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Isolation of Thermophilic Bacteria and Optimizing the Medium Growth Conditions

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A B S T R A C T

The thermophile bacteria that came from hot spring, generally, it is thermostable, which it can produce enzyme. For example, there are protease, lipase, and amylase. Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze starch to sugar. The thermostable of amylase resist to high temperature and pH. It has been used on many industrial productions like foods, fermentation, textile, alcohol, paper, pharmaceutical and detergent. Screening of thermo-amylase bacteria in a hot spring needs to be done because the hot springs got potential as a source of thermostable amylase producing bacteria. Thermostable amylase that produced by natural bacteria (*wild type*) usually has activity that is not too high. The aim of this study is to get thermophile bacteria that produced thermostable amylase and increased the activity of thermostable amylase through optimization the condition of growth medium of thermophile bacteria. The result of this study is found 16 thermophile isolate bacteria and 3 thermophile bacteria that produced amylase. Thermostable amylase activity was high by using the agitation 125 rpm, wheat flour as the substrate at a concentration of 2% and amylase stability for 2 hours at 40-50°C. Thermostable amylase produced SMG9 of bacterial isolates that can be used in various industries.

Introduction

Thermophile bacteria lives at 45°C–80°C and produce enzyme which are thermostable. Enzyme that came from thermophile bacteria also known as thermophile enzyme because of thermostable and thermo-active (Fooladi and Sajjadian, 2012). The thermophile enzyme, like protease, lipase and amylase that are thermostable happened because those werere sistant to high temperature and pH. Thermostable amylase had been used in so many industrial areas, foods, fermentations, textiles, alcohols, papers, pharmaceuticals and detergents (Mageswari *et al.*, 2012). Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze starch to sugar (Megahati *et al.*, 2017). Thermostable amylase that had been used in industry was 30% from all enzymes around the world (Munoz *et al.*, 2011).

Today, the number of bacteria that can produce thermostable amylase is still limited and the screening for this is still less information especially when its sources came

from a hot spring. Indonesia has so many sources of hot spring likes, Rimbo Panti hot spring, Ciater hot spring, Sibiru-biru hot spring and Semurup hot spring. Semurup hot spring was located in Kerinci District, Jambi Province, Indonesia. It had 75°C temperature and pH 8.4. All this time, hot spring of Semurup had not been used yet as source of bacteria producing thermostable amylase.

Thermostable amylase that was produced by nature bacteria (*wild-type*), like on a hot spring, usually have no high activity. Because of it, it needs isolation of thermophile bacteria that can produce thermostable amylase and optimization of growth medium thermophile bacteria. This study has aim to get thermophile bacteria that produced thermostable amylase and increased thermostable amylase activity through optimization growth medium conditions of thermophile bacteria likes the different of agitation speed, carbon sources, source carbon concentration, and amylase stability test.

Materials and Methods

The sample collection and medium

Isolate of bacteria SMG9 was earned at temperature 75°C from Semurup hot spring, Kerinci District, Jambi Province, Indonesia. Medium growth used in this study is Nutrient agar (20 g/l)**,** substrate agar 1%, and medium of production. All mediums were sterilized by autoclave at 121°C for 15 minutes.

Isolation and screening of bacteria

Hot spring sample 250 ml is get into the bottle sample and then labeled. Next, it brings into laboratory and then 1 ml of sample spread into Nutrient agar (20 g/l) medium. After that, it is incubated at temperature 50°C for 24 hours. Thermophile bacteria is growing planted into medium of selective starch agar 1% (10 g/l starch and 15 g/l bacto agar) and incubated at temperature 50°C for 24 hours. Bacteria that was grown on selective medium was dripped with iodine solution. Amylase activity is showed with the creation of clear zone on the selective medium. Bacteria with the widest clear zone was used to the further research.

Enzyme isolation

The thermophile bacteria isolation that has had the widest clear zone then planted into 50 ml medium of production (0.75 g KH_2PO_4, 0.75 g K_2HPO_4, 1.25 g $MgSO_4$, 1.25 g NaCl, 2.5 g starch) with pH 8.5 and shaked with 150 rpm for 24 hours at temperature 50°C. Next, the culture as much 10% moved into 100 ml new medium of production with pH 8.5 and shaker (150 rpm) for 24 hours at temperature 60°C. The bacterial that growth inside the culturing and then centrifuged with 10.000 speed for five minutes. The supernatant moved into new micro-centrifuged tube for amylase test (Teodoro and Martin, 2010).

Amylase testing

The 0.5 ml substrate 1% blended with Pottasium phosphate buffer with pH 7.0 is incubated at temperature 50°C for 5 minutes and added with 0.5 ml thermostable amylase. Then, it is incubated at temperature 50°C for one hour. Enzyme activity was stopped by making heat on substrate-thermostable amylase with boiling the water for 20 minutes.

Then, adding 1 ml Samogyi-Nelson solution (Nelson, 1944). The solution was cooled down on ice for one minute, then adding1 ml Arsenomolibdat solution. Next, it was blended with vortex machine and measured its absorbance on wave length 540 nm. One unite enzyme was definite as numbers of enzyme that release one µmol sugar per minute from the substrate for 60 minutes at temperature 50°C.

The effect of different speed agitation

The medium of production that had been inoculated with thermophile bacteria was shacked with different speed of agitation (100-200 rpm) at temperature 60°C for 24 hours. 10% inoculum moved into new 100 ml medium of production and then it was shacked with other different speed of agitation (100-200 rpm) for 24 hours. Culturing bacteria was centrifuged with speed 10.000 rpm for five minutes. The supernatant, produce during this process, was moved into new micro-centrifuge tube for amylase test.

The effect of different carbon source

So many source of carbon were added into production medium thermophile bacteria likes potatoes flour, rice flour, sago flour, wheat flour, and corns flour each with 1% concentration. Medium was shake with speed 100 rpm (optimized result) on temperature 60°C for 24 hours. 10% of culturing was moved into new medium production and then it was shacked with agitation speed 100 rpm for 24 hours. The culturing bacterial was centrifuged with speed 10.000 rpm for 5 minutes. The supernatant that produced during it was isolated and moved into new micro-centrifuged tube for amylase test.

The effect of different carbon source concentration

Isolate of thermophile bacteria was planted into 50 ml medium production with pH 8.5 and using wheat flour as a carbon source (optimization result). Different concentration of wheat flour was used (1-5%) and shacked with speed 100 rpm at temperature 60°C for 24 hours. Culturing bacteria around 10% was moved into 100 ml new medium production and shacked with speed 100 rpm for 24 hours. Culturing bacteria was centrifuged with speed 10.000 rpm for five minutes. The supernatant that contain extract of amylase was took with micropipette and got into micro-centrifuged tube for amylase test.

Amylase stability test

Amylase was got from optimization result condition of growth medium of thermophile bacteria then tested for its stability. Amylase stability was identified using incubation of amylase at temperature 40-90°C for two hours in the water bath.

Results and Discussion

Isolation and screening bacteria

Isolation of thermophile bacteria had been done at temperature 75°C on hot spring Semurup, Kerinci, Jambi province, Indonesia. The result was found 16 isolate of thermophile bacteria and 3 isolates of amylase producer (SMG9, SMG10, dan SMG11) with the creation of clear zone at around bacteria growth (Figure 1). The clear zone showed that starch was found inside medium has been hydrolyzed with amylase. Another study had also been succeeded to isolate the thermophile bacteria producer of amylase on hot spring in Myanmar and got 4 isolate bacteria producer of amylase (Win *et al.*, 2015). Even though on the hot spring at Saudi Arabia, it was found 3 isolates degrading of starch (Khalil, 2011) and 6 isolates of hot spring Odishi, India (Kumar, 2014). Screening of thermophile bacteria that produce amylase needs to be done on the hot spring for exploring the natural resource and getting new bacteria that produce amylase.

The effect of different agitation speed

Agitation speed had affected on thermophile bacteria growth SMG9 and thermostable amylase production (Figure 2). Agitation speed around 125 rpm can increase thermophile bacteria growing SMG9 and

thermostable amylase production. Agitation speed at under or up to 125 rpm can make decreasing thermostable amylase production. It is different with agitation speed on medium growth *Bacillus licheniformis* BT5.9 where the agitation speed 100 rpm can increase growth of thermophile bacteria and amylase production (Ibrahim *et al.,* 2013). The optimization production of amylase of *Bacillus licheniformis* AH214 was found with agitation speed 160 rpm (Nabey and Farag, 2016). In the meantime, the *Bacillus licheniformis* ATCC6346 had produce amylase with agitation speed 100 rpm (Vengadaramana *et al.,* 2014). Agitation speed is benefit for good mixing through out the fermentation which ensuring sufficient oxygen transfer in aerobic culture, and consequenting improves the cell growth and metabolite synthesis. But too high agitation speed results in intensive shear forces, and in turn causes damage to cell structure and decrease in the yield of secondary metabolite (Gao &Wen-Ying 2007).

The effect of different of carbon source

Production and activity of amylase of thermophile bacteria SMG9 was increase using wheat flour as carbon source then the others (Figure 3). Biosynthesis of the enzyme was took place not only in the presence of starch but also with other carbon sources (Deb *et al.,* 2013). Production and activity of amylase is increase using tapioca flour as carbon source on medium growth of *Bacillus* sp (Sreekanth *et al.,* 2013). On the *Bacillus tequilensis* RG-01, amylase production was increased using wheat bran as carbon source (Tiwari *et al.,* 2014). It is different on *Bacillus subtilis* BI19 where it was using of rice flour as carbon source can stimulate amylase production. Natural carbon source can be used by bacterial as energy source to produced amylase and it can be get with low price (Dash *et al.,* 2015). The different of carbon sources have varied influence on the production of extracellular enzymes especially amylase (Rao and Sathyanarayana 2003).

Fig.1 The clear zone of isolate of thermophile bacteria

Fig.2 The effect of different speed agitation on amylase production

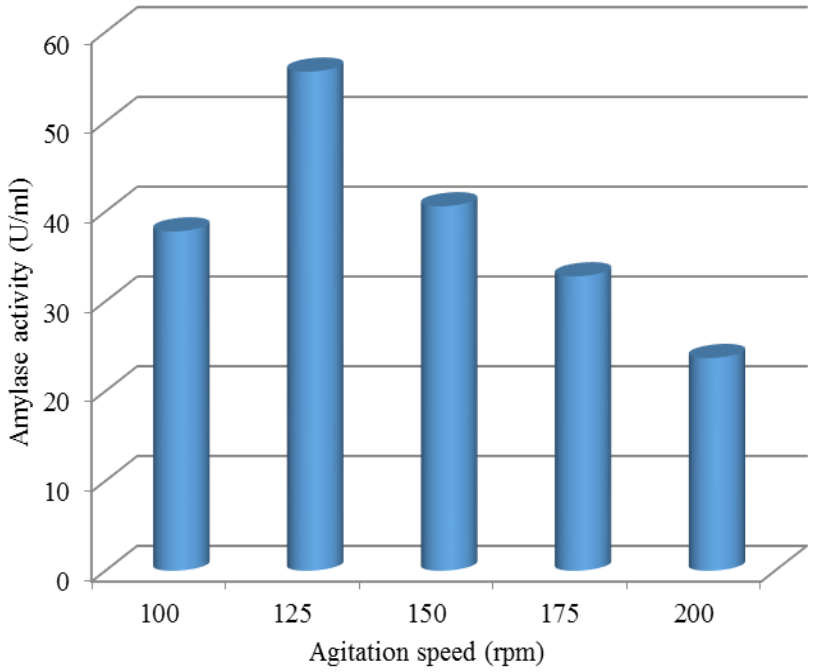


Fig.3 The effect of different carbon sources on amylase production

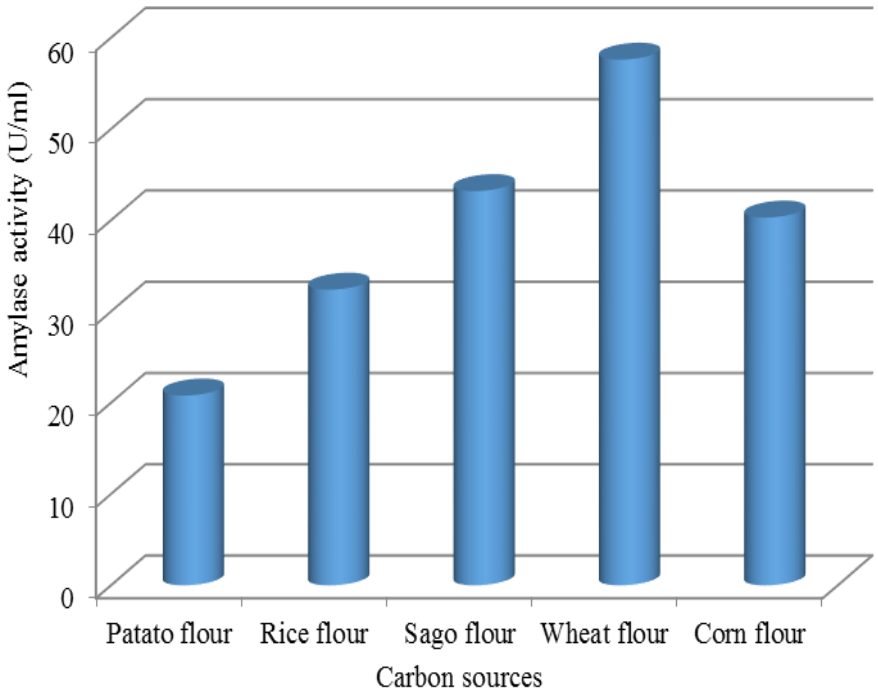


Fig.4 The effect of concentration variation of wheat flour

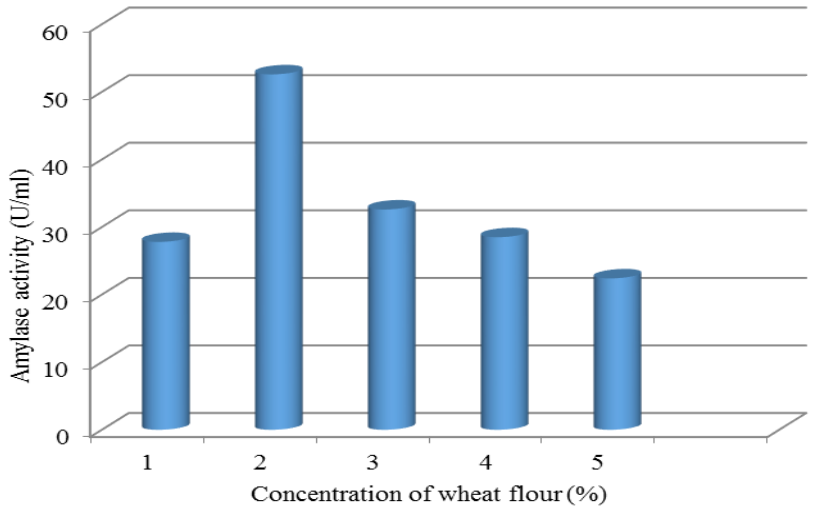
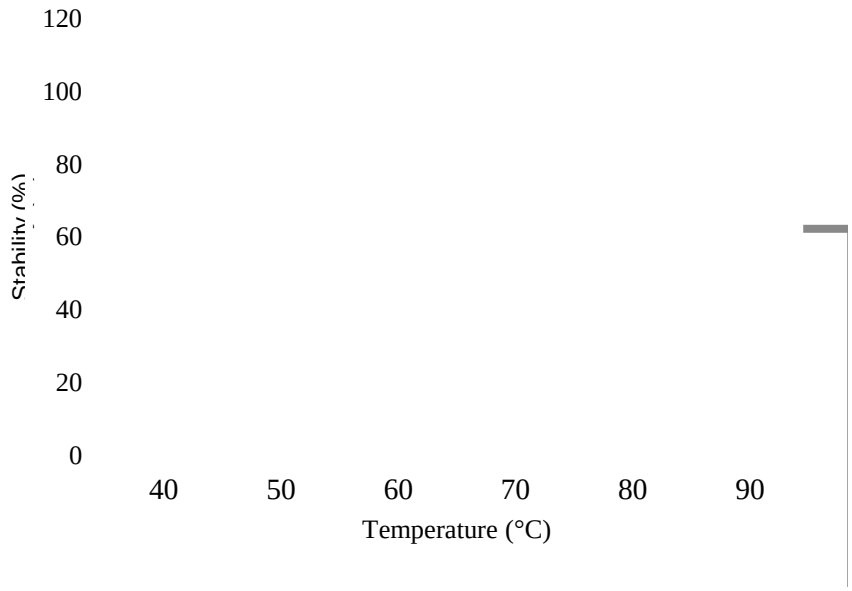


Fig.5 Amylase stability



The effect of variation of carbon source concentration

Concentration of wheat flour is very effecting on amylase activity that produced by thermophile bacteria. High or low concentration of wheat flour can increase amylase production (Figure 4). The result in this study showed that concentration of wheat flour 2% can increase amylase activity. Starch concentration 2% can increase amylase activity of *Cronobacter sakazakii* Jor52 (Samantha *et al.*, 2013). It is different with *Pseudomonas mendocina* where starch concentration 5% can increase amylase activity (Padhiar and Kommu, 2012). The increasing carbon source concentration can also increase amylase activity until a certain level. Too high of carbon source concentration can

make the increasing of medium viscosity until it can disturb transferring O_2 and limiting dissolved oxygen for bacterial growth (Rukhaiyar *et al.,* 1995).

Amylase stability

Generally, some industries need thermostable amylase like amylase to optimize the result of medium growth condition of isolate bacterial (Figure 5). On the Figure 5, it looks that amylase stable for two hours at temperature 40-50°C. In the other hands, at temperature 60-90°C amylase loses its stability. Amylase of *Bacillus* sp strain SMIA-2 also stable for two hour at temperature 40-50°C (Cordeiro *et al.,* 2002). It is different with amylase of *Geobacillus thermoleovorans* strain Rekadwadsis was stable for one hour at temperature 90°C (Rekadwad *et al.,* 2015). Amylase of isolate bacterial PW13, PW11, and PS4 were stable for four hours at temperature 100°C (Sharma *et al.,* 2015). Amylase stability was influenced by pH and temperature. The stable form of amylase is in polypeptide chain covalently bound and fold in the form of three dimentions with its specific pattern. The specific pattern of enzyme results in specific biological activity.

Thermostable amylase activity was high by using the agitation 125 rpm, wheat flour as the substrate at a concentration of 2% and amylase stability for 2 hours at 40-50°C. Thermostable amylase produced SMG9 bacterial isolates can be used in various industries.

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