The Isolation of Termofil Amilolitik Bacterium and Activity Test of Harsh Extract Amylase Enzyme From The Hot Spring at Jailolo Bay in North Maluku

THE ISOLATION of TERMOFIL AMILOLITIK BACTERIUM and ACTIVITY TEST of HARSH EXTRACT AMYLASE ENZYME from THE HOT SPRING at JAILOLO BAY in NORTH MALUKU

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Abstract: *Amylase is a hydrolase group enzyme used in many industrial areas which operate on high temperature. Amylase can be yielded from various organisms. The most common producer of Amylase is Bacterium. Bacterium can live in many extreme environment like hot temperature environment. One of the sources of hot water is Jailolo Bay in North Maluku, on that account it requires to conduct the isolation of thermophile amolytic bacteria from the hot water source of Jailolo Bay in North Maluku Moluccas North and test its amylase activity. Water sample used in this research was taken come from the hot water source with temperature of 75*℃ *and 6,1 water pH. This research was done by isolating the bacteria, by packing 1 mL of the water sample into an add-on medium. Then 1 mL microbe culture was disseminated to a jelly medium and incubated for two days at the temperature of 40* ℃*, 50* ℃ *and 60* ℃*. The resulting Bacterium colony was then processed qualitatively into the amolytic bacterium test in the jelly medium containing 1% sago extract with Iodium. Isolates yielding wide, transparent zone in each incubation temperature were identified. Identifying the bacteria was conducted with colony morphology test, biochemical test, and* the *coloration of Gram. The α- amylase enzyme activity test was relied on the amount of reduction sugar (maltose) which was produced by the extract hydrolysis with DNS method using maltose as standard. The result of the research shows that the source of hot water in Jailolo Bay, North Maluku has potency to yield thermophile amolytic bacteria. The amount of isolates bacteria obtained was 68 isolates from the temperatures of 40*℃*, 50*℃ *and 60*℃ *each resulting 36, 18 and 14 isolates bacteria. Three chosen bacterium isolates (big transparent zone) from each incubation temperature are isolates 51Y222 (40* ^{0}*C), isolates 52B23 (50*℃*) and 52YA (60*℃*) which owns each transparent zone of 9,11 cm; 9,13 cm and 9,20 cm. The microscopic characterization result of Gram coloration indicates that those three isolates are included as the positive Gram bacteria and in form of bacillus. Based on the colony morphology observation macroscopically, microscopically and according to the biochemical test result, isolates 51Y222, 52B23 and 52YA represent bacterium of Bacillus sp. Amolytic enzyme activity of* $51Y2_{22}$*,* $52B2_3$ *and 52YA is each 157,75 mU / mL; 124,07 mU / mL and 1315,61 mU / mL*

Keyword: *amylase enzyme activity, amolytic bacteria, bacterium isolation, bacterium identification*

1. Introduction

Source of hot water represents one of the environment place to live for some organisms which hold up to such temperatures, like thermophile bacteria. Indonesia has several regions containing the source of hot water. One of the place is the source of hot water in Jailolo Bay, North Maluku, with 75 ℃ and 6,1 pH. Around the source of hot water of Jailolo Bay are trees of mangrove. Leaves and fruits of mangrove which fall at the hot spring provide nutrition for the microbes, therefore microbes yielding thermophile enzymes live.

Microbes from common hot water locations generally produce thermostable and thermophile enzymes which are enthused by many enzyme-based on industries. Because industrial process using high temperatures can minimize contamination risks, improve to accelerate the mass transfer, and can shift balance up at forming products [1]. Thermostable enzymes can be yielded by thermophile microbes growing in high temperature places like the hot spring of Lejja, South Sulawesi. There, with temperatures of 40 ^{0}C until 65 ^{0}C bacteria of Bacillus RSAII1B sterothermophillus were found. Enzymes from those bacteria have stability at temperatures of 60ºC and 5,0 pH 5,0 for 3 hours [2]. Thermophile bacteria can live at temperatures above 45ºC and optimally at 55ºC - 65ºC. Because of the ability to live at high temperature environment, this microbe exceeds from other microbes [3].

The first step of the exploration studies about these thermophile bacteria is to identify the bacterium. Identifying the bacterium conventionally is by perceiving the characteristics of its nature of biochemistry, macroscopic and microscopically. One of the ways in identifying bacteria, especially those which produce amylase enzymes is marked with the formation of transparent zone around the bacterium colony in solid medium containing 1% substrate extract after dripped with 0,02 M Iodium, bacterium isolates with high amolytic activity have big transparent zone (4,5).

One hydrolase enzyme group which is sourced from thermophile bacteria and has wide application in so many industrial areas like food, environment and health is -amylase. -amylase enzyme is the enzyme group that has the ability to break the knot of glycoside in the extract and represents industrial enzyme which can contribute to about 30% of enzyme production in the world (6).

Amylase enzyme used in textile industry in Bandung amounts to not less than 4 ton per month or around 2-3 US million dollar per month and all of them are imported, so that the development of biotechnology science especially optimal enzyme production in Indonesia is highly required. One type of the amylase is -amylase [7]. -amylase is one commercial enzyme which is active extract substrates and is very important in the field of biotechnology nowadays. Amylase enzyme produced from microbes in generally enthused by many industries. Spectrum usage of the amylase enzyme is widely used in various areas like medical, analysis chemistry, textile industry, distillation and food industry [8]. Therefore, though -amylase enzyme have been isolated and crystallized, the exploration for more efficient of -amylase source is still required. Thereby in this research, thermophile amolytic bacterium from hot water of Jailolo Bay, North Maluku was conducted.

2. Methods

2.1 Sample Intake and Preparation

Water sample as the source of bacterium isolates was taken from the hot spring of Jailolo Bay, North Maluku. Before the sample was taken, water temperature and the pH were measured at the environment. Each parameter was measured by plunging a grader into some hot water for about 3 minutes. The sample was taken at the coastal part of Jailolo Bay. Then, the water sample was put into a sterile bottle, and labelled afterwards. The water Sample obtained consisted of seawater mixed with freshwater which exists close to the coastal periphery. The obtained sample had the temperature of 75ºC; and 6,1 pH.

2.2 The Isolation of Thermophile Amolytic Bacterium

The water sample was taken from the hot spring in North Maluku which has temperature of 75oC and 6,1 pH. As much as 1 mL sample was enriched in 25 mL of a sterile add-on medium and was incubated at the temperature of 55ºC for 24 hours in a 200 rpm shaker. After that, 1 mL of microbe culture was disseminated into the jelly medium and then was incubated for 24 hours at the temperature of 40ºC, 50ºC, and 60ºC. Bacterium colony which grows and possesses different colony morphology character, each was taken one ose and then was incised into the selective amolytic medium and was also incubated for 24 hours at the temperatures of 40ºC, 50ºC, and 60ºC. The grown colony was then incised by the quadrant until it resulted pure isolates. The pure Isolates then was taken one ose of sample and

poured in the selective amolytic medium and finally incubated within 24 and 48 hours at the temperatures of 40ºC, 50ºC, and 60ºC (Method modification by Arfah et al., 2014) [5].

Bacterium Isolates that grow were then dripped with iodine condensation (2% I_2 and 0,2% KI), if there is transparent zone around the colony, it is indicated as the thermophile bacterium isolates which produce amylase enzymes (amolytic bacterium) due to the extract hydrolysis ability around the colony. Later, the pure bacterium isolates which have transparent zone were re-grown at selective amolytic medium, to determine its amolytic index, and the transparent zone diameter was measured by using calipers. Before each isolate examination, the tissue stock was prepared in the tilted jelly medium. It requires to be done because the iodine test causes the bacterium isolates to die due to the disinfectant characters of iodine condensation (Method modification by Arfah et al) [5]. Thermophile bacterium isolates with big transparent zone diameter and have good growth regularity was then identified for its bacteria.

2.3 Identifying the Thermophile Amolytic Bacterium Isolates

Isolates with big transparent zone was identified. The identification was conducted by both morphology (micro and macroscopy) and through biochemical test. Morphology observation macroscopically from every isolate with wide transparent zone includes colony form and was seen from the surface and the side, the colony edge, colony color, cell coloration, cell form, and cell settlement were all observed the from above. While morphology observation microscopically used microscope and coloration test of Gram bacterium. The Gram Coloration started when the bacterium isolates were put on a preparat glass and then added with NaCl physiology. The preparat was dried in a room temperature continued with hot fixation. After that, violet crystal was added and left to dry for 1 minute. The preparat glass was then rinsed with flowing water, and some lugol liquid was added and left to dry within 30 seconds, and re-rinsed with flowing water. Afterwards, the preparat was enhanced with alcohol, cooled down for 2 seconds and re-rinsed with flowing water again. Then, safranin solution was added, left to dry for 30 seconds and rinsed with flowing water, and finally the preparat was dried. Once dried, the preparat was dripped with immersion oil and then observed with a microscope [5].

Biochemical test includes tests of simon citric jelly (SCA), catalase, triple sugar iron jelly (TSIA), gelatinase, motility, *methyl red* (MR), *voges proskauer* (VP), and carbohydrate fermentation (glucose, lactose, sucrose, maltose) [5].

2.4 Isolation of α-Amylase Enzyme from Thermophile Bacterium Isolates with Wide Transparent Zone.

Amylase enzyme production was done based upon Arfah et al method [9]. Thermophile bacterium Isolates which age 24 hours were taken as much as 1 ose and put into a 250 mL erlenmeyer containing 100 mL inoculum medium mL composed by: yeast extract 0,2%, pepton bakto 1%, NaCl 0,005%, $MgSO_4.7H_2O$ 0,05%, $CaCl_2$ 0,08% and extract 1,5%. After that, the inoculum medium containing bacterium tissue was shaken in an incubator shaker with 200 rpm agitation and temperature of 55 for 24 hours. Then, the active inoculum was taken 10% and poured into a 250 mL erlenmeyer containing 100 mL liquid medium (production medium with the same composition as inoculum medium), afterwards, it was shaken again in a 200 rpm shaker with temperature of 55ºC for 33 hours. Then, it was centrifuged at 3500 rpm, with the temperature of 4ºC for 30 minutes to separate bacterium cell and filtrates. Filtrates which are harsh extract extracellular amylase enzyme (crude enzyme) were later tested for the enzyme activity using the DNS method and protein rate test of Lowry.

2.5 Determination of α-Amylase Enzyme Activity Quantitatively

The principle of -amylase enzyme activity test relied on the amount of reduction sugar (maltose) produced by the extract hydrolysis using DNS method [10]. Mixture of 0,5 mL harsh extract condensation of -amylase enzyme, 0,5 mL phosphate natrium buffer with 7,0 pH and 0,5 mL substrate (sago extract) 2% was incubated at 55ºC temperature for 60 minutes. Afterwards the mixture was added with 1,5 mL DNS reactor, and shaken with vortex for 10 seconds. After that, the mixture was heated in

boiled water for 10 minutes, and cooled in ice water. The result of the reaction was measured for its absorbance at the maximum wavelength. Maltose rate resulted from extract hydrolysis by -amylase enzyme can be calculated by using calibration curve of the standard maltose condensation at around 80-600 ppm. Calculation of the maltose rate was conducted by substituting condensation absorbance obtained from the determination of maltose rate process into the regression equation of the calibration curve from the standard maltose solution. The Maltose rate obtained, was then used to determine enzyme activity by using the calculation formula of -amylase enzyme activity in the equation 1as follows;

$$AE\ (U/mL) = \frac{[Maltose] \times Fp}{BM \times V \times t} \quad \ldots\ldots\ldots (1)$$

Information:
AE = Enzyme Activity (U / ml)
[Maltose] = maltose rate (ppm)
Fp = dilution factor
BM = molecular maltose weight
V = used enzyme volume (mL)
t = incubation time (minute)

2.6 Determination of Protein Rate

Determination of protein rate was based on Lowry method [11] using bovine serum albumin (BSA) as standard solution. Mixture of 1 mL enzyme solution, 5 mL Lowry B reactor, was shaken with vortex for 10 seconds. Later, the mixture was left at room temperature for 10 - 15 minutes, then 1 mL *folinciocalteu* (Lowry A) reactor was added and shaken with vortex for 10 seconds. Afterwards the mixture was left again at room temperature for 30 minutes in order to perfect the reaction, and finally added with 3 mL of distilled water. The concentration of standard protein solution was at around 0,05-1,0 mg /mL and the distilled water as blank. The absorbance was measured by using spectrophotometer at the maximum wavelength (- 645 nm).

The calculation of protein rate in the enzyme was done by substituting the solution absorbance that resulted from the determination of enzyme protein rate into the regression equation of calibration curve standard protein solution. Protein enzyme rate obtained, was then used to determine specific enzyme activity by using the calculation formula of specific activity in the equation 2 as follows;

$$AS\ (U/mg\ protein) = \frac{[Maltose] \times Fp}{BM \times V \times t \times P} \quad \ldots\ldots\ldots (2)$$

Information
AS = Specific Activity (U / protein mg)
[Maltose = maltose rate (ppm)
Fp = dilution factor
BM = molecular maltose weight
V = used enzyme volume (mL)
t = incubation time (minute)
P = total enzyme rate (mg / mL)

3. Result and Disscusions

3.1 Isolation of Thermophile Amolytic Bacterium

The result of thermophile amolytic bacterium isolation obtained from the water sample of the hot spring in Jailolo Bay, North Maluku, was grown with varied temperatures of 40ºC, 50ºC and 60ºC, yielding 68 thermophile bacterium isolates. From those 68 bacterium isolates, there were 36 isolates which grew at the temperature of 40ºC, 18 isolates at the temperature 50ºC and 14 isolates at 60ºC. The Isolates could grow and hydrolize 1% sago extract at 7,0 pH for 24-48 hours, with different hydrolysis ability. Amolytic bacterium was marked by the formation of transparent zone in the bacterium isolates containing 1% sago extract substrate after being dripped with 0,1 M Iodium. The isolates have different

ability when hydrolizing the extract as substrates. This can be seen from the different transparent zone from each isolate. Isolates with high amolytic activity have bigger transparent zone as seen at Table 1;

Table 1. The diameter of big transparent zone from thermophile amolytic bacterium isolates with temperatures at 40ºC, 50ºC and 60ºC and 7,0 pH with incubation time of 24 and 48 hours.

Temperature (ºC)	Isolate Code	Colony Size (cm)		Transparent zone size (cm)
		24 hours	48 hours	
40	51Y222	9,11	9,11	9,11
50	52B23	9,13	9,13	9,13
60	52YA	7,35	7,82	9,20

Bacterium Isolates grown [at] temperatures 40ºC, 50ºC and 60ºC produced isolates with different amount. The amount of bacterium isolates which grow designated that these bacteria can live at such temperatures. The bacterium Isolates represent thermophile bacteria which are capable to live at enough high temperature.

According to Arfah [2], thermophile bacteria can grow in temperatures start from 40oC. The amount of isolates at temperature 40ºC is quite a lot while at temperature 50ºC and 60ºC resulted smaller amount. This happened because thermophile bacteria can live in temperatures start from 40ºC and possibly there are other bacteria which can still live at that temperature. Of all bacterium Isolates, three were selected among the others from the transparent zone with the highest temperature, those were isolate $51Y2_{22}$, $52B2_3$ and 52YA (Picture 1). They each possess transparent zone equals to 9,11 cm, 9,13 cm and 9,20 cm (Table 1). Bacterium colony grown was dripped with Iodium to know bacterium ability in hydrolyzing extract to be glucose or maltose. Detecting was done by observing the formation of transparent zone around the bacterium colony which grew. Bacterium with amolytic activity was marked by the forming of transparent zones (hydrolysis zone) around the bacterium colony in the jelly medium containing 1,0 % starch substrate. The isolates have different ability when hydrolyzing starch as its substrate. Isolates with high amolytic activity have wide transparent zone as seen in Picture 1.

Picture 1. Three chosen isolates based upon the big and regular transparent zone diameter after 48 hours incubation at temperature 40ºC, 50ºC and 60ºC are bacterium isolate $51Y2_{22}$; $52B2_3$ and 52YA

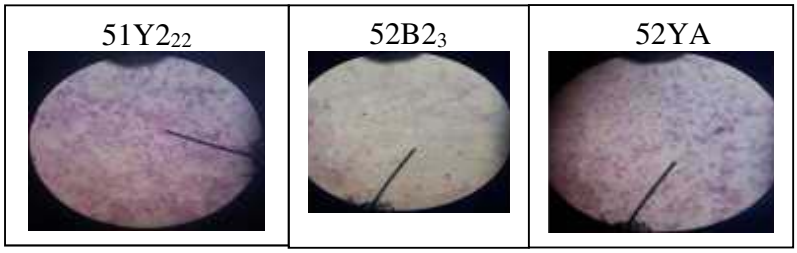
In the picture 1, it is seen that those three isolates have big transparent zone. The existence of transparent zone after addition of iodine designate that thermophile bacterium has the ability to produce amylase enzyme that can hydrolyze starch to be glucose, and the bigger the transparent zone, the bigger amylase enzyme activity as well.

Transparent zone formed from each isolate represents that extract can be hydrolyzed by amylase enzyme. Extract or starch reacted with iodine and turned the color into dark blue. If -amylase enzyme extract is hydrolyzed to be glucose and maltose, this blue color is not formed after the addition of iodine.

The amolytic ability of microbes is marked with the formation of clear zone in the medium containing the extract. The isolation of thermophile amolytic Bacterium of the hot spring sample from Jailolo Bay, North Maluku produced three bacterium isolates ($51Y2_{22}$, $52B2_3$ and 52YA) that have wide transparent zone. Those isolates were identified in a biochemical and morphology test.

3.2 Identification of Thermophile Amolytic Bacterium Isolates

Isolate $51Y2_{22}$, $52B2_3$ and 52YA were the three thermophile bacterium isolates chosen to be identified according to its morphology, and biochemical physiology. The observation result of morphology was conducted through microscope, the bacterium isolate after the Gram coloration was presented at Picture 2. As for the result of morphology examination of the isolates can be seen at Table 2.



Picture 2. Gram coloration of bacterium isolate 52YA, $51Y2_{22}$, and $52B2_3$ with magnification of 1000x

Picture 2 represents the result of Gram coloration of isolate 52YA, $51Y2_{22}$, and $52B2_3$, those isolates have the characters of positive gram bacterium marked with the formation of purple color. Positive Gram bacterium is a bacterium which the cell wall absorbs violet color and has thick *peptidoglikan* layer. The purple color was formed because bacterium cell wall bound the violet crystal color substance. The microscopic observation result of those three isolates showed the shape of bars (*bacillus*).

Table 2. Comparison of morphology characteristic (microscopy and macroscopy) for the thermophile bacterium isolate $51Y2_{22}$, $52B2_3$ and 52YA with *Bacillus sp* genus of $RSAII_{1B}$ isolate, producing -amylase isolate (Arfah et al., 2014)

Test	**Observation Result**			*Bacillus sp.* **$RSII_{1B}$ isolate producing α-amylase**
	52YA	**$51Y2_{22}$**	**$52B2_3$**	
Colony color	cream	cream	cream	cream
Colony shape	Bar with spore	Bar with spore	Bar with spore	Bar with spore
Colony Edge	wavy	wavy	wavy	wavy
Gram Color	Purple	Purple	Purple	Purple
Gram	positive	+	+	+ basil
Colony Surface	Bunched up	spread	Bunched up	spread

Based upon the Table 2, isolate $51Y2_{22}$, $52B2_3$ and 52YA possess morphological similarity with Bacillus sp. RSII1B isolate which had been conducted by Arfah et al [5] and represents a positive Gram bacterium. This shows that thermophile bacterium isolate is grouped in the genus of Bacillus. Similar research was done by Dirmawan et al [12] where they succeeded to isolate 11 thermophile bacteria

producing amylase from the hot spring of Pancar Mountain, Bogor, and 9 isolates with the character of positive gram bacterium displayed the characteristics of Bacillus genus.

Biochemical Test (Table 3) showed that bacterium isolate $51Y2_{22}$, $52B2_3$ and 52YA had similar biochemical test result. Negative citrate test was marked with the absence of color change in the medium, in which it was still green. The catalase test was positive due to the addition of 3% H_2O_2 solution in the medium formed some air bubbles around the colony. The catalase enzyme can elaborate H_2O_2 to be H_2O with O_2 [2]. The TSIA Test (Triple Sugar Iron Agar) was negative because of the color change in the TSIA medium from dark brown into orange or yellow. The bacterium Isolate did not have the character of motile (motility test), and it was marked with the inexistence of bacterium movement in the medium.

The H_2S Test was negative because there was not any formation of black color and no incision marks. Gas forming test was also negative, marked by no formation of cavity beneath the jelly. Methyl Red Test (MR) however showed positive result because some reddish purple ring shape was formed in the medium. Voges Proskauer Test (VP) was again negative because of no formation of such reddish purple ring shape. Carbohydrate fermentation test covering the glucose, sucrose, and maltose test showed positive result due to the color change in the medium from green to yellow after being incubated for 24 hours, while lactose fermentation resulted negatively because the medium color remained green. Based upon the results, it indicates that these bacterium isolates have the ability to use glucose, sucrose, and maltose as the source of carbon.

This biochemical test is one method to determine bacterium genus. Data in the Table 3 indicates that there were mostly same test result equals to RSII1B isolate which had been conducted by Arfah [5], but some tests which resulted differently such as tests of TSIA, motility, lactose, and sucrose. The differences were caused by the differences of bacterium species. Thermophile bacterium Isolates are possibly different species from RSII1B isolates that was conducted by Arfah et al [5], in which represents the bacterium of *Bacillus stearothermophillus*. Therefore, the thermophile bacterium classification only reached the genus level that is Bacillus sp. and further tests phytochemical and molecular PCP 16S RNA test are needed to determine the species.

Table 3. The comparison of physiology and biochemistry characteristic of thermophile bacterium isolates $51Y2_{22}$, and 52YA with the genus of Bacillus sp. RSII1B producing -amylase isolate (5).

Observation	**Results**			*Bacillus sp.*
	$51Y2_{22}$	**$52B2_3$**	**52YA**	**$RSII_{1B}$ isolate**
SCA Test	Negative	Negative	Negative	Negative
Catalase Test	Positive	Positive	Positive	Positive
TSIA Test	Positive	Negative	Negative	Negative
Motility Test	Positive	Negative	Negative	Positive
Sulfide Test	Negative	Negative	Negative	Negative
Indol Test	Negative	Negative	Negative	Negative
Gas Test	Negative	Negative	Negative	Negative
MR Test	Positive	Positive	Positive	Positive
VP Test	Negative	Negative	Negative	Negative
Urea Test	Negative	Negative	Negative	Negative
Glucose Test	Positive	Positive	Positive	Positive
Lactose Test	Negative	Positive	Positive	Positive
Sucrose Test	Negative	Positive	Positive	Positive
Maltose Test	Positive	Positive	Positive	Positive

Extract Hydrolysis	Positive	Positive	Positive	Positive

Based upon the result of analysis in Table 2 and 3, after reconciled with key identification and referred to Bergey's Manual of Determinative Bacteriology' [13], hence thermophile bacterium isolates $51Y2_{22}$, $52B2_3$ and 52YA have similar morphology and biochemical characteristics with Bacillus sp. RSII1B isolate, resulted by Arfah et al [5]. Although they have many similarities in terms of morphology, physiology, and ecology, Bacillus is highly varied in term of biomolecular (12).

3.3 Determination of α-Amylase Enzyme Activity and Protein Rate

Bacterium isolates that have the biggest transparent zone from each incubation temperature that are $51Y2_{22}$, $52B2_3$ and 52YA were analyzed further for the determination of -amylase enzyme activity. The bacterium isolates were inoculated in the production medium and incubated for 33 hours, then they were centrifuged with 3500 rpm speed for 30 minutes, the produced supernatan represents harsh extract of -amylase enzyme (Arfah's method, 2016) [2]. The enzyme was then analyzed for the activity in hydrolyzing extract to be maltose and finally the protein rate was determined. Activity enzyme and protein rate data can be seen in Table 4.

Tables 4. -amylase enzyme activity and protein rate from three chosen isolates

No.	Thermophile bacterium isolates	Protein rate (mg/mL)	Enzyme activity (mU/mL)	Specific activity (mU/mg protein)
1.	51Y222	1,32	157,75	119,50
2.	52B23	0,77	124,07	161,11
3.	52YA	1,72	1315,61	762,22

Data in the Table 4 indicates that the -amylase production in the medium containing 1% sago extract and incubated for 33 hours resulted in harsh extracts of -amylase enzyme with activity equals to 157,75 mU / mL for isolate $51Y2_{22}$, 124,07 mU /mL for isolat $52B2_3$ and 1315,61 mU /mL for isolate 52YA. Transparent zone and enzyme activity data shows that -amylase isolated from bacterium isolate 52YA possess bigger transparent zone and enzyme activity compared to -amylase of bacterium isolate $51Y2_{22}$ and $52B2_3$. The value of enzyme activity and transparent zona was directly proportional. This indicates that the bacterium isolate has greater ability at extract hydrolysis so that redactor sugar like maltose, glucose can be yielded more. Result data of protein rate measurement that was obtained from isolate $51Y2_{22}$, $52B2_3$ and 52YA each as much as 1,32 mU /mL; 0,77 mU /mL, and 1,72 mU /mL.

Data Table 2 displays that protein rate of isolate 52YA is bigger than those resulted by isolates $52B2_3$ and $51Y2_{22}$. This proves that possibly there are other proteins in the isolate which cause high protein rate as well as the protein has not been purified yet. Isolate 52YA also has bigger specific activity than isolate RSII1B resulted in Arfah et al [2] in Lejja hot spring in South Sulawesi that was 370 mU /mg protein.

4. Conclusion

Based on the research result, it can be concluded that the hot spring water from Jailolo Bay, North Maluku has potency to yield thermophile amolytic bacteria. The amount of bacterium isolates obtained is 68 isolates in the temperature 40ºC, 50ºC and 60ºC each represents 36 isolates, 18 isolates and 14 bacterium isolates. Three chosen bacterium isolates (with big transparent zone) from each incubation

temperature are isolate $51Y2_{22}$ (40ºC), isolate $52B2_3$ (50ºC) and 52YA (60ºC) with each transparent zone resulted 9,11 cm; 9,13 cm and 9,20 cm. The characterization result microscopically of Gram coloration indicates that three isolates include positive Gram Bacterium and in form of bacillus. Based upon the colony morphology observation macroscopically, microscopically, and biochemically, it resulted that isolate $51Y2_{22}$, $52B2_3$ and 52YA are bacterium of Bacillus sp. Amolytic enzyme activity of isolate $51Y2_{22}$, isolate $52B2_3$ and isolate 52YA each consecutively 157,75 mU / mL; 124,07 mU / mL and 1315,61 mU / mL.

5. Acknowledgment

We are gratefully thankful for the Ministry of Research, Higher Education and Technology and Universitas Khairun for the research grant with number contract of 003/PEN-PT/PL 2019.

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