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Purification of thermo-stable alkaline protease from *Bacillus sonorensis* ASKL-09.

Asma Farheen, K Lingappa* and Sarfaraz Ahmed.

Department of Post Graduate Studies and Research in Microbiology, Gulbarga University Kalaburagi-585106 Karnataka, India.

ABSTRACT

The present study, focus on purification and evaluating the effect of pH and temperature on protease produced by *Bacillus sonorensis* ASKL-09. The molecular mass of protease was determined to be 44 kDa by SDS-PAGE. The enzyme was active at pH 10 and temperature 50°C indicating the thermo- alkaline nature of the protease. The protease produced by *Bacillus sonorensis* ASKL-09 at high extremity hence the same can be used for industrial applications.

Keywords: Alkaline protease, thermo-stable, *Bacillus sonorensis* ASKL-09, purification

Corresponding author

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INTRODUCTION

Thermo-alkalophilic microorganisms play a key role in the enzyme studies and these microorganisms are coveted for applications in many areas. Among various enzymes, proteases are the most commercialized enzymes with over 65% of total enzyme market [1].

Protease is produced by bacteria and fungi [2] however, the proteases produced by bacteria are extensively used, due to the fact that they can be easily produced in larger quantity, mostly extracellular, and stable at high temperature and pH. The purified protease from the genus *Bacillus showed* significant activity, stability, broad substrate specificity, short period of fermentation, simple downstream process and demonstrated low cost. These properties make the *Bacillus* proteases most suitable for a wider industrial application [3]. In the view of the above the present study focuses on purification and characterization of protease from newly isolated *Bacillus sonorensis* ASKL-09 strain.

MATERIALS AND METHOD

Isolation and identification of *Bacillus sonorensis* ASKL-09 strain

The potential protease producing *Bacillus sonorensis* ASKL-09 strain was isolated from the garden soil sample of Gulbarga University, Kalaburagi, Karnataka, India. The strain was identified based on 16S rRNA sequencing [4].

Production and purification of protease

30 g of wheat bran was dissolved in 300 ml of distilled water taken in 500 ml conical flask. The flask was autoclaved and cooled to room temperature. The flask then inoculated with *Bacillus sonorensis* ASKL-09 strain and incubates at 50°C for 30 h. After incubation cell-free supernatant was collected by centrifugation at 10000 rpm at 4°C. To the cell-free supernatant 70% ammonium sulfate was added slowly with constant stirring. The mixture was incubated overnight at 4°C. The precipitated protein obtained was centrifuged for 20 min at 10000 rpm at 4°C. The pellet was re-suspended in 0.2 M phosphate buffer pH, 7.5 and subjected to dialysis against the same buffer. The dialyzed enzyme was applied to DEAE-cellulose column using phosphate buffer 0.2 M (pH 7.5). The adsorbed protein was eluted with a stepwise NaCl gradient. Five ml fractions were collected assayed for enzyme activity. The active fractions, from DEAE-cellulose step, was loaded to Sephadex G-100 column previously equilibrated with 0.2 M phosphate buffer (pH 7.5) and developed at a flow rate of 1 mL min^{-1}.

The molecular mass of the purified enzyme was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) which was performed as described by Laemmli, 1970 [5]. SDS-PAGE was carried out using 10% polyacrylamide gel and stained with coomassie brilliant blue.

Effect of pH and temperature on enzyme activity

The optimum pH for protease was investigated at pH ranging 6-11 using the buffer system as recommended by Charles *et al.,* 2008 [6]. For pH stability, residual activity was measured after 1 h of incubation in buffers system mentioned above.

The optimum temperature on protease activity was investigated at temperature ranging from 30-60°C. For temperature stability, the residual activity was measured after 1 hour of incubation at different temperature levels.

RESULTS AND DISCUSSION

Purification of alkaline protease

The alkaline protease produced by *Bacillus sonorensis* ASKL-09 using wheat bran as substrate was purified, and the steps involved in the purification were summarized in Table 1. In ammonium sulphate precipitation step the specific activity was 12.7 (IU/mg) with purification fold 1.3 and yield of 53.7%. In dialysis step the specific activity was 21.83 (IU/mg) with purification fold of 2.25. The enzyme was further purified by

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DEAE-cellulose and Sephadex G 100 column resulting specific activity and purification fold of 254.5 and 26.1 respectively. The purified enzyme seemed to be homogeneous, as a single band was observed in SDS-PAGE. The molecular weight of the purified alkaline protease was estimated to be 44 kDa (ture 1). A similar purification result was reported by Charles *et al.,* (2008) using *Aspergillus nidulans* HA-10 with a purification fold of 42. 4 with 42 kDa molecular weight enzyme.

Table 1. Purification steps of alkaline protease by *Bacillus sonorensis* ASKL-09

Purification steps	Total protein (mg)	Total enzyme Activity (IU)	Specific activity (IU/mg)	Purification fold	Yield (%)
Crude	2388	23362	9.7	1	100
Ammonium sulphate (70%)	1022	12567	12.7	1.3	53.7
Dialysis	544	11,880	21.83	2.25	50.8
DEAE-cellulose	124	10624	85.6	8.82	45.4
Sephadex G 100	34	8654	254.5	26.1	37.0

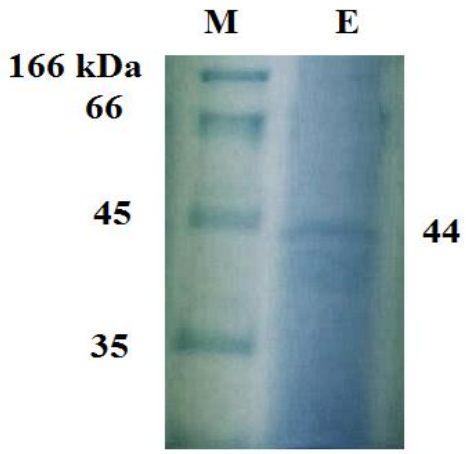

Figure 1: SDS-PAGE of purified alkaline protease from *Bacillus sonorensis* ASKL-09

Effect of pH and temperature on enzyme activity

The effect of pH on purified enzyme was determined at pH ranging 6-11. Figure 2 show the effect of enzyme at various pH. The enzyme activity gradually increased from pH 6- 10, with optimum pH at 10 and then decreased. The enzyme was active in alkaline condition when compared to acidic range with 87% residual activity at pH 11 (Figure 3). Our result correlates with Ibrahim *et al.,* (2015) [7] and Khan *et al.,* (2011) [8] showing enzyme at alkaline condition by *Bacillus* sp.

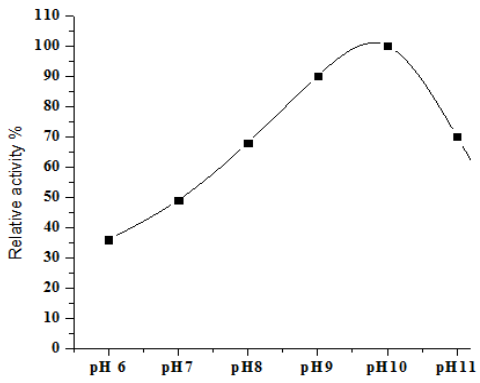

Figure 2: Effect of pH on enzyme activity

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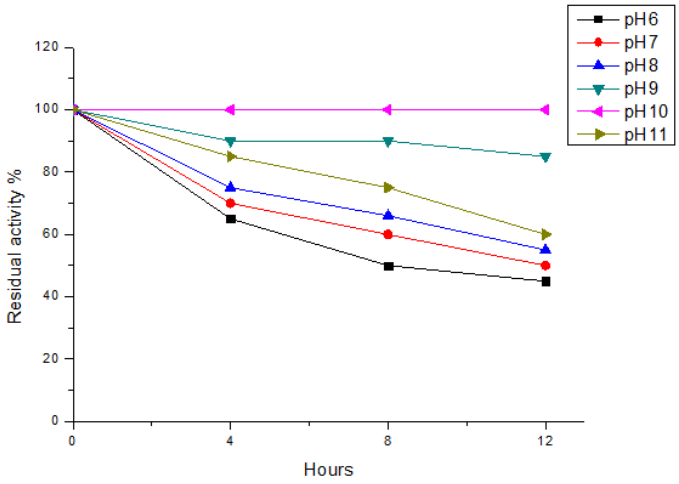

Figure 3: Effect of pH on enzyme stability

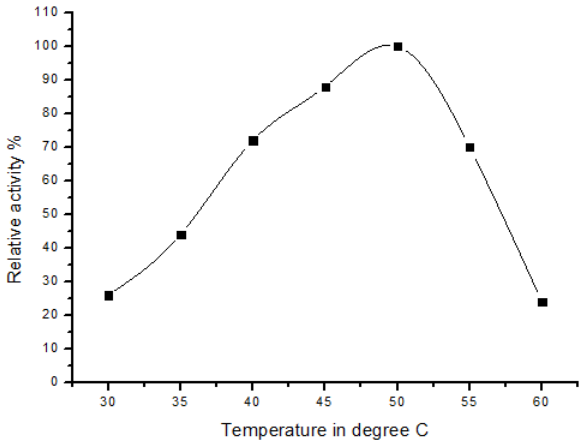

Figure 4: Effect of temperature on enzyme activity

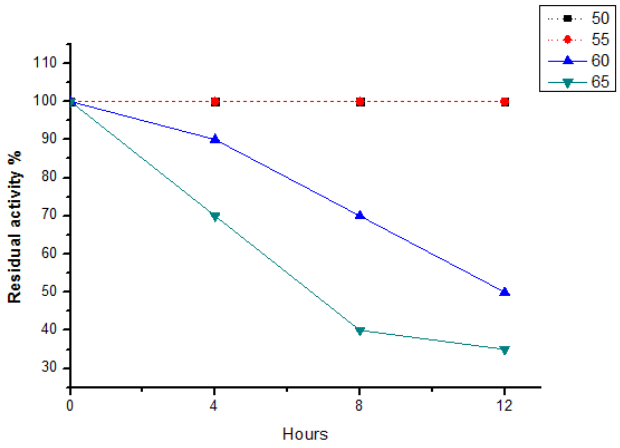

Figure 5: Effect of temperature on enzyme stability

The effect of temperature on enzyme activity was determined at temperature ranging from 30-60°C. The enzyme activity was gradually increased from 30°C and reached maximum at 50°C (Figure 4). Figure 5 suggest the enzyme was thermo-stable showing 100 % stability till 55°C. The enzyme activity of *Bacillus sonorensis* ASKL-09 was high (40°C) when compared with other *Bacillus* strain [7] suggesting thermo- stability.

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CONCLUSION

A theromo-alkaline stable protease was produced by *Bacillus sonorensis* ASKL-09 having a molecular mass of 44 kDa. The enzyme was active at pH 10 and temperature 50°C and produced in low cost substrate (wheat bran) hence, the same can be used for large scale production for industrial use.

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