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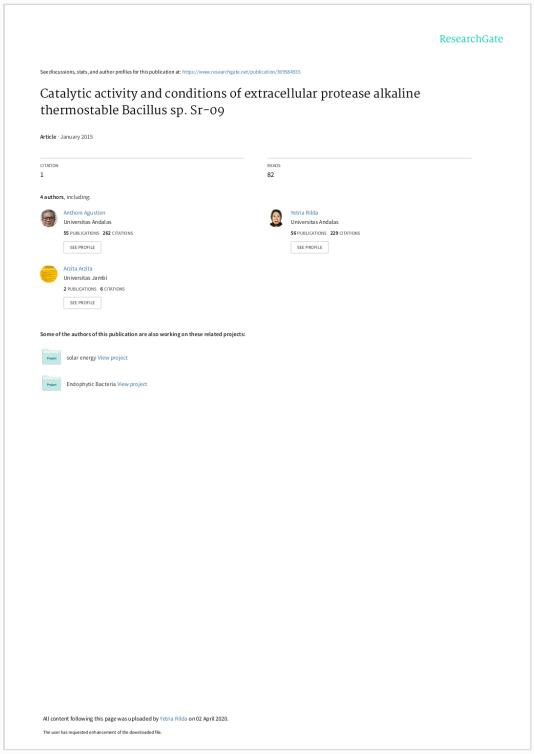

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Catalytic activity and conditions of extracellular protease alkaline thermostable *Bacillus* sp. SR-09

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ABSTRACT

Has done research on the catalytic activity and the condition of alkaline thermostable proteases were isolated from the thermophilic bacterium Bacillus sp. SR-09. Measurement activities crude extract of alkaline protease is determined by the method of Walker, an enzyme protein content was determined by Lowry method. Results showed that maximum alkaline protease produced at 18 hours of incubation. The optimum working conditions enzyme crude extract at a temperature of 70^0 C, pH 8.5 and substrate interaction time E-S for 15 minutes and the enzyme is stable in hot temperatures for 30 hours.

Keywords: catalytic; alkaline protease; thermostable; *Bacillus sp*. SR-09

INTRODUCTION

Alkaline protease (EC3.4.21 - 24, 99) is a protease active at neutral to alkaline pH interval, having the active serine (serine protease) or the type of metal (metalloprotease) and has a very important commercial value [1]. Sixty percent of the industrial production worldwide enzyme is a protease and 25% of them are thermostable. *Bacillus* spp. can produce alkaline protease which is widely used as detergent additives that have economic value [2]. Alkaline proteases contained in detergents serve to hydrolyze ingredients comprising proteins when washing [3]. 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 [4]. *Bacillus* sp. SR-09 was isolated from a Kerinci hot spring, produce thermostable alkaline protease enzyme with a specific activity of the highest of the other bacterial isolates and stable at high temperature and alkaline protease from *Bacillus* sp. SR-09 was indicated potentially be used as detergent additives [5]. Character alkaline thermostable proteases from *Bacillus* sp. 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. Based on the above description, it conducted research on catalytic activity and extracellular conditions thermostable alkaline protease from *Bacillus* sp. SR-09.

EXPERIMENTAL SECTION

A source of enzymes and proteolytic *Bacillus* sp. SR-09

The source of the enzyme in this study is *Bacillus* sp. SR-09 that has been available in the laboratory. Rejuvenation of bacteria performed as it has been routinely performed in microbiology laboratories. Single colonies of bacteria was inoculated on a petri dish containing medium Nutrient Agar pH 8.5 containing 1% casein, incubated at 60 °C for 24 hours. If formed clear zone around bacterial colonies indicated bacteria, positive produce of alkaline protease.

Production and isolation of protease alkaline

After the rejuvenation of the bacteria in the medium NA, conducted by making inoculum with bacterial inoculated into 50 ml of production medium with the composition: NaCl 0.5 g, K_2HPO_4 0.3 g; KH_2PO_4 0.3 g; $MgCl_2$. 6 H2O 0.1 g; casein 10 g and 1000 ml of distilled water at pH 8,5. Culture was incubated at a temperature of 50^0 C, agitation 150 rpm for 24 hours. A total of 5 ml inoculum inoculated in Erlenmeyer containing 95 ml of production medium. Then incubated at a temperature of 50^0 C, agitation 150 rpm for 24 hours. Bacterial cultures were centrifuged at 5000 rpm for 10 min at room temperature. Supernatant obtained a crude extract solution of alkaline protease. Assay of enzyme specific activity in the rough solution of alkaline protease.

Determination of the specific activity of alkaline protease

Protease activity is determined according to the method of Walter [6]. A total of 0.25 ml casein in 0.25 ml 0.05 M buffer pH 8.0 Tris.Cl be included in the test tube and in pre-incubation at 60^0 C for 5 minutes. Added 0.05 ml enzyme solution and incubated at 60^0 C for 15 minutes. The enzymatic reaction was stopped by addition of 1.25 ml of 10% TCA. The same procedure is done for a standard solution of 5 mmol / L-tyrosine and blank. The mixture was incubated at room temperature for 20 minutes, then centrifuged at 6000 ×g for 20 minutes. A total of 0.375 ml of the supernatant was transferred into a clean tube, then added 1.25 ml of 0.5 M Na_2CO_3 and 0.25 ml of 1 N Folin-Ciocalteu's reagent. Absorbance is read at a wavelength of 578 nm. Determination of enzyme protein content was performed according to the method of Lowry [7], which as has been routinely done in the laboratory. Specific activity enzyme (U/mg) = enzyme activity (U/ml) / protein concentration (mg/ml)

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: 50, 60, 70, 80 and 90 ^{0}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 and 10 with optimum incubation temperature and time interaction E-S for 15 minutes.

The effect of the interaction time E-S to the activity of the enzyme

Testing the effect of the interaction time ES enzyme activity is done by varying the time interaction E-S on the enzyme activity test for: 10, 15, 20 and 25 minutes with incubation temperature and pH optimum substrate.

Enzyme stability against heat

Testing the stability of the enzyme to heat conducted by crude enzyme solution was incubated at 60 ^{0}C for a certain time. Interval time 2 hours testing the activity of enzymes in the enzyme catalytic optimum conditions.

RESULTS AND DISCUSSION

The effect of incubation on the enzyme activity

Enzyme activity at different incubation temperature, pH of 8.0 and a fixed substrate interactions ES for 15 minutes is presented in Fig. 1.

Fig. 1 shows that the enzyme activity is at an incubation temperature range of 50 to 90 ^{0}C, with optimum enzyme activity at 70 ^{0}C. 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 50 to 90 ^{0}C heat, where the catalytic hydrolysis enzyme alkaline protease optimum at 70 ^{0}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.

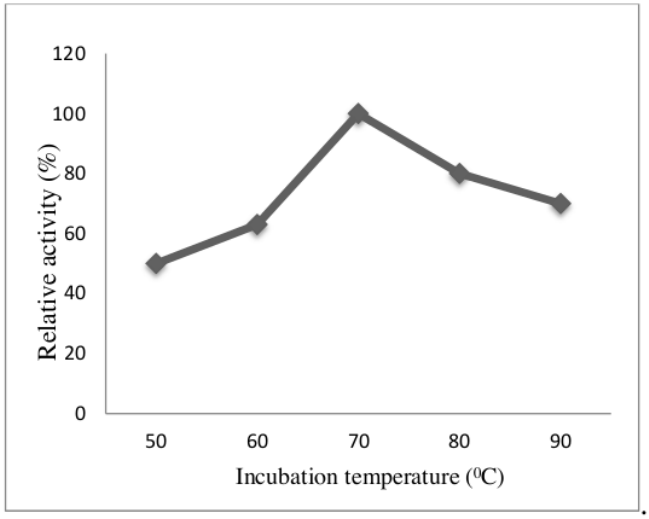

Fig. 1 Effect incubation temperature on the enzyme activity

The effect of pH on the activity of the enzyme substrate

Enzyme activity on substrates of different pH, temperature and time of incubation of 70 ^{0}C for 15 minutes E-S interactions are presented in Fig. 2. On the substrate pH range between 8.0 to pH 10, the enzyme has its activity, this means that the enzyme works on alkaline or alkaline atmosphere (Fig. 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 [8].

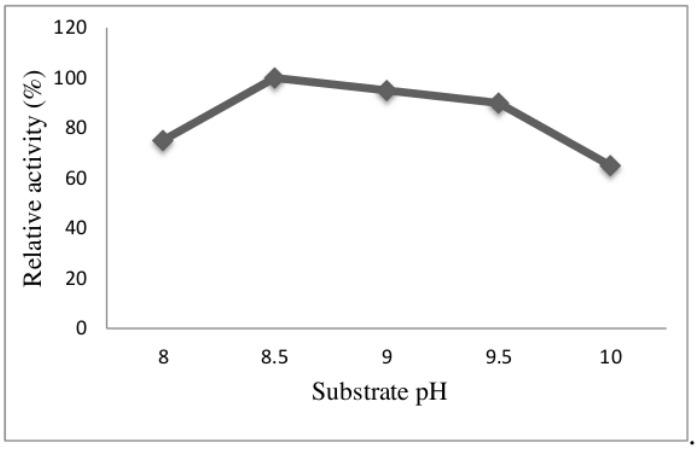

Fig. 2 Effect substrate pH on the enzyme activity

Effect of E-S interaction times on the an enzyme activity

Enzyme activity at different times ES interaction, incubation temperature of 70 ^{0}C and pH 8.5 substrate is presented in Fig. 3 and it 3 shows that the product is formed as a result of enzymatic reaction, starting 10 minutes contact the enzyme and the substrate. But time interaction optimum E-S is 15 minutes away, where the highest enzyme activity. Time interaction E-S on an enzyme is very important to know exactly, because it relates to the product formed per times.

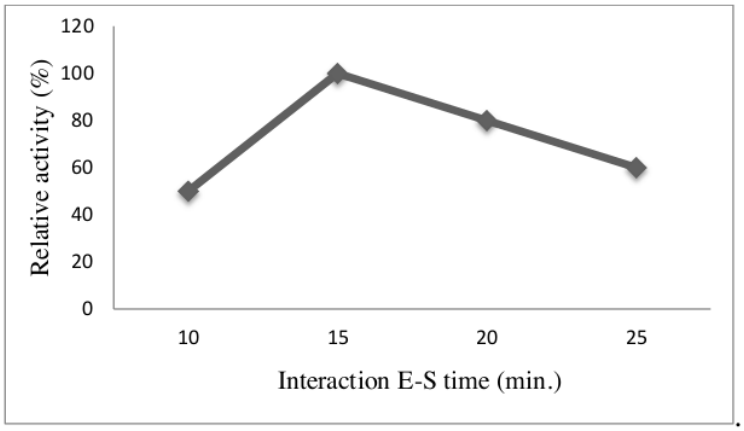

Fig. 3 Effect interaction E-S time on the an enzyme activity

Stability enzyme against heat

Testing the stability of an enzyme to heat is very important to do, in Fig. 4 alkaline protease activity to heat for 40 hours. The relative activity of the alkaline protease enzyme remains 100% to heating 60 ^{0}C for 30 hours, this means that the catalytic power of enzymes remained stable despite the hot environmental conditions, so the alkaline protease is said to be thermostable. 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. Conformation of the enzyme in the reaction enzymatic plays an important role, the folding of the three-dimensional structure of the enzyme due to their chemical bonds between amino acids forming enzyme.

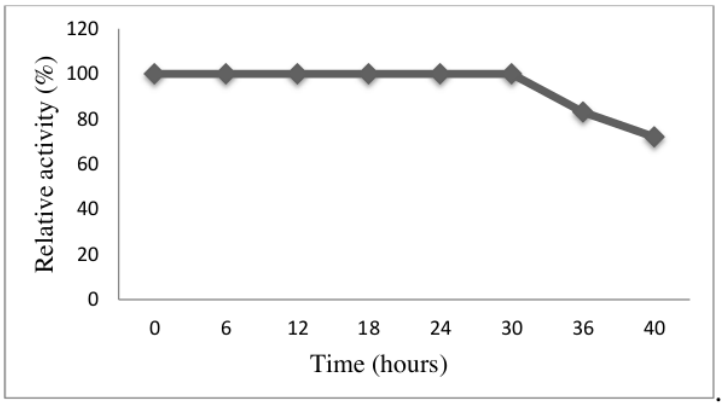

Fig. 4 Enzyme activity during 40 hour at 60 ^{0}C

CONCLUSION

From the research that has been done, it can be concluded that the optimum working conditions of the crude enzyme at a incubation temperature of 70^0 C, substrate pH 8.5 and interaction E-S time for 15 minutes and the enzyme is stable at 60^0 C for 30 hours.

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