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Research Article

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Physicochemical Characterization of Purified Alkaline Protease from *Aneurinibacillus thermoaerophilus* SR-09

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ABSTRACT

Research has been done on the physicochemical characterization of purified alkaline proteases were isolated from A. thermoaerophilus SR-09. Measurement activities of alkaline protease are determined by the method of Walker, enzyme protein content was determined by Lowry method. Results showed that the optimum enzyme alkaline protease is at a temperature of 70°C with a pH of 8.5. The enzyme is stable to heat and alkaline conditions, alkaline protease enzyme belonged to the serine protease and where casein as a substrate specific; the enzyme is stable against surfactants and oxidants.

Keywords : Physicochemical; Characterization; Alkaline protease; *Aneurinibacillus thermoaerophilus* SR-09

INTRODUCTION

Bacillus bacterial is a major concern in biotechnology because it is relatively easy for the isolation of various environments and able to grow in synthetic media [1]. *Aneurinibacillus thermoaerophilus* SR-09 was isolated from a Sumerup hot spring, Jambi province of Indonesia, produce crude alkaline protease enzyme with a specific activity of the highest of the other bacterial isolates and stable at high temperature and alkaline protease was indicated potentially be used as detergent additives [2]. Sixty percent of the industrial production worldwide enzyme is a protease and 25% of them are thermo stable, where the alkaline protease produced of *Bacillus* spp. which is widely used as detergent additives [3]. Alkaline protease having the active serine (serine protease) or the type of metal (metalloprotease) and active at alkaline pH, and has a very important commercial value [4]. Alkaline proteases contained in detergents serve to hydrolyze ingredients comprising proteins when washing [5]. Physicochemical characterization of alkaline proteases from *A. thermoaerophilus* SR-09 which is a local isolate is very important to know before the enzyme is applied and commercialized as an additive in the detergent industry.

EXPERIMENTAL SECTION

Sources of Alkaline Protease

Purified alkaline protease of *A. thermoaerophilus* SR-09 used for physio chemical characterization of the enzyme.

Determination of the Activity and Protein Content of Alkaline Protease

Measurement activities of alkaline protease are determined by the method of Walker [6] with solution of 5 mMol / L-tyrosine as standard and blank used distilled water. Determination of enzyme protein content was performed according by Lowry method [7], which as has been routinely done in the laboratory.

The Effect of Incubation Temperature on the Enzyme Activity

Testing the effect of incubation temperature on the enzyme activity is done by varying the incubation temperature on the enzyme activity test: 60, 65, 70, 75, 80, 85 and 90°C with a pH of 8.0 and time interactions E-S for 15 minutes.

The Effect of pH on the Activity of the Enzyme Substrate

Testing the effect of pH on the activity of the enzyme substrate is done by varying the pH of the substrate in the enzyme activity test: pH 8.0; 8.5; 9.0; 9.5; 10.0; 10.5 and 11.0 with optimum incubation temperature and time interaction E-S for 15 minutes.

Temperature Stability of Enzyme

Testing the stability of the enzyme to heat conducted by enzyme solution was incubated at 60°C for a certain time. Interval time 2 hours testing the activity of enzymes in the enzyme catalytic optimum conditions.

pH Stability of Enzyme

Testing the stability of the enzyme to alkaline pH conducted by enzyme solution was incubated in pH 8.5 at 60°C for a certain time. Interval time 2 hours testing the activity of enzymes in the enzyme catalytic optimum conditions.

Substrate Specificity of Enzymes

The substrate specificity of the enzyme to the substrate in the enzymatic reaction by varying the kind/type of substrate used in the enzyme activity assay. The substrate used in this test is casein, bovine serum albumin, albumin, and gelatin respectively at the rate of 1%. Furthermore, the specific enzyme activity tests [8].

Classification of Enzyme

Effect of inhibitors against the enzyme is done by the use of inhibitors such as phenyl methyl sulfonyl fluoride (PMSF), para-chloro mercury benzoate (pCMB), and ethylene diamine tetra acetic (EDTA on each substrate (pH optimum) with a concentration finally 10 mM. Then added a solution of enzyme and incubated at the optimum temperature for 15 minutes, so that the data obtained specific activity of alkaline protease [9].

Effect of Surfactant and Oxidant

Tween solution Tween-20, Tween -80, TritonX-100, CTAB, SDS and H_2O_2. At each level of 5%, each treatment included 1 ml in a test tube. Then pipetted 1 ml of an enzyme solution into the tubes and incubated at room temperature for 1 hour. Furthermore, from each treatment determined of the specific activity of the enzyme [10].

RESULTS AND DISCUSSION

The Effect of Incubation Temperature on the Enzyme Activity

Enzyme activity is at an incubation temperature range of 60 to 90°C, with optimum enzyme activity at 70°C (Figure 1). This indicates that the enzyme can work or have a catalytic power to hydrolyze proteins into peptides or amino acids in the temperature range of 60 to 90°C heat, where the catalytic hydrolysis enzyme alkaline protease optimum at 70°C.

Temperature plays an important role in the catalytic enzyme to hydrolyze a substrate, this is because the enzyme is composed of a variety of amino acids, where the nature or type of amino acids making up the enzyme greatly affects the catalytic activity as a result of the effects of incubation temperature in hydrolyze a substrate into a product.

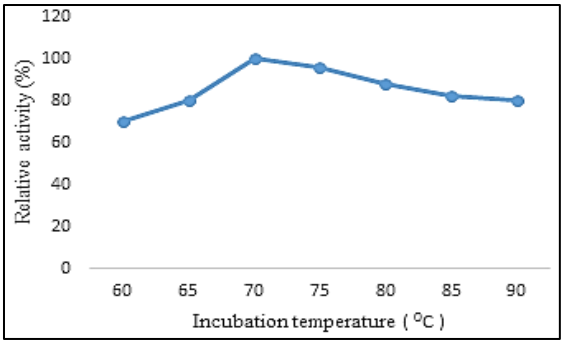

Figure 1: Effect incubation temperature on the enzyme activity

The Effect of pH on the Activity of the Enzyme Substrate

Enzyme activity on substrates of different pH, the substrate pH range between 8.0 to pH 11, the enzyme has its activity, this means that the enzyme works on alkaline or alkaline atmosphere (Figure 2). Based on the pH of the enzyme, the enzyme is included in a group of enzymes that are alkaline and enzyme activity optimum at pH 8.5. pH is a factor that greatly affects the catalytic action of enzyme, this is due to the protein in the form of amino acids making up the enzyme. pH is a crucial factor of enzymatic reactions, so that the pH can give the effect of the charge on the ion, or the nature of the amino acids forming enzyme in catalyzing a substrate into a product.

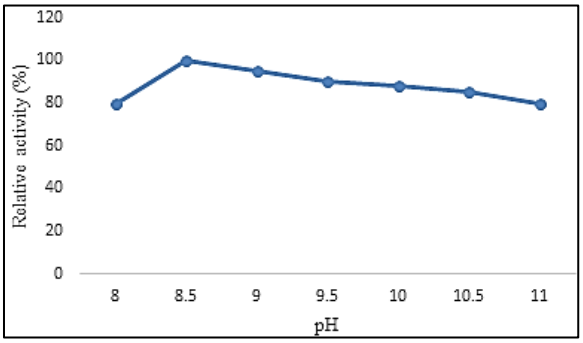

Figure 2: Effect substrate pH on the enzyme activity

Stability of Enzyme at Heat and Alkaline pH

Testing the stability of an enzyme to heat and alkaline pH is very important to do, in Figure 3 alkaline protease activity to heat for 50 hours.

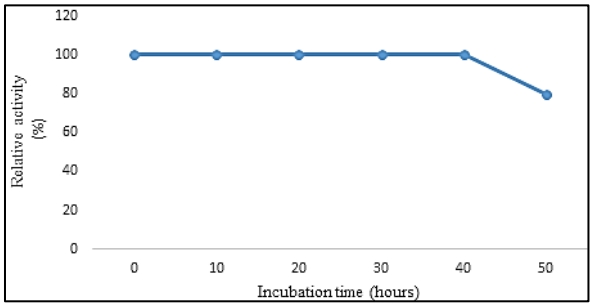

Figure 3: Enzyme activity during 50 hours at 60°C

The relative activity of the alkaline protease enzyme remains 100% to heating 60°C for 40 hours, this means that the catalytic power of enzymes remained stable despite the hot environmental conditions, so the alkaline protease is said to be thermo stable. Its resistance to heat enzyme, it is because these enzymes are built from hydrophobic amino acids, and amino acids that possible have a disulfide bond.

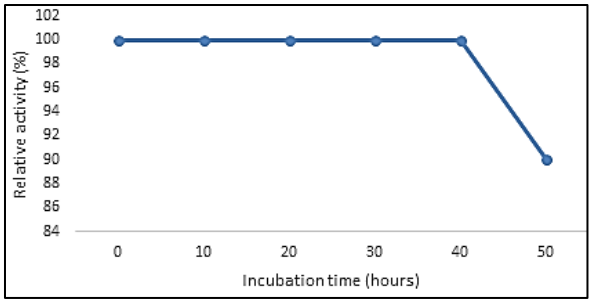

Figure 4: Enzyme activity during 50 hours at pH 8.5 and 60°C

Figure 4 showed the enzyme was stable at alkaline pH 8.5 condition during 40 hours, the activity remains 100%. Fifty hours incubation, the enzyme activity is remaining 90%. Conformation of the enzyme in the reaction enzyme plays an important role, the folding of the three-dimensional structure of the enzyme due to their chemical bonds between amino acids forming enzyme. Stabilized of the enzyme at high temperature and alkaline condition is indicated the enzyme to using as additive detergent.

Substrate Specificity of Enzymes

Alkaline protease produced by *A. thermoaerophilus* has a specific substrate that is casein, which is activity relatively enzyme is 100% (Table 1). Although the substrate bovine serum albumin (BSA), Albumin and Gelatin is a protein, but not a specific substrate for the alkaline protease, it is characterized by lower enzyme activity compared with casein substrate. This indicates that the alkaline protease enzyme can hydrolyze BSA, albumin, and gelatin, but non-specific. Protease has a high degree of hydrolytic activity on casein as a substrate and a low to moderate if hydrolyze BSA and albumin.

Table 1: The relative activity of the enzyme on different substrates

Substrate (1%)	Relative activity (%)
Casein	100
BSA	77
Albumin	50
Gelatin	30

Enzyme Classification

Classification is done by adding protease inhibitor in protease enzymatic reaction and presented in Table 2. The table shows that the addition of the inhibitor PMSF protease enzymatic reaction resulted in the lack of enzyme activity, is being marked by a specific protease activity of 0.0 U/mg. While the addition of inhibitors p-CMB and EDTA, the specific activity of the enzyme is similar to the treatment of enzymatic reactions without inhibitor. This suggests the enzyme inhibitor PMSF is perfectly whereas p-CMB chemical compounds and EDTA cannot inhibit the protease enzymatic reaction.

Table 2: Relative activity of enzyme at different inhibitor

Inhibitor	Relative Activity (%)
PMSF	0
p-CMB	96
EDTA	98
without inhibitor	100

Based on this, including the class of serine protease produced protease of the effect of pH on the activity of the enzyme is known that the enzyme alkaline, therefore protease produced by *A thermoaerophilus* including serine-alkaline group. It has been reported the alkaline protease from *Bacillus* including most types of groups of serine proteases [9]. Enzymatic reactions protease from *Bacillus clausii* I-52 greatly inhibited by PSMF, indicating a serine protease [11]. Inhibitor PMSF 5 mm 100% inhibited of alkaline protease from *Bacillus licheniformis* RP1, indicating alkaline proteases produced by bacteria that includes a group of serine proteases [12]. PMSF combination with amino acid residues in the catalytic serine protease resulted in the lack of enzyme activity, PMSF molecules will react with the OH groups of the amino acid serine thus causing an inhibitory effect irreversible [13].

Effects of Surfactant and Oxidant to Enzyme

The provision of anionic surfactant compounds, ionic surfactant and oxidant does not affect the activity of alkaline protease enzyme activity which is 90-100% (Table 3). SDS can give the effect of reducing the hydrophobic interactions that play an important role in maintaining the tertiary structure of the enzyme [14]. Alkaline protease from *Bacillus clausii* I-52 is stable against anionic surfactant compounds such as Triton X-100 and Tween, but also to the anionic surfactant like SDS which still have the enzyme activity of 97.5% (SDS 1%) and 72.6% (5% SDS) [11].

Table 3: Surfactant and oxidant effects to relative activity

Surfactant/Oxidant	Relative activity (%)
Control	100
Tween-20	100
Tween-80	98
TritonX-100	98
CTAB	95
SDS	95
H_2O_2	90

CONCLUSION

Optimum enzyme alkaline protease is at a temperature of 70°C with a pH of 8.5. The enzyme is stable to heat and alkaline conditions, Alkaline protease enzyme belonged to the serine protease and where casein as a substrate specific; the enzyme is stable against surfactants and oxidants.

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