RESEARCH ARTICLE

Application of Protease Produced from an Actinomycete Strain as a Detergent Additive

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Abstract

Proteases have important biotechnological applications, the largest being in laundry detergents, where they help removing protein based stains from clothing. In view of the same, the present work was conducted to study protease activity of an actinomycete strain from Sambhar Salt Lake, Rajasthan and its applicability in removing blood stains from cloth pieces. The results show that the washing performance of the detergent with enzyme was comparatively better than using detergent alone. Being a native of saline environment the isolated protease can be used as an additive in detergents to increase their washing ability and removal of proteinaceous stains as it is expected to remain stable in harsh conditions of alkaline pH and high salt concentrations characteristic of detergents.

Keywords

Actinomycete, Blood stain, Detergent, Halophilic, Protease

Introduction

Enzymes are well known biocatalysts that perform a multitude of chemical reactions and are commercially exploited in many industries like detergent, cosmetic, food, pharmaceutical, diagnostics and dairy industries. About 3000 enzymes have been isolated and described till today and majority of them belong to mesophilic organisms.The properties of enzymes isolated from these organisms does not make them useful in processes requiring extreme conditions of temperature, pH and ionic strength (Kumar and Takagi, 1999). In order to suffice the needs of the enzymes exploited in diverse conditions, researchers are gaining interest in enzymes produced from organisms thriving in extreme environments, the extremophiles.

Among the large number of enzymes used in biotechnological processes, proteases account for about 40% of total enzyme sales in industrial market sectors, like, detergent, food, tannery, dairy, diagnostics, hair and personal care, silver recovery, waste management and pharmaceutical industries (Gupta *et al*, 2002) and this is expected to rise in future. As detergent additives, proteases were first introduced in 1914 and since then they have been used widely (Gupta *et al*, 2002). The worldwide enzyme market is dominated by alkaline protease accounting for two-third of the share of the detergent industry (Agarwal *et al*, 2004). To act as a detergent additive protease should be stable and active in the presence of typical detergent ingredients like surfactants, builders, bleaching agents, bleach activators, fillers, fabric softeners (Najafi and Deobagkar, 2005).

The present investigation was conducted with the aim of testing the applicability of protease produced by an actinomycete strain isolated from Sambhar Salt Lake, Rajasthan, in detergent industry.

Material and Methods

Microorganism

A halotolerant and alkaliphilic actinomycete, A1F1, was isolated from waters of Sambhar Salt Lake, Rajasthan, India. The isolation was carried in Actinomycete Isolation Agar (AIA) media containing cyclohexamide (50µg/ml), nystatin (25µg/ml) and NaCl (3%) at 28°C. The culture was maintained on AIA at 4°C.

Screening for proteolytic activity

The isolate was streaked on milk agar media containing (g/l): 33.33 skim milk powder, 1.18 potassium dihydrogen phosphate, 1.0 disodium hydrogen phosphate, 1.25 glycine, 0.66 sodium hydroxide, 30.0 sodium chloride, 16.67 agar, adjusted to pH 8.1 and incubated for 48hrs at 28°C and observed for zone of hydrolysis.

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Table 1. Treatment of blood stained cotton cloth pieces

Reagents \ Groups	Control	Group A	Group B	Group C	Group D
100ml water	-	+	+	+	+
1ml partially purified enzyme	-	-	+	-	+
1ml detergent solution (7mg/ml)	-	-	-	+	+

Production media and culture conditions

The culture was inoculated with seed inoculum having absorbance 0.25-0.30 at 600nm and incubated for 48 h in 250 ml of erlenmeyer flasks containing 100 ml of M1 medium (Gohar *et al*, 2006) at 120 rpm at 28°C.

Protease assay

Production medium was centrifuged at 4°C at 10,000 rpm and proteolytic activity in the supernatant was determined spectrophotometrically using the method of Cupp-Enyard (2008) taking casein as substrate.

Wash performance test

250ml skim milk broth was used as the production media for testing the wash performance of the extracellular protease produced under the same culture conditions. Crude protease was harvested by centrifuging the culture at 10,000 rpm for 20mins at 4°C. The enzyme in the supernatant was partially purified by precipitation with ammonium sulphate at 50% saturation. All subsequent steps were carried out at 4°C. The protein was resuspended in 0.1 M Tris-HCl buffer, pH 7.8 for further use.

The partially purified enzyme was applied to blood stained white cotton cloth pieces (4X4 inches) in four groups (A-D). Group A was treated only with water, Group B with combination of water and partially purified enzyme, Group C with water and detergent solution (7mg/ml) and Group D with a combination of water, partially purified enzyme and detergent solution (Table: 1). Untreated blood stained cloth piece was taken as control. The treatment was carried out for 30 minutes at room temperature (Banerjee *et al*, 1999).

After incubation, the cloth pieces were taken out, rinsed with water and dried and were examined for stain removal. Untreated cloth pieces stained with blood were taken as control.

Results and Discussion

Actinomycete strain A1F1 thrives well in alkaline and halophilic environment as it showed growth and activity at pH 8.1±2 and 3% NaCl. It showed a clear zone of hydrolysis on milk agar plate (Fig.1) confirming the production of extracellular protease. The protease activity was found to be 12.856 U/ml against casein (Fig.2).

Fig. 1. Skim milk agar assay of A1F1

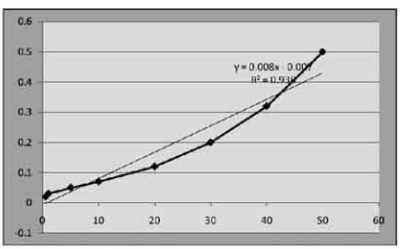

Fig. 2. Standard curve of tyrosine for protease activity assay

Table 2. Comparative account of blood stain removal

Group	Destaining efficiency
A	+
B	+++
C	++
D	++++

*+: slight, ++: moderate, +++: good, ++++: best destaining

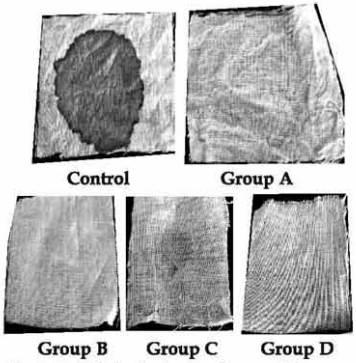

Fig.3. Dried cloth pieces after washing test

The partially purified enzyme obtained after centrifugation at 4°C when applied to blood stained white cotton cloth pieces showed that the enzyme when used alone was removing the blood stains better than detergent (Fig.3). Moreover, best destaining was observed when both were used in combination. (Table: 2).

Similar results were also reported by Anwar and Saleemuddin in 1997 using protease from *Spilosoma oblique* and Najafi and Deobagkar in 2005 from *Pseudomonas aeruginosa* PD100 highlighting the importance of microbial proteases in the removal of proteinaceous stains from clothes. The optimization of the culture conditions for protease production are currently being studied and we hope that in future protease showing good enzyme yield from this strain will be available to be used as an additive in detergents.

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