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Potency of Amylase-producing Bacteria and Optimization Amylase Activities

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Abstract. Enzymes are capable to act as biocatalyst for a wide variety of chemical reactions. Amylase have potential biotechnological applications in a wide range of industrial processes and account for nearly 30% of the world's enzyme market. Amylase are extracellular enzymes that catalyze the hydrolysis of internal α-1,4-glycosidic linkages in starch to dextrin, and other small carbohydrate molecules constituted of glucose units. Although enzymes are produced from animal and plant sources, the microbial sources are generally the most suitable for commercial applications. Bacteria from hot springs is widely used as a source of various enzymes, such as amylase. But the amount of amylase-producing bacteria is still very limited. Therefore it is necessary to search sources of amylase-producing bacteria new, such as from hot springs Pariangan. The purpose of this study was to isolation of amylase-producing bacteria from Pariangan hot spring, West Sumatera and amylase activity optimization. The results were obtained 12 isolates of thermophilic bacteria and 5 isolates of amyalse-producing bacteria with the largest amylolytic index of 3.38 mm. The highest amylase activity was obtained at 50°C and pH 7.5.

1. Introduction

Thermophilic bacteria can be isolated from various places such as crater areas, volcanoes and hot springs. In the field of food industry, amylolytic enzymes play a role in the manufacture of glucose syrup, bread making, and baby food. In the field of non-food industry, amylolytic enzymes play a role in the paper industry, leather tanning, pharmaceuticals, textiles and as detergent additives. This industrial process requires an amylolytic enzyme that is resistant to high temperatures of about 70-80° C [4]. Amylase has been used in the field of biotechnology, in industrial processes the use of amylase already reaching 30% of the world's enzyme market [10]. Amylase is an extracellular enzyme that hydrolyzes the internal bonds of α-1,4-glycosidic starch into dextrin and other carbohydrate molecules of the glucose unit [5]. Amylases are generally produced by animals and plants, but amylases of thermophilic bacteria are more attractive to be used commercially.

Pariangan hot springs is one hot spring located in *Nagari Pariangan Tanah Datar* District of West Sumatera province, Indonesia. Temperature of *Pariangan* hot spring between 47-50°C and pH ranged between 8.4-9.0. This caused *Pariangan* hot springs has a high level of bacterial diversity. In addition, bacteria from high pH environments will generally produce stable enzymes that are high in pH, such as amylase. Stable amylase at high pH is very attractive for use in various industries, especially the detergent industry. This study aims to isolate amylase-producing bacteria from *Pariangan* hot spring, West Sumatera and optimization of amylase activities.

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2. Methodology

1. Isolation of Thermophile Bacteria

The hot water samples inside the bottle are shaken to homogeneous. Hot water samples are poured into a liquid NA medium and leave to freeze. Subsequently incubated at 50°C for 24 to 48 hours. Characteristics of the colony morphology observed include shape, edges, and elevation [3].

2. Purification of Thermophile Bacteria

Growing bacterial colonies were inoculated into NA medium that had been densely packed with quadrant methods. Subsequently incubated for 24 to 48 hours at a temperature of 50°C. Thus seen single growing colonies. Any pure isolate that can be grown is assumed to produce amylase on the starch-containing medium and each sample is repeated twice (duplo).

3. Selective Media of Thermophile Bacteria

Bacteria that have been successfully purified, then grown on a selective medium or medium for starch. Each Petri dish was placed in a different bacterial colony with one source of the same isolate then incubated for 24 hours at 50°C.

4. The Activity of Thermophilic Bacteria

Bacteria that successfully grow on selective medium then droping with iodine solution and waited a few minutes until really fused between iodine solution with starch so clear zone clear.

5. Isolation of Amylase

The amylase-producing isolates were grown into 25 ml basal medium (3 g/l K_2HPO_4, 3 g/l KH_2PO_4, 3 g/l $MgSO_4$, 5 g/ NaCl, and 10 g/l starch) with pH 7.5 and dishaker at 150 rpm at 50°C for 24 hours.
When the bacterial growth is subsequently removed 5 ml bacterial cultures into 100 ml basal medium and centrifuged at 150 rpm for 24 hours at 50°C. Bacterial culture formed centrifuged at 5000 rpm for 10 minutes. The supernatant containing the thermostable amylase extract was taken by micropipette and inserted into the microcentrifius tube for the activity test.

6. Amylase Activity Test

The amylase activity test was performed by incubating 0.5 ml of starch 1% for 5 min at 50°C and then adding 0.5 ml of amylase then incubated again for 1 hour at 50°C. To stop the hydrolysis process heating the boiling water (100°C) for 20 minutes. Then added 1 ml of Samogy Nelson solution then divortex and reheated to boiling water for 20 minutes. Further cooled in ice water and added 1 ml of arsenomoliblad solution then shaken then sufficient volume to 10 ml by adding 7 ml aquadest. Measure absorbance at wavelength 540 nm [8].

7. Determination of Ph Optimum Amylase

Amylase activities was tested with pH variation with 1% starch in buffer pottasium phosphate (pH 6.5-8.5).

8. Determination of Temperature Optimum Amylase

Optimum temperature for amylase activities was determined by incubating amylase at different temperature (45-60°C) using substrate with 1.5% concentration and amylase activities were tested.

3. Results and Discussion

1. Isolation of Thermophile Bacteria

The morphological characterization of thermophilic bacteria showed that the bacteria originating from Pariangan hot spring were cream colored, smooth shallows, and flat elevation (Table 1).

Table 1. Morphology characterization of termophile bacteria.

Location	Isolates code	Colour	Shape	Shallows	Elevation
S1 (Temperature 50°C, pH 8)	S1.1	Yellowness	Round	Smooth	Arise
	S1.2	Crem	Round	Smooth	Flat
	S1.4	Crem	Round	Smooth	Arise
S2 (Temperature 48°C, pH 9)	S2.1	Yellowness	Round	Smooth	Arise
	S2.2	Crem	Round	Smooth	Arise
	S2.3	Crem	Round	Smooth	Flat
	S2.4	Yellow	Round	Smooth	Flat
S3 (Temperature 47°C, pH 8)	S3.1	Yellowness	Round	Smooth	Flat
	S3.2	Crem	Round	Unorderd	Flat
	S3.3	Crem	Round	Smooth	Arise
	S3.4	Yellowness	Round	Smooth	Arise

The results of amylase activity tests obtained 4 isolates of amylase-producing bacteria from 11 isolates (Figure 1). The amylolytic enzyme activity test was conducted qualitatively by considering the measurement of the amylolytic index (Table 2). S1.2 isolate have amylolytic index is 3.83 mm, S2.3 isolate have amylolytic index is 1.91. S3.1 isolate have amylolytic index is 3,38, S3.2 isolate have amylolytic index is 2,42. The largest of amilolytic index was 3.83 mm that produced of S1.2 isolate and the lowest activity was 1.91 mm that produced of S2.3 isolate.

Table 2. Amylolytic Index of bacteria isolates

Location	Isolates code	Clear zone diameter (mm)	Colony diameter (mm)	Amylolytic indeks (mm)
S1 (Temperature 50°C, pH 8)	S1.1	-	-	-
	S1.2	1,61	0,42	3,83
	S1.4	-	-	-
S2 (Temperature 48°C, pH 9)	S2.1	-	-	-
	S2.2	-	-	-
	S2.3	1,17	0,61	1,91
	S2.4	-	-	-
S3 (Temperature 47°C, pH 8)	S3.1	1,59	0,47	3,38
	S3.2	1,02	0,42	2,42
	S3.3	-	-	-
	S3.4	-	-	-

11 isolates of thermophilic bacteria originating from Pariangan hot spring 7 isolates did not produce amylase activity. According to [11] iodine solutions do not provide colour with carbohydrate polymers of less than five monosaccharide groups, eg glucose. The media surrounding bacteria colonies that do not produce amylase will be blue when iodine solution drops. This shows that the starch in the medium is not degraded to simple sugars which means that bacteria do not produce amylase.

According to [2] the small diameter of the clear zone formed from each isolate is different. This is caused the ability to hydrolyze the starch of each isolate is different. The difference between clear zones is also due to differences in the amylolytic gene that each thermophile bacterium possesses. According to [9] the magnitude of the resulting clear zone depends on the amount of glucose monomer

produced from the process of starch hydrolysis. The larger the amount of glucose monomers produced the larger the clear zone formed around the colony.

S1.3 isolate S2.3 isolate S3.1 isolate S3.2 isolate

Figure 1. Thermophilic Bacteria Isolates

2. Amylase Activity Test from Thermophilic Bacteria

From the research that has been done, obtained the results of different amylase activity of four different bacterial isolates. The highest amylase activity was obtained by S2.3 isolate that is 66,68 U/ml, followed by S1.3 isolate that is 64,54 U/ml, S3.2 isolate that is 43,76 U/ml, and S3.1 isolate that is 37.04 U/ml (Figure 2). The highest activity isolate was selected for further research that is S2.3 isolate.

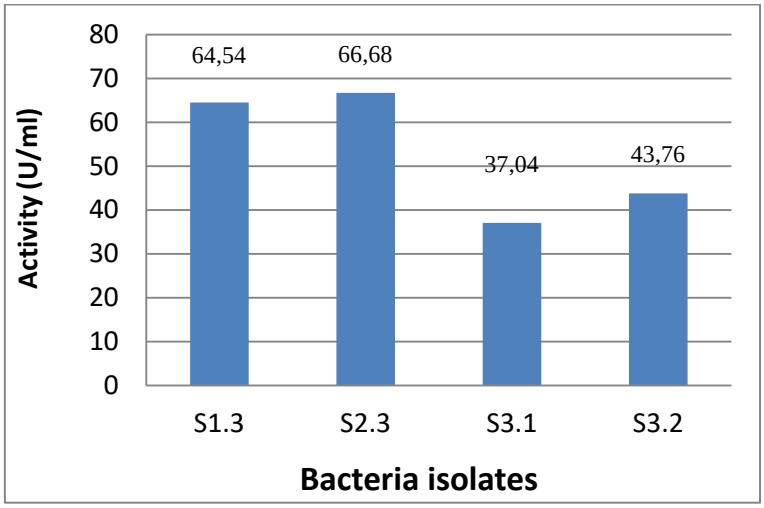

Figure 2. Amylase Activity Test of Four Bacterial Isolates

The presence of enzyme activity of different values each isolate caused by enzymes produced each type of different microorganisms will produce different enzymes of the amount and sequence of amino acids that form the enzyme. According to [1] that the specific activity of different enzymes from *Bacillus* sp. probably due to the amount of enzyme and amino acid enzyme protein produced by each isolate of *Bacillus* sp. different from each other.

a. pH Optimum

The highest amylase activity was obtained at pH 7.5 which was 81.9281,92 U/ml (Figure 3). According to Kristjonson (1999) the optimum pH of amylase-producing thermophilic bacteria lies between pH 6.0-9.0. The catalytic activity of enzymes within cells may be partially regulated by changes in the pH of the environment medium [7]

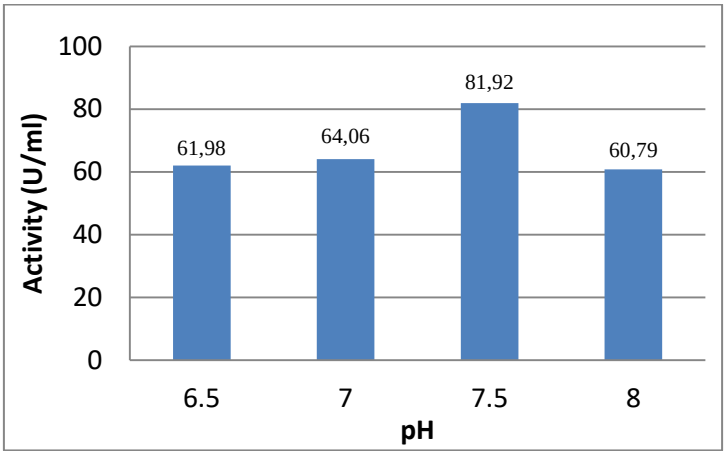

Figure 3. Effect of pH to Amylase Activity

b. Temperature optimum

The result of temperature optimization on amylase activity was obtained that the highest amylase activity was obtained at temperature 50°C (Figure 4).

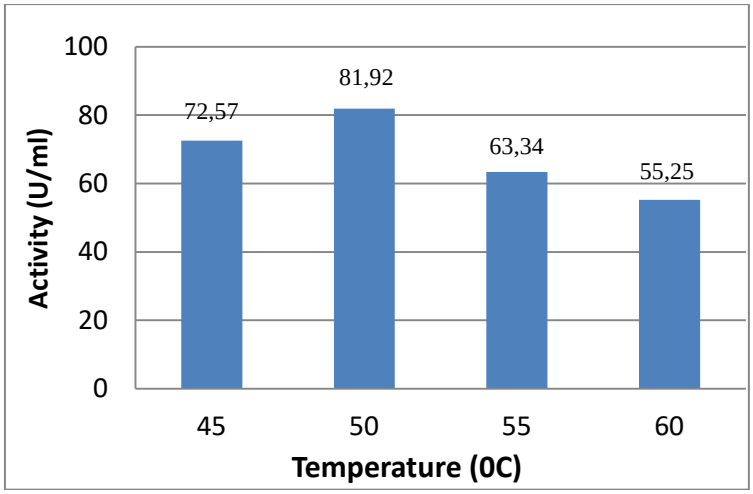

Figure 4. Effect Temperature to Amylase Activity

The highest activity was obtained at 50°C incubation temperature, because it was the temperature suitable for growth of thermophilic bacteria to produce amylase. According to [6] the optimum temperature of amylase-producing thermophilic bacteria lies between 40-70°C. The optimum temperature of amylase is usually always the same as the ambient temperature

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