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Potential Application of Protease Isolated From Bacillus Sp JS1

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ABSTRACT:
A continuous research is attempted to fulfil the highest industrial demands of natural protease presenting special properties A protease isolated from Bacillus SP JN1could be useful for degradation ofnatural proteins and blood stains. With respect to properties of the enzyme and its capability for degradation of different protein sources, this protease finds potential application for waste treatment, used in detergents and leather industry. Further to exploit its application, contact lenses were incubated with the crude enzyme solution, and the protein debris on it was found to be scrubbed at an optimum time of 60 minutes. Therefore, it increases the transmittance of the contact lens indicating its use as a potential contact lens cleansing agent.The application of the enzyme in the removal of blood stains and digestion of the egg white was checked which clearly showed the potential property of the enzymes. This enzyme can be used as detergent additive as it removed the blood stain effectively and digested the egg white in 24 hours at room temperature.
Keywords: Protease, Blood, Contact, lens, Enzyme

I. INTRODUCTION:

Proteases execute a large variety of functions and have important biotechnological applications and find application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes (Anwar and Saleemuddin, 1998; Gupta et al. 2002). Proteases are widely used in laundry detergents, where they help removing protein based stains from clothing (Banerjee et al. 1999) . For an enzyme to be used as a detergent additive it should be stable and active in the presence of typical detergent ingredients, such as surfactants, builders, bleaching agents, bleach activators, fillers, fabric softeners and various other formulation aids. In textile industry, proteases may also be used to remove the stiff and dull gum layer of sericine from the raw silk fibre to achieve improved luster and softness.Proteases are also useful and important components in biopharmaceutical products such as contact-lens enzyme cleaners and enzymic debriders1 (Anwar and Saleemuddin, 2000). The proteolytic enzymes also offer a gentle and selective debridement, supporting the natural healing process in the successful local management of skin ulcerations by the efficient removal of the necrotic material. Although proteases are widespread in nature, microbes serve as a preferred source of these enzymes because of their rapid growth, the limited space required for their cultivation and the ease with which they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their various applications.Nowadays the enzymes are used in the commercial market in large quantity. Especially the proteases are taking up the 60% of the market value among the commercially used enzyme by industries. Proteases are very inexpensive enzymes that are used for hydrolyzing proteins. Specifically microbial proteases are the most essentially used in different sectors, such as textile, detergent, leather, feed, waste, and others. These proteases are exclusively used in laundry as detergent additives as these enzymes can survive the extreme pH and temperatures (Gupta et al.,2002). In this paper we present potential application of the protease for different industrial proposes.

II. MATERIALS AND METHODS:

Bacterial Strain

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The protease was isolated from Bacillus SP JS1(ACC NO: MZ345631) and was used for this work. This strain was cultured with production medium, after incubation period of 48 h, the cells were harvested at 10,000 rpm at 4 ℃ and the supernatant was used as further application studies.

Removing blood stain

A clean piece of cloth was soaked in blood and allowed to dry the blood cloth. Then the cloth was soaked in 2% formaldehyde for 30 min and washed with water to remove excess formaldehyde. The cloth was cut to equal sizes and they were incubated with the enzyme at 45- 50ºC for different time incubation. After incubation time, each piece was rinsed with water for 2 min and then dried. The same procedure was done for the control except incubation with the enzyme solution.

Blood Stain Removal:

As the main industrial application of the protease enzymes is the ability of them to remove or hydrolyze the biological stain, these enzymes are used as detergent additive to remove tough biological stains. So these enzymes are tested against different concentration of blood stains. The effect of the stain removal was noted down. The blood stain was treated with the crude enzyme and also with detergent mixed with the crude enzyme. After the treatment of the stained cloth in the enzyme, they are rinsed off with the tape water and the treated stained cloth is compared with a standard cloth which is not treated with any detergent or enzyme.

Preparing the cloth:

For this application, a white cotton cloth is taken. Five pieces is taken and labelled as A, B, C, D and E. All these clothes were stained with different concentrations of blood- 12.5%, 25%, 50% and 100%. Each cloth was tested with the following: Cloth A: treated with water only, as Negative Control.Cloth B: treated with only the enzyme without any detergentCloth C: treated with only the detergent without any enzyme.Cloth D: treated with both the detergent and the enzymes

Making the Serial Dilutions:

Four 125 mL cups were obtained and labelled as 100%, 50%, 25% and 12.5%. 4 mL of blood was pipetted out in the cup labelled as 100%. Out of this 100% blood, 2mL was pipette out in 50% cup and 2 mL of deionized water was added. 2mL of blood from 50% dilution was taken and added to 25% cup with 2 mL of deionized water. 2mL of the 25% solution was pipetted out and added in 12.5% cup and 2 mL of deionized water is added to this. 2mL of the 12.5 % dilution was then discarded.

Egg white Digestion:

Protease enzyme has the basic activity of hydrolyzing the peptide bonds of amino acids in protein. Therefore their application or ability of hydrolyzing the egg white albumin is checked. Here the egg white is carefully separated from the raw egg. To the egg white, a considerable amount of the enzyme was added and incubated for 24 hours at room temperature. After the incubation time the egg white was observed. Because of the enzyme on the albumin, the egg white gets coagulated in the bottom of the test tube. For the positive control, egg white without adding any enzyme is used.

Application of protease in contact lens cleansing

To study the efficiency of the enzyme in removing proteinaceous wastes deposited over the lenses, the enzyme was used as a cleansing agent. The lenses were coated with artificial tear solution before enzymatic treatment, and the % transmittance was recorded (Table 1). Before treatment, the uncoated, and coated lenses showed 97%, and 68% transmittance respectively. After the enzyme treatment, the transmittance of the coated lenses started increasing from 68% until the final transmittance (95%). The final transmittance was recorded after 60 minutes of incubation. However, the coated lens treated with phosphate buffer recorded 70% transmittance. Thus, the enzyme can be further used as a potent contact lens cleansing agent.

III. RESULTS AND DISCUSSION

Many protease that are in the market currently are types of proteases that are isolated from the microorganism sources. It can also be noted that the most commercially important proteases are isolated from the Bacillus sp as these proteases are isolated from bacillus sp

Blood stain removal

The sample was tested on the blood stain. The blood was stained in the cloth with different concentration such as 12.5%, 25%, 50% and 100% which was achieved by serially diluting the blood. These stains were treated with the crude enzyme with detergent, detergent without enzyme and water for comparison. The stained cloth was treated with enzyme and detergent for 10 minutes and then rinsed off with tap water. The effect of the stain removal was noted. Hence these enzyme removed stains with detergent, this can be used as a

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detergent additive and has a potential to be used for industrial purposes.

Treatment	Blood Stain Concentration (Intensity 0, +, + +, + + +, + + + +)			
	12.5%	25%	50%	100%
Water only	0	0	0	0
Water + detergent w/enzyme	++++	++	+++	+++
Water + detergent w/o enzyme	++++	+++	+++	+++

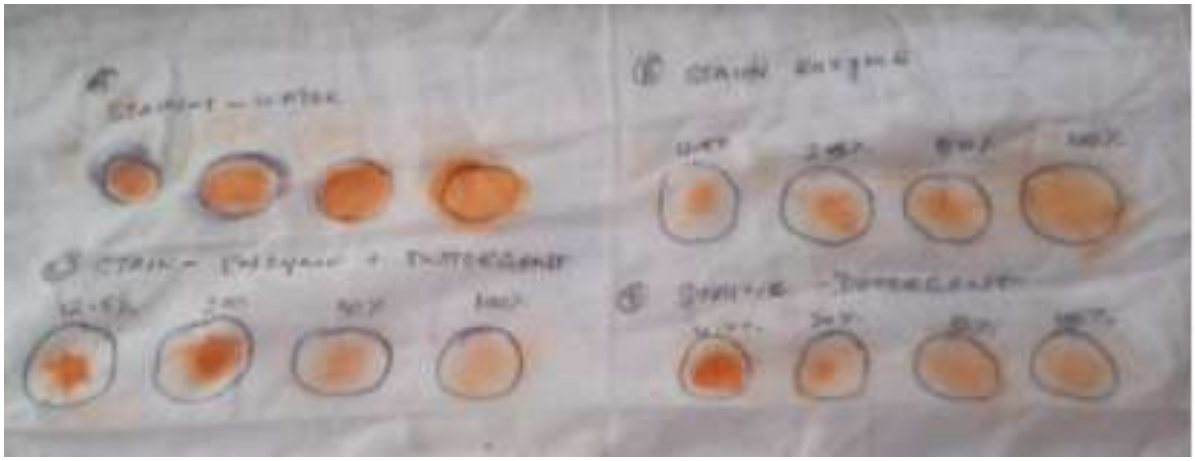

Fig 1:The blood stain removal

Egg White Digestion:

The crude enzyme was then subjected to egg digestion. As egg white consists of protein, the enzyme is added to it in order to find out the ability of the enzyme to hydrolyze it. 10 mL of the enzyme was added to100 mL of egg white and incubated for 24 hours. After 24 hours the settling down of the egg white was observed hence proving the ability of the enzyme of digesting egg white.

Fig 2: Egg white digestion

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Assay for lens cleansing

The enzyme was used for cleansing protein-coated contact lenses. Protein removal was assayed by the method used by Jadhav et al. with minor modifications (Jadhav et al. 2014). The contact lenses were coated with an artificial tear solution. The artificial tear solution was prepared with 0.2% lysozyme in electrolyte solution consisting of 0.22 g Na2CO3, and 0.7 g NaCl (pH – 7.5). The solution was incubated at 50ºC for 20 minutes for the proper denaturing of protein. The solution was filter-sterilized and used for the coating of the lenses. The light transmission reading was taken for all the contact lenses before initiating the experiment using a spectrophotometer at 500 nm. The contact lenses were incubated in 3 ml of artificial tear solution for 20 min at 30 ºC. The readings for the coated lenses were recorded followed by the enzyme treatment for 10, 30, 60, and 90 min. Readings were taken post enzyme treatment. A set of control lenses were treated with phosphate buffer (pH − 7), and readings were recorded at similar intervals (Jadhav et al. 2014)

Table 1: Comparison of transmittance (%) and removal of protein debris from contact lenses

	Transmittance (%)			
Lenses in different enzyme solutions	0 min	15 min	30 min	60 min
Untreated lenses	60	60	62	62
Lenses in phosphate buffer	60	60	63	64
Lenses in an enzymaticsolution	61	65	68	69

IV. CONCLUSION

The fact that the optimum protease production was achieved with medium containing inexpensive substrates, is a strong indication of achieving cost effectiveness for its maximum utilization. Furthermore, its waste degradation potential directly confirms its exploitation for commercial use as proteolytic enzyme for environmental clean-up. This enzyme property, if considered, will help to alleviate the problem of waste management, which is chemically expensive. Therefore, the study gave an insight on the adequate materials to use for its maximum production and its biotechnological exploitation as a hydrolytic enzyme. This result is very interesting and encouraging because most previously reported enzyme production by Bacillus spp. was achieved with more refined and expensive substrates. However, there is still needed to try these substrates in other strains of Bacillus species for protease production.

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