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Effect of Temperature and Medium pH on Amylase Activity of SMG9 Thermophile Bacteria

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Abstract:- Thermo-alkaliphile bacteria derived from hot springs that have a pH above 8. Thermo-alkaliphile currently used as a source of various enzymes such as, lipase, protease, and amylase. Bacteria amylase is an enzyme that can hydrolyze starch into sugar. Amylase produced by thermo-alkaliphile bacteria role in industrial fields various, such as the leather tanning industry, biscuits, textiles, and as a detergent additive. In the detergent industry needed amylase resistant to temperature and high pH. Amylase needs in industry various increase up to 30% of the enzyme needs worldwide. Isolation of thermo-alkaliphile bacteria has been successfully carried out in a Semurup hot spring, Kerinci (Jambi) and obtained bacteria isolate thermo-alkaliphilel SMG9 with highest activity is 41.92 U/ml. The purpose of research is to determine the optimum temperature and pH of amylase activity through media optimization (temperature and pH). The results of the optimization of bacterial growth media temperature thermo-alkaliphile show that the highest amylase activity was obtained at 60°C. The results of the optimization of the bacterial growth medium pH thermo-alkaliphile shows that the highest amylase activity was obtained at pH 8.5.

Keywords:- *amylase, optimization, activity assay, and thermo-alkaliphilic bacteria.*

I. INTRODUCTION

The source of hot water is one of the habitats of microorganisms, such as archaea and bacteria. Bacteria can live in extreme environments (extremist), such as at 45°C-80°C (thermophile), above 100°C (hyperthermophiles), low temperature (psychrophile), high saline ((halophila), pH low (acidophile), high pH (alkaliphile). Bacteria-derived from hot springs (thermophile) are the source of various enzymes Enzymes derived from the thermophile bacteria are termed as thermophile enzymes because they are thermostable and thermo-activity [6] Thermophile enzymes, such as lipase, protease, and amylase are widely used in industry because it is stable at high temperature and pH. Amylases play a role in the textile, pharmaceutical, beer, sugar production, and detergent industries. [9] Amylase bacteria are enzymes that can hydrolyze starch into sugars [11]. The need for amylase continues to increase to 30% of all the world (8). Amylase derived from hot springs bacteria with high pH (thermo-alcaliphile) is needed as an additive in the detergent industry because the detergent industry requires stable enzymes at high temperature and pH. Generally, hot springs in Indonesia have neutral and acidic pH. Unlike the Semurup hot spring has a temperature of 80°C and pH 8.4 or alkaline. Semurup hot spring is the source of various amylase-producing thermo-alkaliphile bacteria. Isolation of amylase-producing thermo-alcaliphile bacteria was successfully performed and SMG9 isolates were obtained with the activity of 41.92 U/ml [11]. Therefore, it is necessary to increase amylase activity through media optimization (temperature and pH). Optimization of media to temperature and pH is done to determine the optimum condition of amylase activity.

II. METHODE

A. Temperature

Isolates of SMG9 thermo-alcaliphile bacteria were planted into 10 ml of liquid starch medium (3 g K2H2PO4, 3 g KH2PO4, 3 g MgSO4, 10 g NaCl and 10 g starch) with pH 7.5 and shaker at 150 rpm at varying temperatures (50°C-90°C) for 24 hours. When the bacterial growth is subsequently transferred 2, 5 ml bacterial culture into 50 ml of liquid starch medium and centrifuged at 150 rpm for 24 hours. Bacterial culture formed centrifuged at 5000 rpm for 10 minutes. The supernatant containing the thermostable amylase extract is taken with the micropipette and inserted into the microsentrifuge tube for the activity test [10].

B. pH medium

Isolates of SMG9 thermo-alcaliphile bacteria were implanted into 10 ml of liquid starch medium (3 g K2H2PO4, 3 g KH2PO4, 3 g MgSO4, 10 g NaCl and 10 g starch) with varying pH (pH 5.5- pH 9.0) and shaker with a speed of 150 rpm at the optimum temperature obtained for 24 hours. When the bacterial growth is subsequently transferred 2,5 ml bacterial culture into 50 ml of liquid starch medium and centrifuged at 150 rpm for 24 hours. Bacterial culture formed centrifuged at 5000 rpm for 10 minutes. The supernatant containing the thermos table amylase extract is taken with the micropipette and inserted into the microsentrifuge tube for the activity test [10].

C. Amylase Activity

The amylase activity test was performed by incubating 0.5 ml of starch solution 1% for 5 min and added 0.5 ml of amylase then incubated at 50°C for 1 hour. To stop the reaction is done warming on boiling water for 20 minutes. Plus 1 ml of Samogy Nelson solution, vortex and heated in boiling water for 20 minutes. Cooled in running water and added 1 ml arsenomolibdat solution then sufficient volume to 10 ml. Measure absorbance at 540 nm wavelength [14]. One enzyme unit is defined as the amount of enzyme needed to remove 1 µmol of sugar during the experiment.

III. RESULTS AND DISCUSSION

Bacterial growth is affected by temperature, changes in ambient temperature can cause changes in bacterial

morphology and physiology. Therefore every bacteria needs optimum temperature for growth and every bacteria has the optimum growth range and temperature. The result of temperature optimization (50°C-90°C) on growth media of amylase-producing thermo-alkaliphile bacteria showed an increase in amylase activity at 60°C and decreased amylase activity at 90°C (Figure 1). Amylase activity increases at 60° C because it is the temperature suitable for growth of thermo-alcaliphile bacteria for amylase production. Optimization of bacterial growth medium was conducted to determine the optimum temperature of amylase activity [7]. The growth media temperature plays an important role in bacterial growth and amylase production [1,3,13]. It has been reported that the temperature range of 35°C-80°C is the optimum temperature of growth and amylase production in bacteria [12]. Temperature optimization of growth media of Semurup thermo-alcaliphile bacteria isolate, Bacillus sp PA-05 [2] and bacterial isolate TPT-20 [4] resulted in amylase at 60°C.

The optimum result of the growth of amylase thermo-alcaliphile bacteria with varying pH (5.5-9.0) showed that pH 8.5 was optimum pH of enzyme activity with activity 39.27 U/ml. The lowest amylase activity was obtained at pH 5.5 with an activity of 8.92 U/ml (Figure 2). Each bacteria has a growth medium with the right pH because the pH of the media plays a role in morphological changes and enzyme secretion [5]. Each bacteria has an optimum growth media pH for growth and production of the enzyme with the highest activity. Bacillus generally has an optimum pH of 6.0-9.0 for growth and amylase production ([14] pH 7 is the optimum pH of amylase production in *Bacillus licherniformis* thermo-alcaliphile bacteria and *Bacillus subtilis* [6].

IV. CONCLUSION

The optimum temperature and pH of growth medium of thermo-alcaliphile bacteria showed that the highest amylase activity was obtained at 60°C and 8.5 pH.

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