM B-326 were inoculated into a medium containing 1% starch, 1% soybean meal, 0.3% $CaCO_3$ (pH 9.0). The flasks were kept under shaking conditions for 36 h at 30 ^{0}C. After 36 h the culture filtrate obtained was centrifuged at 13,000 g for 10 minutes to remove the biomass [8]. The supernatant was partially purified by ammonium sulphate precipitation (60% saturation) and the enzyme thus obtained was used for the evaluation of its activity on casein, azocoll and hide powder azure substrates and bating efficacy on chemically dehaired buffalo hide.

2.3. Enzyme assays

2.3.1. Caseinolytic assay

Protease activity was measured using caseinolytic assay [9]. The culture supernatant (1 mL) was incubated in 4 mL of 0.625% casein at 37 ^{0}C for 30 min. The reaction was stopped by 5 mL of trichloroacetic acid (5%) and

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the casein hydrolysis product was measured by modified Folin Ciocalteu method [10], against inactive enzyme. A standard graph was generated using standard tyrosine of 10- 50 μgmL^{-1}. One unit of protease activity was defined as the amount of enzyme, which liberated 1μg tyrosine per min at 37 ^{0}C.

2.3.2. Azocoll assay

Azocoll assay was determined using method described by Ensign & Wolfe [11]. Ten mg of azocoll was added to 2 mL of Tris-HCl buffer (pH 8.0); to it 0.5 mL of suitably diluted enzyme was added and incubated for 10 min at 37^{0}C. The reaction mixture was centrifuged and the absorbance of the supernatant was measured at 580nm relative to a blank without enzyme. One unit of activity was defined, as the amount of enzyme required to increase the absorbance by 0.001absorbance unit under assay conditions.

2.3.3. Hide powder azure (HPA) assay

Hide powder azure assay was determined using method described by Puvankrishnan & Dhar [7]. 25 mg of HPA substrate was incubated with different concentrations of enzyme (0.01, 0.02, 0.03 and 0.04 mL) at 37^{0}C for 20 min. The mixture was centrifuged and the supernatant was read at 595nm. The graph was plotted for different enzyme concentrations and absorbance at 595nm. The slope was directly proportional to the enzyme activity.

2.4. Bating application

The bating action of the enzyme was evaluated on the laboratory scale. Three buffalo hide pieces (3 cm x 3 cm) were soaked in 300% (w/v) tap water; dehairing was done by traditional chemical- 10% lime and 2% sodium sulphide. The limed, experimental pieces were washed thoroughly and delimed with 150% water and 1% ammonium chloride for 40 min and then added bating agent- animal dung (1%), commercial bate (1%) and *B. cereus* protease (0.15%) separately. All the other operations were performed together [7].

2.5. Bating efficiency tests

For thumb impression test, the time taken by the bated pelt to regain its original shape after a thumb was pressed on the grain side, and the intensity of the impression was noted [5]. The scud (hair root, degraded protein. etc.) removal by scrapping with fingernails was assessed. The degree of slipperiness, cleanliness of the bated pelt pieces was categorized.

2.6. Histopathology of hides

Histopathology of cattle dung, enzymatically and commercial bate powder bated pelt were studied using a method described by John & Merriline [12]. The hide pieces were dehydrated with 80, 95 and 100% (v/v) of alcohol gradients followed by xylene and then embedded in paraffin. The longitudinal sections of hide embedded in paraffin wax were obtained using microtome. The sections were fixed on slides using starch paste containing thymol, which acts as preservative. The sections were stained using Harris's haematoxylin stain followed by 0.5% (v/v) HCl and diluted ammonia. The slides were observed microscopically for opening up of collagen fibers.

[III] RESULTS AND DISCUSSION

The protease from *B. cereus* MCM B-326 was assessed for its potential use in bating step of leather processing. The specificity of protease from *B. cereus*, ComBate and cattle dung with casein, azocoll and hide powder azure substrates is summarized in **Table–1**.

Table 1: Enzyme activities of different bating agents with different substrates

Bating agents	Substrates and enzyme activity (Ug^{-1}min^{-1})		
	Casein	Azocoll	HPA
Protease from *B. cereus*	947.69	1935	310
ComBate	675.95	1180	243.3
Cattle dung	692.93	1725	153.3

HPA- Hide powder azure

From **Table–1** it was observed that the ComBate and cattle dung have enzyme activities with casein, azocoll and hide powder azure, as substrates but it was less as compared with protease from *B. cereus*. Likewise hide powder azure and azocoll were evaluated for their susceptibility as collagen-rich substitutes for casein in assaying for proteolytic activity of bate [13]. Various proteolytic activities of bate were measured in drum liquor by Mozeersky *et al.* [13-17]. Casein was a general protease substrate while azocoll and HPA were recommended substrates for elastase type of enzymes.

A qualitative assessment of the effect of the protease as a bating enzyme gave a highly slippery, silkier and clean pelts with a very fine grain texture. Scud loosening was very easy and the intensity of the thumb impression was very deep. Taking 22 seconds for treated with animal dung, 18 seconds for ComBate and 26 second for *B. cereus* protease to reshape after imprint. These tests give an indication of the extent of removal of the interfibrillary material that leads to better opening up of the fiber structure. Hameed *et al.* [5, 18] reported qualitative assessment of the effect of the crude protease from *B. subtilis* as a bating enzyme gave a slippery feel to the leather with a very fine texture of the grain. Scud loosening was very easy and the intensity of thumb impression was very deep taking 25 sec to reshape after imprint. Mozersky *et al.* [17] reported bating with a halophile protease active in 4M NaCl and found to yield leather with satisfactory physical characteristics. This high salt concentration will be used to loosen the tight bonding of decorin to collagen, thus rendering the proteoglycan more susceptible to proteolysis and removal from the hide.

The collagen fiber opening was confirmed by histological technique followed by microscopy. The microscopy of bated pelts with the bating agents is shown in **Figure–1**. This reveals a greater opening up of the fiber structure in the case of the pieces bated with *B. cereus* protease than with the cattle dung and ComBate. Collagen fibers were not damaged because of protease action and are shown in **Figure–2**.

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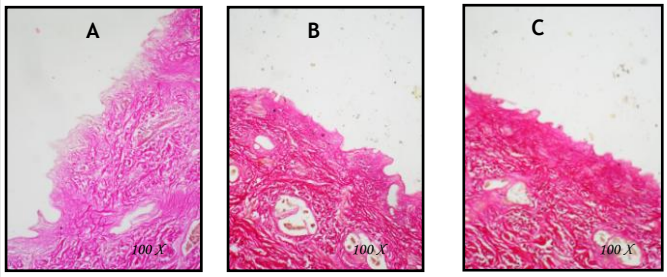

Fig: 1. Micrographs of pelt bated with A: animal dung, B: ComBate and C: protease from *Bacillus cereus* MCM B-326 (A and B showed disintegrated hair follicles, C showed empty follicles)

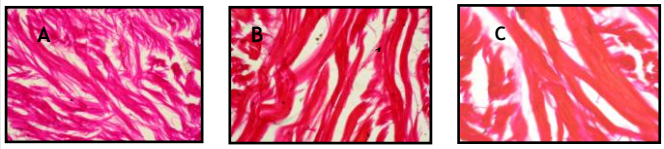

Fig: 2. Micrographs of pelts collagen bated with A: animal dung, B: ComBate and C: protease from *Bacillus cereus* MCM B-326 (A showed less opened collagen fibers, B showed moderate opened collagen fibers, C showed well-opened collagen fibers)

Scanning electronic microscopy of goat pelts bated with protease from *Streptomyces* sp. G_{157} reveled a greater opening up of the fiber structure than the commercial enzyme bate [19]. Ding *et al.* [4] reported histological study of goatskin with pancreatin bates performing a function of removing epidermal matter, eliminating scud and cleaning grain surface. Zuo et al. [20] used surfactants for enhancing enzymatic bating of goat skin. Sirvaityte et al. [21] reported commercial enzymes LITHUDAC L and Novo Bate WB for bating of pelts after deliming with peracetic acid. The results presented indicated that *Bacillus cereus* protease can work as a very good bating agent in leather processing.

[IV] CONCLUSIONS

The extracellular protease from *Bacillus cereus* MCM B-326 was found to be an effective bate as compared to cattle dung and ComBate.

FINANCIAL DISCLOSURE

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