Production and Characterization of Protease from TP2 isolate from Plant Swamp Silage

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Submission date: 29-May-2019 11:48PM (UTC+0700)
Submission ID: 1137479232
File name: Research_J_of_Biotechnology-Ace_Baehaki.pdf (168.94K)
Word count: 1836
Character count: 9501

Production and Characterization of Protease from TP2 isolate from Plant Swamp Silage

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Abstract

The purpose of this research was production and characterization of protease from TP2 isolate of Plant Swam Silage. The optimum pH and temperature of protease from TP2 isolate were 11.0 and 45ºC respectively. Na^+ and Mg^{2+} increased TP2 protease whereas K^+, Fe^{2+} and Zn^{2+} inhibited protease from TP2 isolate inhibiting the enzyme. Study on the effect of metals ion indicated that protease from TP2 isolate was metaloenzyme. Moleculer weight of protease by using SDSPAGE from TP2 isolate was 34,75 kD to 185,51kDa.

Keywords: Production, Characterization, Protease, TP2.

Introduction

Proteases are a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins and break them down into polypeptides or free amino acids. In the last decade, a concern on protease as medicinal target for overcoming bacterial diseases and viral diseases has rapidly increased because of the obvious involvement of this enzyme in the molecular of the diseases mechanism[1]. Proteases are divided into two major groups namely exopeptidase and endopeptidase, depending on their actions. Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate whereas endopeptidases cleave peptide bonds distant from the termini of the substrate.

Based on functional groups present in the active site, proteases are grouped into four important groups namely serine proteases, aspartic proteases, cysteine proteases and metalloproteases. In this research, we reported production of protease from TP2 isolate from Plant Swam Silage and characterization of the extracellular protease.

Material and Methods

Assay of protease Activity: Protease activity was measured according to the Bergmeyer method[2] using either casein at 1% w/v concentration in buffer Tris-HCl 0.05 M. As much as 50 µl enzyme filtrate was mixed with 250 µl substrate and incubated for 10 minute at 37^{0}C. Trichoracetic acid (TCA) 0.2 M was added and incubated at 37^{0}C for 10 minutes followed by centrifugation at 4000 g for 10 minutes.

The supernatant was mixed with Na_2CO_3 0.4 M followed by Folin Ciocalteau reagent (1:2) and incubated further at 37^{0}C for 20 minutes. The reaction products was measured at λ 578 nm. Substrate solution without enzyme and enzyme solution without substrate were used as control. One unit (U) was defined as the number of enzyme producing 1 µmole of tyrosine per min.

Effect of pH and temperature on protease activity: Protease activity of the enzyme was measured using buffer universal pH 6.5 –9.0 containing 0.029 M of A solution (citrate acid, phosphate acid, borate acid and dietilbarbiturate acid) and B solution (NaOH 0.2 N) at a temperature of 50°C with casein (0.5%) as the substrate. The effect of temperature on protease activity was measured at 35; 40; 45; 50; 55; 60 and 65 °C at pH 7.0 with casein (0.5%) as the substrate.

Effect of metal ions on protease activity: The effects of various metal ions were tested on the activity of enzyme at 50 °C in universal buffer pH 7.0, with casein 5% (w/v) as the substrate. The metal ions such as Na^+, K^+, Mn^{2+}, Zn^{2+}, Fe^{2+} and Mg^{2+} at the final concentration with 5 mM were applied in the reaction mixture.

Molecular weight determination: Molecular weight was estimated by electrophoresis under denaturating polyacrylamideSDS (SDS-PAGE) with 8 % polyacrylamide gels[3]. The standard moleculer weight markers were phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa) and lysozyme (14.3 kDa).

Results and Discussion

Protease Production: TP2 Isolate of Plant Swam Silage grew well in Lauria Bertani (LB) Broth containing 1% triptone; 0.5% yeast extract and 1% NaCl. The optimum fermentation time of TP2 Isolate is shown in figure 1.

The optimum production of protease from TP2 isolate was 40 h incubation. Protease production from *Bacillus subtilis* increased gradually from 0 to 36 h at which it was maximal, at 243.28 U/mL per min; then decreased with time[4].

Effect of pH on enzyme activity: The enzyme exhibited greatest activity in the pH range of 9.0 to 11.0 with an optimum pH of 11.0 (figure 2). Maximum protease activity was observed at pH 11.0. Vazquez et al found that Pseudoalteromonas sp strain P96-47 showed high protease production at pH 9. Miyamoto et al[6] found pH 10.0 was the optimum pH for the protease production from Alteromonas sp strain O-7.

Effect of temperature on enzyme activity: A temperature range between 35°C and 65°C was used to study the effect of pH on protease activity. Enzyme activity increased with temperature within the range of 45°C to 50°C. A reduction in enzyme activity was observed at values above 50°C. Fig. 3 showed the effect of temperature on protease activity from TP2 isolate.

The optimum temperature of 45°C was recorded for the protease in this study. It was previously reported that 30°C was the optimum temperature for the protease production from *B. subtilis*[7] and 50°C of *Bacillus licheniformis* F11.4[8].

Effect of metal ions and specific inhibiotor on enzyme activity: Some enzymes require metal ions as cofactors to support the catalytic efficiency of the enzyme. The metal helps catalytic reactions by binding to the substrate cutting side. Besides acting in enzyme binding with a substrate, some metals can also bind to enzymes directly to stabilize the active conformation or induces formation of a binding site or an active site enzyme. Table 1 showed effect of metal ions on protease activity.

Ions Na^+ and Mg^{2+} increased protease whereas K^+, Fe^{2+} and Zn^{2+} inhibited protease from TP2 isolate inhibiting the enzyme. The results indicated that the protease from TP2 isolate was metalloprotease. Ion Mg^{2+} increased while ion Fe^{2+} inhibited protease from *Bacillus caseinilyticus*[9]. Protesae from *Bacillus subtilis* DR8806 was stimulated by K^+, Ca^{2+}, Mg^{2+} and Fe^{2+} at 10 mM concentration up to 134, 129, 128 and 112 % respectively[10].

Molecular weight determination: Molecular weights were determinated by using SDS-PAGE and zymogram technique. Molecular weights protease from TP2 isolate are given in figure 4.

SDS-PAGE indicated that the molecular mass of the protease from TP2 Isolate was 34,75 kDa to 185,51 kDa. It was previously reported 66 kDa from *Bacillus caseinilyticus* protease[9], 34 kDa serine protease from *B. pumilus* CBS[11] and a 35 kDa manganese-dependent alkaline serine protease from *B. pumilus* TMS55[12].

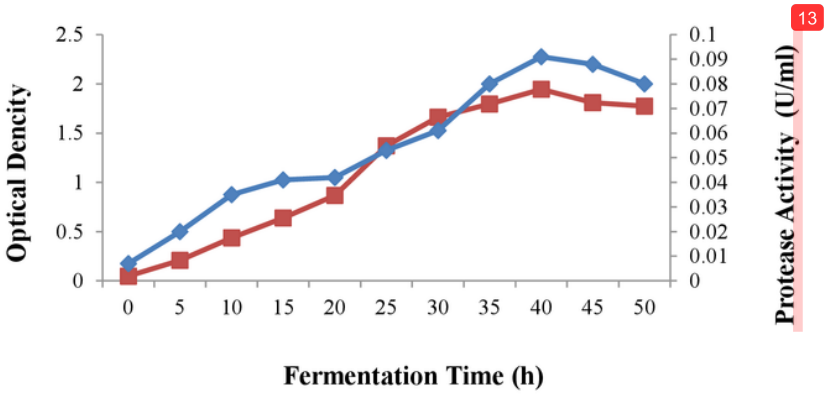

Figure 1: Optimum fermentation time of protease from TP2 Isolate (- ◆ - Bacteria growth, - ■ -Protease activity).

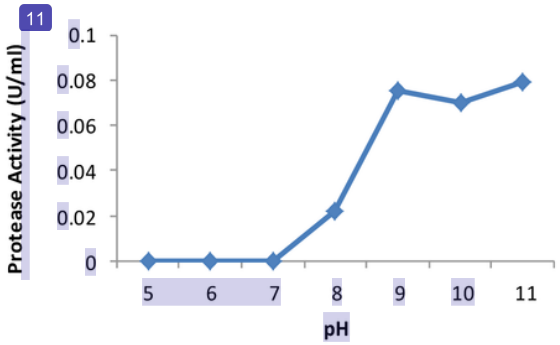

Figure 2: Effect of pH on protease from TP2 isolate. Buffer used 0,05 M universal buffer

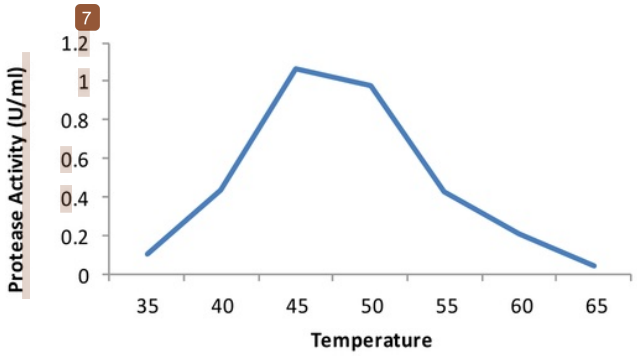

Figure 3: Effect of temperature on protease from from TP2 isolate

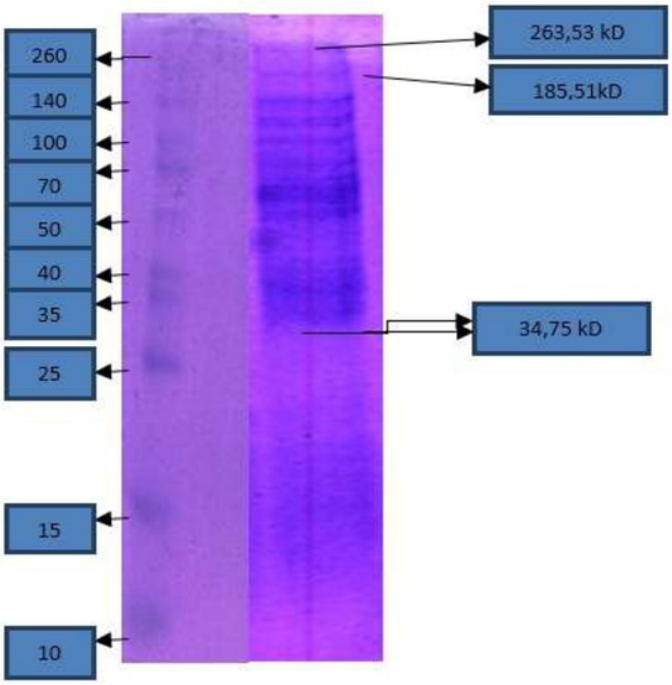

Figure 4: SDS PAGE and zymogram protease from *Bacillus cereus* VBE16. 8 % polyacrylamide gel as used for analysis. SDS-PAGE stained with silver: M, relative molecular mass standards.

Table 1
Effect of metal ions on protease activity

Treatment	Concentration (mM)	Relative Activity (%)
None	-	100
Na^{+}	5	125.0
K^{+}	5	58.2
Fe^{2+}	5	0.0
Zn^{2+}	5	41.6
Mg^{2+}	5	175.0

Conclusion

The optimum pH and temperature of protease from TP2 isolate were 11.0 and 45°C respectively. Na^{+} and Mg^{2} increased protease whereas K^{+}, Fe^{2+} and Zn^{2+} inhibited protease from TP2 isolate inhibited the enzyme. Study on the effect of metals ion indicated that protease from TP2 isolate was metalloenzyme. Molecular weight of protease by using SDSPAGE from TP2 isolate was 34.75 kD to 185.51kDa.

Acknowledgement

This research was supported by Competitive Grant (Hibah Profesi 2016, Universitas Sriwijaya) from Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Kementristekdikti).

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(Received 20th November 2017, accepted 27th September 2018)

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