Stain removal and Dehairing using protease enzyme from *Bacillus cereus*

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ABSTRACT

In the present study, an attempt was made to examine the potential of Bacillus cereus for stain removal in fabric clothes and dehairing on animal skin. Microorganisms from marine soil were subjected to acclimatization in the production media obtained from nutrient broth. The most promising isolated bacteria was used for the experiment. After an incubation period of 48 hours, the optimum pH and temperature for destaining and dehairing by Bacillus cereus was 5.0 and 40°C respectively. This potential increased the applicability of this microorganisms for stain removal as well as hair removal. The result promises the application of Bacillus cereus in detergents and leather industry.

Keywords-;Dehairing, Stain, Bacillus cereus.

I. INTRODUCTION

Microorganisms are living microscopic organisms which are ubiquitous in nature. They are discovered by Antoni Van Leeuwenhoek.They exist in unicellular or colonial form. Microbes are massive range of organisms including bacteria, fungi, viruses, algae, archaea, and protozoa. Microorganisms can obtain energy from carbohydrates, alcohols, and amino acids. Microorganisms will only occur in a medium if appropriate nutrients are provided. The study of microorganisms is known as microbiology. Microorganism can produce certain enzymes which are known as microbial enzymes which are complex chemicals produced by bacteria (Tyrell,Schopf*et al*.,2017). Microorganisms are capable of producing enzymes. They are proteins which perform catalytic operations. Enzymes are highly specific as they are able to distinguish between slightly different molecules. They can operate at optimum temperature, pressure, and pH which makes them attractive catalysts for diverse processes. The first reports on industrial use of enzyme products go back to the beginning of the last century. It was the German scientist Rohm who introduced the use of bovine pancreas extracts for the removal of stains in dirty clothing. Hypersensitivity is combatted by developing dust free granulates in which the enzyme is incorporated into an inner core containing inorganic salts and sugars as preservative bound with fibers of colloid. This core is coated with waxy material which gets later dispersed in the wash; this combination of materials prevents dust formation and protects the enzyme against damage. Enzymes are used only in small amounts in most detergent preparations, only 0.4-0.8% crude enzyme by weight and follows the ability to withstand the conditions of use than extreme cheapness. Once released from its granulated form the enzyme must withstand anionic and non-ionic detergents, soaps, oxidants etc. Lipases and proteases process any remnants of protein, starches and fats in the clothing which make them great for getting rid of stains. Whereas non biological detergents are tough on stains but gentle on skin. Biodetergents may be used to protect environment and

save energy because it is biodegradable and can work efficiently at low temperature (JT Dulaney*et al*., 1970). In the present study, stain removal in fabric cloth using the protease enzyme from Bacillus cereus has been studied. It was screened from new habitats for protease production with novel properties to meet the needs of rapidly growing detergent industry. The enzyme has been extracted in media like nutrient broth. After an incubation period of 72 hours, the optimum temperature and pH was 40° C and 5.0 respectively. The enzyme is also utilized for dehairing in goat skin with respect to the enzyme suspension of 10, 20 and 30 ml respectively after an overnight incubation.

2. 5.MATERIALS AND METHODS

5.1 SAMPLING SITE:-

Marine soil from Kollam Beach (Kerala) was collected from the shore in sterile polythene bags, which contained some amount of seawater to maintain the moisture content.

5.2 SCREENING OF THE MICROBES FOR PROTEASE ACTIVITY:-

The soil sample was serially diluted to 10^{-3} and the dilutions were spread plated on Tributyrin Agar medium. The plates were then incubated for 48hours at 37°C. The colony that showed the zone was taken and pure culture was made on Nutrient agar plates by quadrant streaking, and incubated overnight. The bacterial characterization was done using Bergey's manual.

5.3 CHARACTERIZATION OF PROTEASE PRODUCING BACTERIAL CULTURE

The characterization of lipase producing bacterial culture was done by biochemical tests. Biochemical tests were used for the identification of isolates bacteria culture for lipase enzyme production. Different Biochemical tests were performed to prove the identity of isolated bacterial culture. Catalase test, glucose fermentation test, mannitol fermentation test and Novobiocin sensitivity test were used for identification.

5.4PREPARATION OF PRODUCTION MEDIA

Optimum pH of 5.0 is adjusted for the preparation of production media which may enhance the amount of enzymes obtained. Add 20 ml nutrient broth to the cooled production media and placed inshaking incubator for 48 hours at 40°C. Finally, the production media is centrifuged at 5000 rpm for 15 minutesand stored in refrigerator for further use.

5.5 Staining

Five fabric clothes (6×6) are taken per stain and soaked well. The stained clothes were taken in separate five beakers. Control is set up by putting the stained cloth in 100 ml distilled water and it is named as A. The experiment is carried out in the remaining beakers, each filled with 100 ml distilled water. Beaker B is set up by adding the stained cloth along with 0.14 tide detergent without enzyme. Beaker C is placed with stained cloth, tide detergent with enzyme. Beaker D is placed with stained cloth, tide detergent with enzyme and 2 ml crude enzyme. Beaker E is placed with stained cloth, tide detergent without enzyme and 2 ml crude enzyme. Beaker F is placed with stained cloth and crude enzyme only. The following beakers are placed in the hot air oven overnight.

5.6 DEHAIRING ON GOAT SKIN

The dehairing efficacy of the same enzyme is assessed by applying it on goat skins. Four wet salted goat skins (4×4) were taken. Liming was done on the goat skins. The control was set up by soaking the goat skin on the beaker which has a combination of 20% water , 7% lime and 2.5% sodium sulfide. The experiment was done on three beakers namely A, B,and C. These beakers contained 20% water and 7 % lime and enzyme paste having the enzyme

suspension of 10 ml, 20 ml and 30 ml respectively. The beakers were incubated overnight in a shaking incubator at 40°C.

6. RESULTS AND DISCUSSION

6.1 SCREENING

The bacterial colonies from serial dilution were then screened by quadrant streaking and pure cultures were obtained.

The lipolytic bacteria was isolated from bakery waste and identified as Staphylococcus epidermis according to Bergey'smanual. The bacterial strain was purified by sub-culturing on agar slant. Characterization of bacterial strain was done by various biochemical tests. The production of lipase activity by staphylococcus sps was screened on tributyrin agar medium which was incubated for 24 hours.

Gram staining was done for identifying the morphology and nature of the bacteria. It was identified as gram positive cocci as it retained the colour of crystal violet.

6.2 BIOCHEMICAL AND MORPHOLOGICAL CHARACTERISTICS

Catalase test, glucose fermentation test, mannitol fermentation test and novobiocin sensitivity test were performed for characterization of the bacterial isolates. The results are listed in the table below.

6.3 STAIN REMOVAL

Visual examination of various pieces of fabric cloth after incubation exhibits the effect of enzyme in removal of stains. The beakers named C, E and F showed a better result that the stains was completely removed and the fabric clothes retains their white colour. Earlier studies revealed that microbial biosurfactants are considered safer alternative to chemical or synthetic surfactants owing to lower toxicity, ease of biodegradability and low ecological impact. Strains of bacillus subtilis can be employed in laundry detergents. (AK Mukherjee, 2007).

6.4 DEHAIRING ON GOAT SKIN

The experiment includes the set up of four beakers. Control has a combination of lime and sodium sulfide and the rest carrying out the experiment with the enzymatic paste having 10 ml enzyme suspension, where 100% removal was found. Enzyme yield was maximized by optimizing the culture conditions of a low-cost culture medium under submerged condition with a lab scale fermentor of 5 L capacity (Tanmay Paul *et al*.,2014).

7. TABLES AND FIGURES

7.1 Table

Table 1: Characterisation of isolated microbes.

SL NO.	PARAMETRES	RESULT
1	Gram nature	Positive
2	Morphology	Cocci
3	Motility	Non- motile
4	Catalase test	Positive
5	Glucose fermentation test	Negative
6	Mannitol fermentation test	Negative
7	Novobiocin sensitivity test	Positive

7.2 FIGURES

Fig-1. Control for dehairing experiment(4×4).

Fig-2 Goat skin in 10 ml enzyme suspension after an overnight incubation.

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