



Characterization of Extracellular Protease Lactic Acid Bacteria From Shrimp Paste

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ABSTRACT: Protease is an enzyme that is able to hydrolyze peptide bonds of proteins and enzymes is one of the most widely used in industry. The aim of this research is to investigate the characterization of extracellular protease lactic acid bacteria (LAB) isolated from shrimp paste. Treatment used in this research is optimization condition of pH, temperature, the effect of metal and enzyme kinetics toward extracellular protease LAB. The result showed isolation and identification of lactic acid bacteria is *Lactobacillus plantarum*. Extracellular protease has enzyme activity 3,89 IU/ml, specific enzyme activity 0,34 IU/mg and protein content 4,16 mg/ml. Extracellular protease from *L. plantarum* has optimum activity at pH 7, 47°C, activator Mg²⁺, Km 0,0797 mM and Vmax 0,0026 mmol/minutes/mg, and molecular weight range 25,35-69,64 kDa.

Key words: Protease, LAB, Shrimp Paste

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INTRODUCTION

Protease is an enzyme that is able to hydrolyze peptide bonds of proteins [1] and one of the most widely used in industry [2-4]. One of the bacteria that are known to produce proteases that lactic acid bacteria.

Lactic acid bacteria found in many fermented fish products. One of this product is the shrimp paste. Studies of protease sources from lactic acid bacteria have been carried out. Research on the isolation of lactic acid bacteria that have proteolytic activity, including of the type *L. plantarum*, *L. brevis*, and *Pediococcus* [7]. Lactic acid bacteria isolated capable to producing protease activity by 4,09±0,13 mm and enzyme activity by ≤ 20 nmol (*leusine arilamidase*, *Sistine arilamidase*, *valine arilamidase*) [8].

The aim of this study is to determine the characteristics of protease produced by lactic acid bacteria isolated from shrimp paste with optimization condition of pH, temperature, the effect of metal and enzyme kinetics.

MATERIAL AND METHODS

Sample:

The main material used is shrimp paste that has been packaged in accordance with the standards of packaging raw materials that are coated with plastic packaging aluminum foil.

Isolation and identification of lactic acid bacteria:

5 grams of mashed shrimp paste, put in MRSB 45 mL was incubated for 48h at 37°C in incubator. One of the liquid culture streaked into a petri disk containing MRSA using quadrant method was incubated for 48 hours at 37°C. Each colony that grew back then grown on agar slant test MRSA sterile and estimation of lactic acid bacteria that produce protease using SMA sterile medium [9]. Lactic acid bacteria which showed the largest clear zone on medium then identified. Identification was conducted on the morphological observation [10], Gram staining and cell shape [11], bacterial motility [12] and biochemical tests with kits Microbact [5].

Growth curve:

As much as 3 ose *Lactobacillus plantarum* proteolytic carved into MRSA, and then incubated at 37°C for 24 hours. 1 mL of *L. plantarum* had been incubated subsequently and pure colonies were inoculated into 50 mL MRSB, incubated for 18 h in a shaker incubator at a 37°C with a speed of 150 rpm, and 0.1 Å OD cells calculation with a wavelength of 620 nm. Growth chart seen through the optical density value (OD) of the culture colonies in MRSB [9].

Extraction and purification of extracellular protease:

Extraction of crude extracellular protease using centrifugation method [13]. Extraction of crude extracellular protease enzyme centrifugation method harvested at logarithmic phase, then centrifuged at 12,000 rpm for 15 min 4°C [14]. The supernatant ammonium sulfate was added gradually while stirring with magnetic stirrer with a concentration up to 50% and left for one night at 4°C. Furthermore, centrifuged 12,000 rpm, 4°C, for 15 minutes [1]. The supernatant was carried out the result of precipitation dialysis at 4°C. Results of dialysis used

for protease activity test and protein content [19]. After dialysis performed enzyme centrifuged at a speed of 12,000 rpm at 4°C for 15 min.

Characterization of extracellular protease:

Characterization of extracellular proteases includes the effect of temperature, pH, metal and kinetics. The effect of temperature on protease activity using a temperature 27°C, 37°C, 47°C and 57°C [16]. pH which used 6, 7, 8, and 9 [17] [18]. To determine the influence of metal, using varied metal were consist of Mn²⁺, Ca²⁺, Mg²⁺ and Fe²⁺ concentration of 5 mM [18]. While the enzyme kinetics using casein substrate concentration 0,25%; 0,5%; 0,75% and 1% (v/v).

Determination of Molecular Weight extracellular protease:

Determination of the molecular weight of protein using gel electrophoresis method (SDS-PAGE) [19].

RESULTS

Isolation and identification of lactic acid:

Based on the results of isolation and identification bacteria from shrimp paste, the type of bacteria found Gram-positive group, it is shown in Table 1. In order that Gram observations based on motility and catalase test to indicate type of lactic acid bacteria. Then estimation of protease test using skim milk agar medium.

Lactic acid bacteria which produce the largest clear zone identified using Microbact kit, which is identified as *Lactobacillus plantarum*. Then, the growth curve is known that the early exponential phase and the stationary phase at 32.

Table 1. Results of Identification of LAB

Isolate	Morphology Test			Catalase Test	Protease Test
	Forms of bacteria	Gram	Motile		
T1	Rod	+	+	+	-
T2	Rod	+	+	+	-
T3	Rod	+	+	+	-
T4	Rod	+	-	-	+(1,32)
T5	Rod	+	+	-	+(0,52)
T6	Rod	+	+	+	-
T7	Cocci	+	-	-	-
T8	Rod	+	-	+	-
T9	Rod	+	+	-	+(1,25)
T10	Cocci	+	-	+	-
T11	Cocci	+	+	-	+(0,75)

Extraction and purification of extracellular protease:

1,11 IU/ml crude extracellular protease enzyme activity, 0,27 IU/mg specific enzyme activity and of 11,22 mg/ml protein content. Result of pure extracellular protease enzyme activity is 3,89 IU/ml, 0,34 IU/mg specific enzyme activity and 4,16 mg/ml protein content.

Characterization of extracellular protease:

Determination of optimum pH: Determination of optimum pH using a pH value of 6, 7, 8 and 9. In Figure 1 shows optimum enzyme activity with activity value of 1,9259 IU/mL, pH 7 and the value of specific enzyme activity is 0,1716 IU/mg.

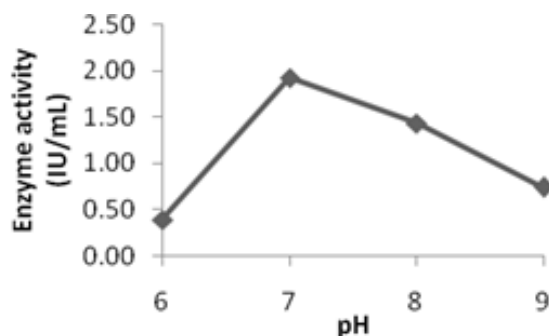


Figure 1. Effect of pH on the extracellular protease activity *L. plantarum*

Determination of optimum temperature: In Figure 2 chart shows that the optimum protease activity of *L. plantarum* that is at a temperature 47°C with the value of enzyme activity 9,7619 IU/ml and 0,87 IU/mg specific enzyme activity.

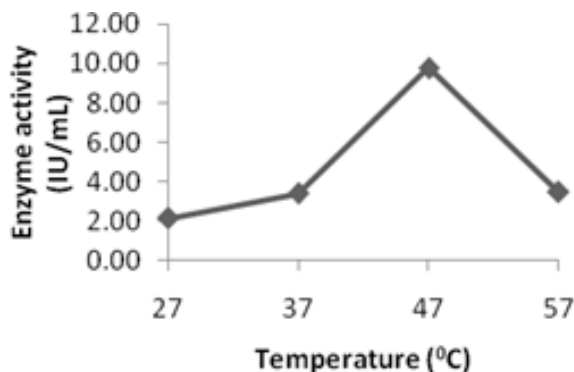


Figure 2. Effect of temperature on the extracellular on protease activity *L. plantarum*

Effect of Metal: In characterization the effect of metal, used various metal ion Mn^{2+} , Ca^{2+} , Mg^{2+} and Fe^{2+} with 5 mM concentration. Figure 3 shown $MgSO_4$ and $FeSO_4$ have functions as an activator. While $MnSO_4$ and $CaSO_4$ have function as an inhibitor. The highest value of enzyme activity using $MgSO_4$ metal with 7,5 IU/mL enzyme activity values and 0,6461 IU/mg specific enzyme activity.

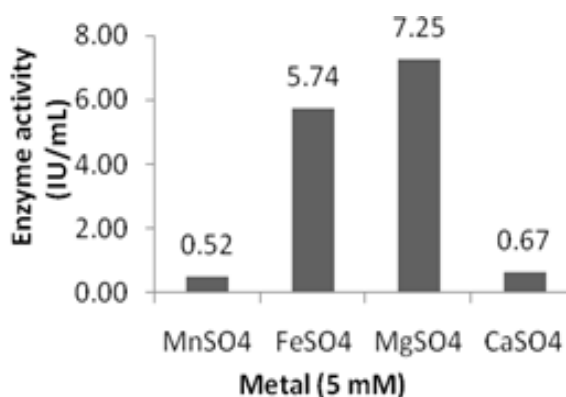


Figure 3. Effect of metals on the activity of extracellular proteases *L. plantarum*

Kinetics of extracellular proteases *L. plantarum*. The enzyme kinetics using casein substrate concentration 0,25%; 0,5%; 0,75% and 1% (v/v). Lineweaver-Burk graphic linear equations obtained above $y = 30.596x + 384$ where the value of K_m and V_{max} , respectively $V_{max} = 0.0026$ mmol/min/mg and $K_m = 0.0797$ mM.

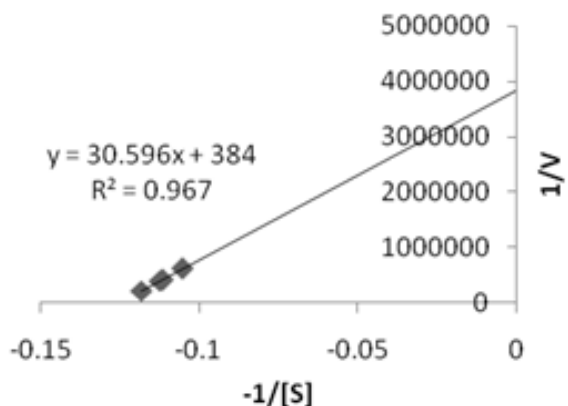


Figure 4. Graphic of Lineweaver-Burk of extracellular protease activity *L. Plantarum*

Determination of Molecular Weight extracellular protease. Based on Figure 5 can see that extracellular protease of *L. plantarum* has a molecular weight of 25-70 kDa.

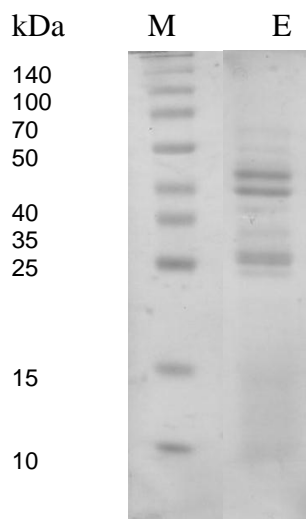


Figure 5. SDS-PAGE electrophoresis test of Extracellular Protease

DISCUSSION

The results of isolation and identification of bacteria obtained types of lactic acid bacteria *L. plantarum*. This is consistent with research [20]. In his research found lactic acid bacteria *L. plantarum* on shrimp paste. This is supported by research [21], approximately 64% of *Lactobacillus* species found in fish and shrimp. Extraction of extracellular proteases performed at 32nd which is the end of the exponential phase and early stationary phase. Proteolytic enzymes in *L. delbrueckii*, *L. bulgaricus* and *S. thermophilus* were isolated at the end of logarithmic phase [22].

Total and specific enzyme activity increasing extracellular protease activity of crude into pure extracellular proteases. This suggests that the use of 50% ammonium sulfate (w/v) precipitation able to increasing protease activity. This is in accordance with the nature of protein will precipitate due to the salt presence. The deposition of this protein causes increasing enzyme activity this is due to decreasing number of contaminants that block active site of enzyme to bind substrate.

The enzyme activity is influenced by pH, temperature, and magnitude of metal kinetics. In optimum pH value, active sites enzyme have conformations corresponding to casein substrate, resulting in that formation is cause the maximum cluster proton donor and recipient are important in ionization catalytic enzyme is at the desired level, so as to produce the maximum also [6]. The optimum pH obtained in the study is a neutral pH, pH 7. The enzyme activity is affected by the high and low temperatures, where at low temperature makes the enzyme reaction is slow, the faster there action temperature is raised. Power ion stability against proteases depends on kind of metal used and the concentration of a given magnitude [23]. At a certain concentration of a particular metal ion can act as an inhibitor, but can also act as an activator in other concentrations [19]. Km values stated dissociation constant of complex enzyme-substrate. If the small Km value indicates complex enzyme-substrate more stable so that the enzyme has a affinity communities to the substrate and vice versa. Pure enzyme which has allowed the active side can react better, thus increasing the activity which impact on the value of Km.

CONCLUSION

Lactic acid bacteria were isolated from shrimp paste and has made the identification of the bacterium *Lactobacillus plantarum*. Pure extracellular protease enzyme activity higher than the extracellular proteases are still rough with values respectively 4,43 U/mg and 1,26 U/mg. Extracellular protease of *L. plantarum* has optimum activity at pH 7, temperature of 47°C, with a metal activator Mg²⁺ and has a Km of 0.0797 mM and Vmax 0.0026 mmol/min/mg and has a molecular weight distribution from 25.35 to 69.64 kDa.

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