Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):776-780

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Production and characterization of protease from patogenic bacteria *Escherichia coli*

Ace Baehaki[a*], Tati Nurhayati[b], Nurheni Sri Palupi[c] and Maggy T. Suhartono[c]

[a]*Department of Fisheries Product Technology, Faculty of Agriculture, Sriwijaya University, Indralaya Ogan Ilir, South Sumatera, Indonesia*
[b]*Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science,, Bogor Agricultural University, Bogor, West Java, Indonesia*
[c]*Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Bogor, West Java, Indonesia*

ABSTRACT

The purpose of this research was to production and characterization protease from patogenic bacteria Escherchia coli. The optimum pH and temperature of protease from Escherichia coli were 7.5 and 40 ºC, respectively. Co^{2+} (5 mM) and Ba^{2+} (5 mM) increased E. coli protease whereas Na^{+} (1 and 5 mM), K^{+} (1 and 5 mM), Mn^{2+} (1 and 5 mM), Co^{2+} (1 mM) and Ba^{2+} (1 mM) inhibited protease from E. coli inhibited the enzyme. Study on the effect of metals ion and specific inhibitors indicated that protease from E.coli was serin metaloenzyme. Moleculer weight of protease by using SDSPAGE and zymogram technique from E. coli was 36 kDa.

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 been rapidly increased because of the obvious involvement of this enzyme in the molecular of the diseases mechanism [1].

Escherichia coli typically colonizes the gastrointestinal tract of human infants within a few hours after birth. Usually, *E. coli* and its human host coexist in good health and with mutual benefit for decades. These commensal *E. coli* strains rarely cause disease except in immunocompromised hosts or where the normal gastrointestinal barriers are breached — as in peritonitis, for example. The SPATEs (serine protease autotransporters of enterobacteriaceae) are a subfamily of serine protease autotransporters that are produced by diarrhoeagenic and uropathogenic *E. coli* and *Shigella* strains [2].

In this research, we reported that production of protease from *Escherichia coli*. Characterization of the extracellular protease was presented.

EXPERIMENTAL SECTION

Protease Production

The LB media contained tryptone1 %, NaCl 1 % dan yeast extract 0.5 % (w/v). The cell growth was monitored turbidimetrically through absorbance at l = 620 nm. As much as 10 % of seedling culture with optical density of 0.8 (at 620 nm) was sub-cultured into the same media for enzyme production. Incubation was conducted at 37 °C and samples were taken for analysis of enzyme activity.

Assay of protease Activity

Protease activity was measured according to the Bergmeyer method [3] 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 centrifuged at 4000 g 10 minutes. The supernatant was mixed with Na_2CO_3 0.4 M, followed by Folin Ciocalteau reagent (1:2) and incubated furthur 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 which produce 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. Plot of enzyme relative activity against pH was constructed to determine the optimum pH for the reaction. The effect of temperature on protease activity was measured at 30; 40; 50; 60; and 70 °C at pH 7.0, with casein (0.5%) as the substrate.

Effect of metal ions and specific inhibitor 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+}, Co^{2+}, and Ba^{2+} at the final concentration with 1, and 5 mM were applied in the reaction mixture. Specific inhibitor such as EDTA and PMSF (Phenyl methyl sulfonyl fluoride) were applied in the reaction mixture.

SDS-PAGE and Zymogram analysis

Molecular weight was estimated by electrophoresis under denaturating polyacrylamide- SDS (SDS-PAGE) with 8 % polyacrylamide gels [4]. Enzymes activity *in situ* was determined by zymogram following Choi *et al.* [5]. Acrylamide gel was copolymerized with the 1 % protein (collagen) substrates. Following electrophoresis, the gel was soaked in Triton X-100 2.5 % for 1 h and further incubated for enzyme substrate reaction in buffer *Tris*- Cl 10 mM, pH 8, 37 °C for 24 h. The activity bands was visualized after incubation in staining solution (50 % methanol + 10 % acetic acid + 0.06 % coomassie brilliant blue R-250) for 30 min followed by incubation in destaining solution (5 % methanol + 7.5 % acetic acid). Positive result was seen as clear bands. 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

Escherichia coli grew well in Lauria Bertani (LB) Broth containing 1% triptone; 0.5% yeast extract and 1% NaCl. Figure 1 show the optimum fermentation time.

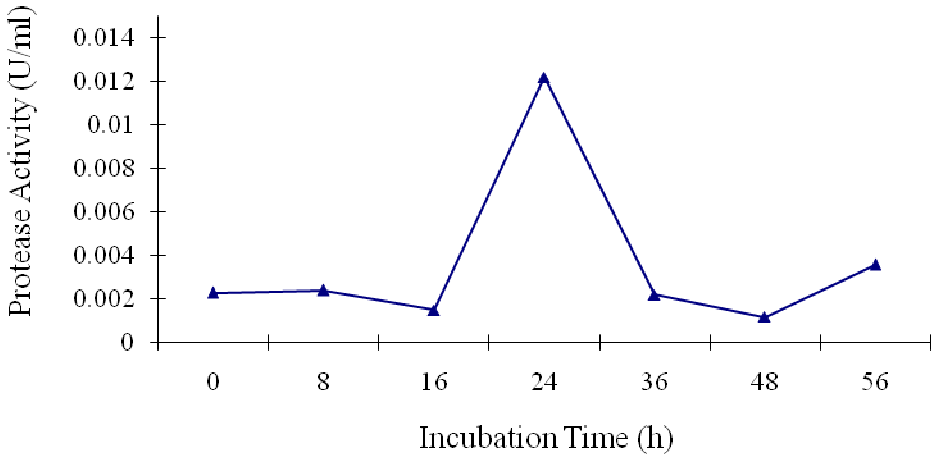

Figure 1. Optimum fermentation time of *E. coli* protease
Each value represents the mean ±SE of three independent Experiments

The optimum production of *E. Coli* protease was 24 h incubation. This was similar to *Bacillus* sp. [6]. *Bacillus* sp. exhibited maximum ability to biosynthesize proteases within 24 h. Moreover, Johnvesly *et al.* [7] found that a high

level of extracellular thermostable protease activity was observed after 24 h incubation and hence our results are in complete accordance with earlier reports.

Effect of pH on enzyme activity

The enzyme exhibited greatest activity in the pH range of 7.0 to 8.0, with an optimum pH of 7.5 (Figure 2).

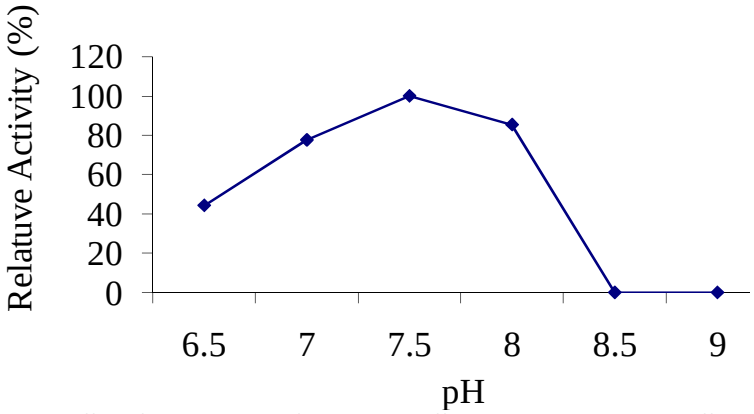

Figure 2. Effect of pH on protease from *E.coli.* Buffer used 0,05 M universal buffer

Each value represents the mean ± SE of three independent Experiments.

Maximum protease activity was observed at pH 7.5. The result was similar to most reports which were pH 7.0 to 7.5, but lower than pH 8 of *Bacillus aryabhattai* K3 [8], pH 9.0 of *Bacillus licehniformis* F11.4 [9], and pH 10 of *Bacillus licheniformis* KBDL4 [10].

Effect of temperature on enzyme activity

A temperature range between 30 ^{0}C and 70 ^{0}C was used to study the effect of pH on protease activity. Enzyme activity increased with temperature within the range of 30 ^{0}C to 40 ^{0}C. A reduction in enzyme activity was observed at values above 40 ^{0}C. Fig 3 showed the effect of temperature on protease activity from *E. coli*.

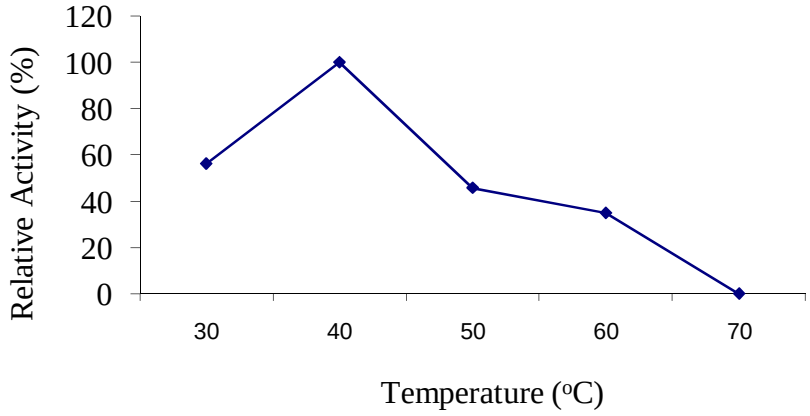

Figure 3. Effect of temperature on protease from *E.coli*

Each value represents the mean ± SE of three independent Experiments

The optimum temperature of 40°C was recorded for the protease in this study. This was similar to *Bacillus* sp. [6], and higher than with the 35°C optimum temperature reported for *Bacillus aryabhattai* K3 [8], but lower than 50^{0}C of *Bacillus licheniformis* F11.4 [9], 60°C of *Bacillus licheniformis* UV-9 [11] and 80 ^{0}C of *Bacillus* HUTTBS62 [12].

Effect of metal ions and specific inhibiotor on enzyme activity

Effect of metal ions, Co^{2+} (5 mM) and Ba^{2+} (5 mM) were the activator whereas Na^{+} (1 and 5 mM), K^{+} (1 and 5 mM), Mn^{2+} (1 and 5 mM), Co^{2+} (1 mM) and Ba^{2+} (1 mM) inhibited protease from *E. coli*. Table 1 showed effect of metal ions and specific inhibitors on protease activity.

Table 1. Effect of metal ions and specific inhibitors on protease activity

Treatment	Concentration (mM)	Relative Activity (%)
None	-	100
Na^+	1	28.8
Na^+	5	24.8
K^+	1	36.2
K^+	5	20
Mn^{2+}	1	24.8
Mn^{2+}	5	23.8
Co^{2+}	1	69.5
Co^{2+}	5	179.1
Ba^{2+}	1	41.9
Ba^{2+}	5	129.5
EDTA	1	57.1
EDTA	5	0
PMSF	1	0
PMSF	5	0

Most metal ions affect the enzyme activity. Na^+ (1 and 5 mM) and K^+ (1 dan 5 mM) as monovalent ions showed inhibitory effect on protease in our case, which was similar to the results claimed that the enzymes from *Bacillus aryabhattai* K3 [8] which was inhibited by Na^+ and K^+. However, Co^{2+} (5 mM) and Ba^{2+} (5 mM) enhanced its activity.

EDTA, a chelating agent for metal ions inhibited and activated the enzyme consistent with the finding that (Table 1) indicating this protease was metalloprotease. The protease from *E. coli* was inhibited by PMSF completely, indicating this protease was serin protease. The results indicated that the protease from *E.coli* was serin metalloprotease.

Molecular weight determination

Molecular weights were determinated by using SDS-PAGE and zymogram technique. Molecular weights protease from *E. coli* are given in Figure 4.

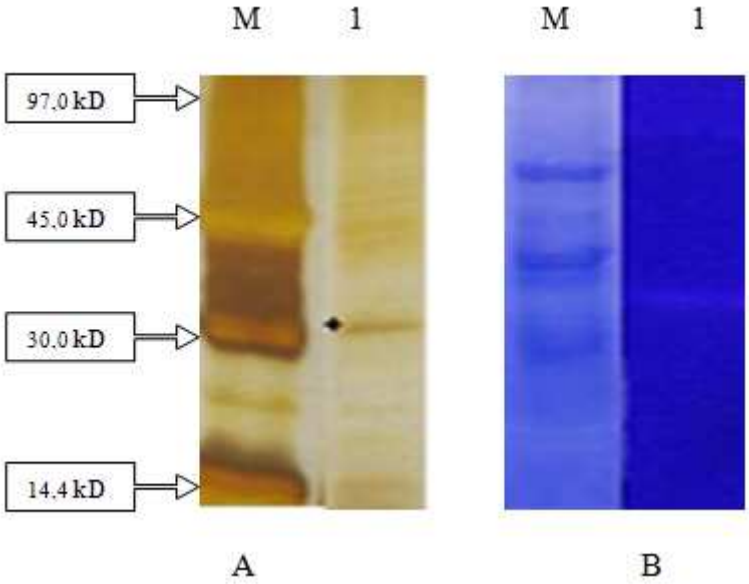

Figure 4. SDS PAGE and zymogram protease from *E.coli*. 8 % polyacrylamide gel as used for analysis. A, SDS-PAGE stained with silver: line 1, relative molecular mass standars: phosporylase b (97 kD), bovine serum albumin (66.0 kDa), ovalbumin (45 kD) carbonic anhydrous (29.0 kDa), soybean trypsin inhibitor (20 kDa), lysozime (14.4 kD). line 2, *E.coli* protease. B, Zymogram: 1ine 1, relative molecular mass standars. line 2, *E.coli* protease

Both of SDS-PAGE and zymography indicated that the molecular mass of the protease from *E. coli* was 36 kDa. This is similar to protease VII from *E.coli* [13] and *Bacillus licheniformis* UV-9 protease [11]. Different molecular masses for different protease have been reported: 48 kDa for *Bacillus* HUTTBS62 [12]; 30,9 kDa for B*acillus* sp. HS08A [14]; 75.0 kDa for *Bacillus sp.* S17110 [15].

REFERENCES

[1] A Baehaki; MT Suhartono; NS Palupi; T Nurhayati, *J. Technol Industri Pangan*., **2008**, 19(1), 80-89

[2] JB Kaper; JP Nataro; HLT Mobley, *Nature Review Microbiol*., **2004**, 2, 123-140
[3] HU Bergmeyer; J. Bergmeyer; M. Graβl. Methods of Enzymatic Analysis. Verlag Chemie, Weinheim, 1983, 1007-1009.
[4] UK Laemmli, *Nature*., **1970**, 227, 680-685.
[5] NS Choi; KS Yoon; JY Lee; KY Han; SH Kim, *J. Biochim Mol Biol*., **2001**, 34, 531-536.
[6] FS Josephine; VS Ramya; N Devi; SB Ganapa; KG Siddalingeshwara; N Venugopal; T Vishwanatha, *J Microbiol Biotech Res*., **2012**, 2 (1), 163-168
[7] Johnvesly B; BR Manjunath; GR Naik, *Bioresour Technol*., **2002**, 82(1):61-4.
[8] KM Sharma; R Kumar; S Vats; A Gupta, *Int J. Adv Pharm, Biol Chem.*, **2014**, 3(2), 290-298
[9] A Baehaki; Sukarno; D Syah; S Setyahadi; MT Suhartono, *Asian J. Chem.*, **2014**, 26(10), 2861-2864
[10] AP Pathak; KB Desmukh. 2012, *Indian J Exp Biol*., **2012**, 50, 569-576.
[11] M Nadeem; JI Qazi; Q Syed; M Gulsher, *Songklanakarin J. Sci. Technol*., **2013**, 35(2), 187-195
[12] H Aqel; F Al-Quadan; TK Yousef, *J. BioSci. Biotech*., **2012**, 1(2), 117-123.
[13] K Sugimura; T Nishihara, *J Bacteriol*., **1988**, 170(12), 5625-32.
[14] GR Huang; TJ Ying; P Huo; JX Jiang, *Afr. J. Biotechnol*., **2006**, 5, 2433-2438
[15] SC Jung; H Pai; MS Kim; KS Baik; W Lee; CN Seong; SK Choi, *J. Microbiol*., **2007**, 45, 402-408