Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (18):192-196
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Enhanced Thermo-stability of Bacterial Alkaline protease by calcium ions

Bhuvaneshwari Veerapandian, V. Ponnusami* and K. R. Sugumaran*

Bioprocess Engineering Laboratory, School of Chemical and Biotechnology, SASTRA University, Thanjavur – 613401, Tamilnadu, India

---

ABSTRACT

The purpose of this study is to improve the thermo-stabilty of alkaline protease produced by Bacillus sp. using rice bran during solid state fermentation. After partial purification of alkaline protease, various parameters namely effect of temperature and metal ions on enzyme activity was investigated. The suitable temperature for alkaline protease activity was observed at 50°C. Among various metal ions examined, calcium ion improved the thermal stability of the protease considerably. At 50 °C, addition of 1 mM calcium chloride in enzyme solution resulted in 26% increase in enzyme activity. Influence of calcium ions on half life of alkaline protease were compared at temperatures above optimum.

Keywords: Alkaline protease, Rice bran, Thermostability, calcium ions.

INTRODUCTION

Owing to the versatile applications in food, silk, leather and detergent industries, 60% of world enzyme market sale is contributed by protease. Though protease is extensively applied in the determination of peculiar structural characteristics in oligopeptides, proteins, and polypeptides [1-2], its potential applications in an industrial field is yet to be satisfied due to the lack of stability. Industrially important enzymes posses enough storage stability and reaction stability under prevailing condition. Several researchers had paid more attention for improving thermal stability of protease [2-5].

In this present study, alkaline protease production was investigated in solid state fermentation from rice bran. The partial purification of produced alkaline protease was subjected to various characterization studies. Effects of temperature, initial pH and metal ions on enzyme activity were investigated. Finally, Thermo stability of alkaline protease was improved by the selected of metal ion.

MATERIALS AND METHODS

2.1. Fermentation

In this study, *Bacillus* sp. MTCC 511 was procured from Microbial Type Culture Collection, Chandigarh, India. The stock was preserved in nutrient broth of composition yeast extract: 2g/dm^3, peptone: 5g/ dm^3, beef extract: 1g/ dm^3 and NaCl: 5g/ dm^3 at pH 7.8 and it was stored at 4°C.

Twenty grams of rice bran was taken in 250 ml conical flask, in which basal medium[21] containing NaCl: 0.5 g/ dm^3, NH_4Cl: 0.5 g/ dm^3, $MgCl_2$: 0.1 g/ dm^3 and Yeast Extract: 0.1 g/ dm^3 with 70% moisture content. The initial pH of the heterogeneous production medium was adjusted to 10 using suitable buffer solution. Seed culture (10% v/v) was inoculated to sterilized heterogeneous medium. The solid state fermentation was investigated at 30°C without agitation. The sample was extracted from the production medium as described by Nagamine et al., 2003 after fermentation [6]. Specific activity of protease is defined as one micromoles of tyrosine released per unit time per g dry

solid substrate.

2.2. Effect of temperature and metal ions

This study was performed by measuring the enzyme activity at different temperature between 30-100 ºC. Then effect of metal ions like Mg^{2+}, Na^{+}, Cu^{2+}, Hg^{2+} and Ca^{2+}on the protease activity were investigated at optimum temperature (50 ºC) and pH 10. After finding the suitable metal ion according to activity of enzyme, half life of the enzyme was studied in the presence of metal ion and results were compared above the optimum temperature.

RESULTS AND DISCUSSION

3.1. Characterization of alkaline protease

Thermo-stable enzyme can be employed at higher temperatures, resulting in faster enzymatic reaction rates [7-9]. In general, alkaline proteases used in a detergent formulation should have a high catalytic activity over a range of alkaline pH and high temperatures. It has been shown in literature that thermostability of alkaline protease from *Bacillus cereus* BG1 was improved by Ca^{2+} ions [10].

3.5.1 Effect of temperature on enzymatic reaction rate

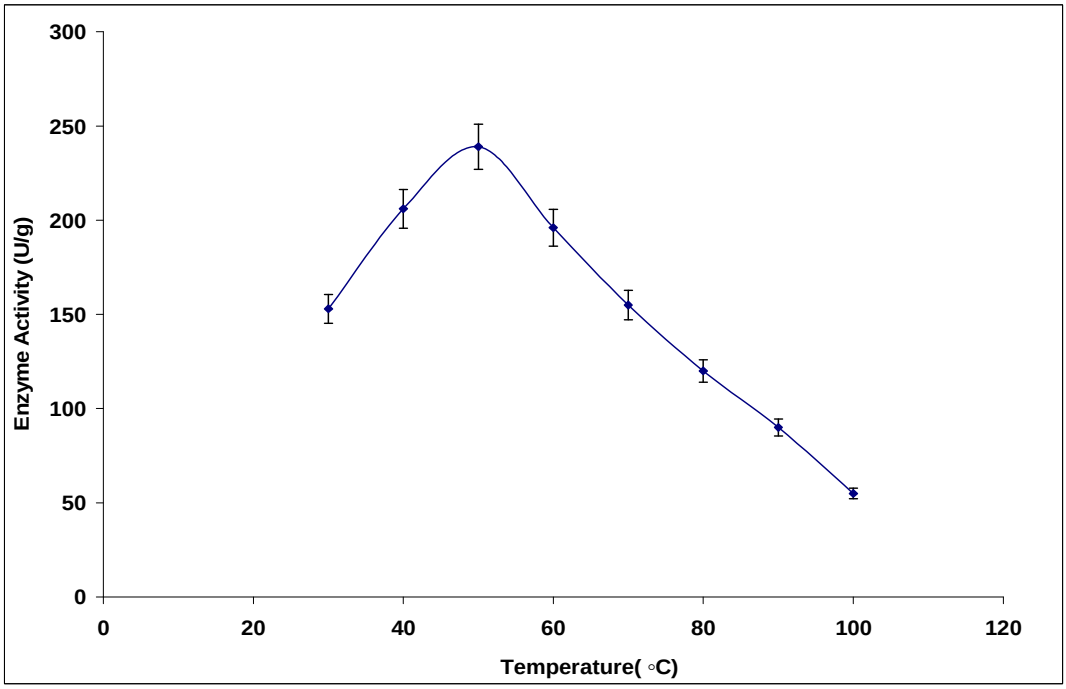

Fig.1. Effect of Temperature on enzyme activity

From the Fig. 1, it was found that the activity of alkaline protease increased from 60 to 100 U/ml as temperature was increased from 30 °C to 50 °C in the absence of metal ions. However, beyond 50 °C enzyme activity decreased. Earlier report showed that optimum temperature for alkaline protease from *Bacillus thuringenesis* was about 50°C [5]. Similarly, the appropriate temperature for alkaline stable protease produced by *Bacillus* sp. was found to be 60°C [10, 11].

3.2. Screening of metal ions on enzymatic reaction rate

The enzyme activity is either enhanced or inhibited by the addition of metal ions like Mn^{2+}, Ca^{2+}, Na^{+}, Fe^{2+}, Co^{2+} etc., [12, 13]. In this study, effect of various metal ions viz. Mg^{2+}, Na^{+}, Hg^{2+}, Ca^{2+} , Cu $^{2+}$and Fe^{2+}on enzyme activity was studied at optimum temperature. Among these metal ions, Fe^{2+}, Mg^{2+} and Ca^{2+} enhances enzyme activity and alkaline activity was maximum in the presence of Ca^{2+} ions in the solution at optimum temperature (Table 1). Other metal ions such as Cu $^{2+}$, Hg^{2+} and Na^{+} inhibited alkaline protease activity. Our report is consistent with previous literature on protease activity from *Bacillus laterosporus*-AK1, in which alkaline protease activity was improved by Ca^{2+}, Mg^{2+} ions , where as reduced by Hg^{2+}, Na^{+} ions[48]. Ca^{2+} in enzyme solution improved thermostability of the enzyme [10]. Suresh Babu Naidu and Lakshmi Devi, 2010 reported that alkaline protease activity was improved by Mg^{2+}, Mn^{2+} and Ca^{2+} ions where as inhibited by Zn^{2+} , Hg^{2+}, Cu^{2+} ions [14].

Table 1: Screening of metal ions on enzyme activity

S.NO	METAL IONS	%CHANGE IN ACTIVITY
1	Control	-
2	Na^{+}	-5
3	Mg^{2+}	9
4	Hg^{2+}	-7.2
5	Ca^{2+}	26
6	Fe^{2+}	16
7.	Cu^{2+}	-15

3.3. Effect of Ca^{2+} ion concentration on enzyme activity

To study the effect of Ca^{2+} ion concentration on enzyme activity Ca^{2+} ion concentration was varied from 0 to 5 mM in the enzyme solution. During the enzymatic reaction, it was observed that Ca^{2+} ion at a concentration of 1 mM was effective for enhancing the activity of protease (Fig. 2). Alkaline protease activity from *Bacillus licheniformis* ATCC 21415 was increased by 26.6% in the presence of Ca^{2+} ions [15]. Influence of Ca^{2+} ion concentration on alkaline protease was investigated and maximum alkaline protease activity was obtained at 2 g/L $CaCl_2$ concentration [10].

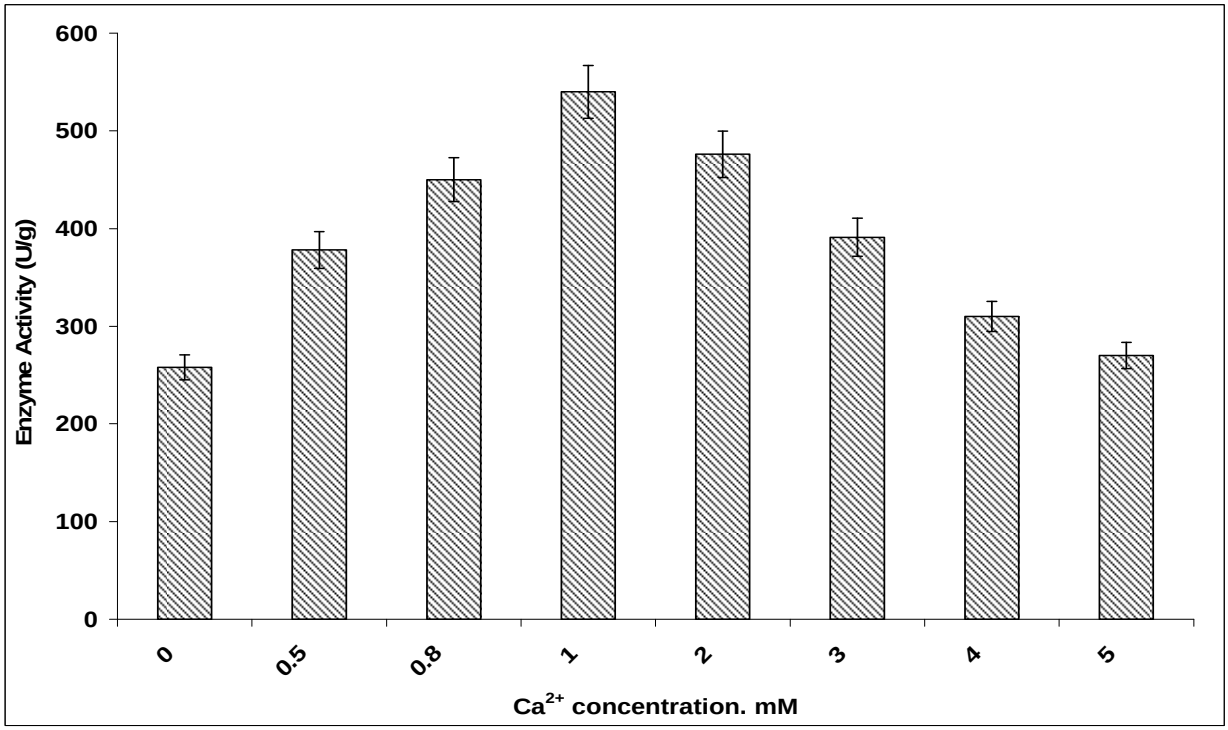

Fig. 2. Effect of Ca^{2+} ion Concentration on Enzyme Activity (Error bar±5%)

3.4. Thermo-stability study

Table 2: Half life period of alkaline protease above optimum temperatures with and without Ca^{2+} ions

S No	Temperature (ºC)	Half Life With Ca^{2+} ions (min)	Half Life Without Ca^{2+} ions (min)
1	60	210.5	176
2	70	161.4	131.8
3	80	112.4	82.4
4	90	86.2	55

The main function of the calcium is to increase the thermo tolerance of the enzyme and prevent thermal unfolding and proteolytic attack [16]. Cationic metal ions were used to improve the thermal stability of alkaline proteases from *Bacillus* sp. [17]. The half life period of alkaline protease was determined at different temperatures both in the presence of 1 mM calcium ion and absence of calcium ion. The results are shown in Fig 3. It was observed that half life of enzyme in the presence of Ca^{2+} was higher than that of enzyme without Ca^{2+} at any temperature. At 90°C, 33% enzyme activity was lost after 1 hour of heating the enzyme solution with Ca^{2+} whereas the loss of activity in the absence of Ca^{2+} was 44.7% during the same period. Comparison of half life period of alkaline protease at different temperatures is shown in Table 2. In this study, alkaline protease half life period at 60°C was 210.5 min (1mM Ca^{2+}) and 176 min (without Ca^{2+}). This is comparable or better than previous reports. Earlier, Ghorbel-Frikha et. al., 2005

found that at 60°C, half life period of alkaline protease was 23 min and 53 min with 1 mM Ca^{2+} and 2 mM Ca^{2+} concentrations respectively [10].

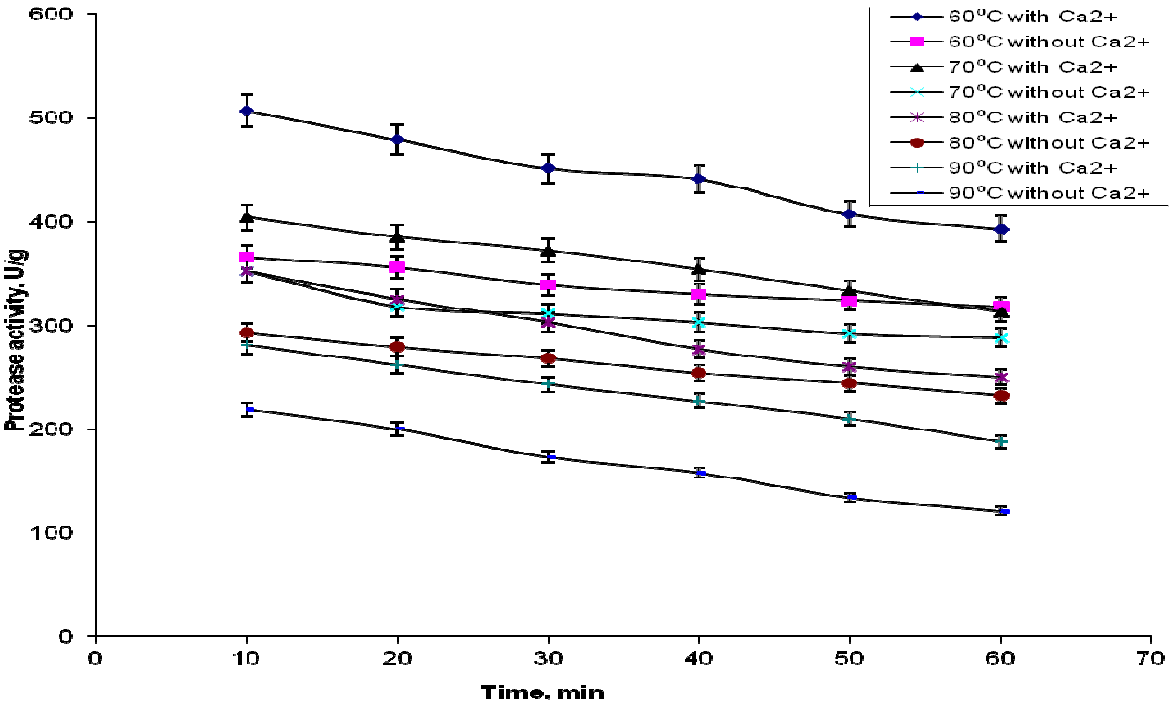

Fig. 3. Effect of heat on the half life of the enzyme with and without Ca^{2+} ions

CONCLUSION

Industrially important enzyme, alkaline protease was produced by solid state fermentation and then partially purified. The effect of different parameters, temperature and metal ions on alkaline protease activity was investigated. Calcium ions increased the thermal stability of the enzyme. Even small concentration Ca^{2+} ion (1mM) was sufficient to increase the stability of the enzyme at high temperatures. Half life period of alkaline protease was found to increase by 20% at 60 °C and by 56.7 % at 90 °C. Thus, alkaline protease enzyme has been produced successfully using the low cost substrate rice bran successfully.

REFERENCES

[1] C. G. Kumar, H. Takagi, *Biotechnol. Adv*., **1999**, 17, 561–594.
[2] N. P. Nirmal, R. Seeta Laxman, *Enzyme Res.*, **2014**, Article ID 109303, 8 pages http://dx.doi.org/10.1155/2014/109303.
[3] K. Ikegaya, S. Sugio, K. Murakami, K. Yamanouchi, *Biotechnol. Bioeng*., **2003**, 81(2), 187-192.
[4] K. K. Doddapaneni, R. Tatineni, R. N. Vellanki, S. Rachcha, N. Anabrolu, V. Narakuti, L. N. Mangamoori, *Microbiological Res*., **2009**, 164, 383-390.
[5] K. R. Sugumaran, V. Ponnusami, D. Gowdhaman, V. Gunasekar, S. N. Srivastava, *International J ChemTech Res.*, **2012**, 4(1), 198-202.
[6] K. Nagamine, K. Murashima, T. Kato, H. Shimoi, K. Ito, *Bio.sci.Biotechnol. Biochem*., **2003**, 67, 2194–2202.
[7] W. C. Nascimento, M. L. Martins, *Braz.J. Microbiol*., **2004**, 35, 91-96.
[8] J. Gomes, W. Steiner, *Food Technol. Biotechnol*., **2004**, 42, 223-235.
[9] J. G. Zeikus, C. Vieille, A. Savchenko, *Extremophiles*., **1998**, 1, 197-183.
[10] B. Ghorbel-Frikha, A. Sellami-Kamoun, N. Fakhfakh, A. Haddar, L. Manni, M. Nasri, J.*Ind. Microbiol. Biotechnol.*, **2005**, 32, 186–194.
[11] D. Jain, I. Pancha, S. K. Mishra, A. Shrivatsav, S. Mishra, *Biores. Technol*., **2012**, 115, 228-236.
[12] S. M. Abu Sayem, M. J. Alam, Md. M. Hoq, Proceedings of the Pak. Academy of Sci., **2006**, 43(4), 257- 262.
[13] H. Akel, Farouk Al-Quadan, T. K. Yousef, *Eur.J. Sci. Res*., **2009**, 31(2), 280-288.
[14] K. S. B. Naidu, K. Lakshmi Devi, *J. Pure Appl. Microbiol*., **2010**, 4(1), 83-90.
[15] M. Anvari, G. Khayati, *Trends in Appl. Sci. Res*., **2011**, 6(10), 1206-1213.
[16] T. D. Spurway, C. Morland, A. Cooper, I. Sumner, G. P. Hazlewood, A. G. O'Donnell, R. W. Pickersgill, H. J. Gilbert, *J. boilogical chem*.,**1997**, 272(28), 17523-17530.

[17] R. N. Z. A. Rahman, C. N. A. R. Razak, K. Ampon, M. Basri, W. M. Z. Wan Yunus, A. B. Salleh, *Appl. Microbiol.Biotechnol*., **1994**, 40, 822-827.