Biosaintifika 10 (2) (2018) 291-297

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Isolation of Thermophilic Bacteria from Bora Hot Springs in Central Sulawesi

✉Slamet Ifandi[1], Muh. Alwi[2]

DOI: http://dx.doi.org/10.15294/biosaintifika.v10i2.14905

[1]Departement of Biology, Faculty of Mathematics and Natural Sciences, Universitas Billfath, Indonesia
[2]Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tadulako, Indonesia

History Article

Received 5 April 2018
Approved 12 June 2018
Published 30 August 2018

Keywords

Isolation; Thermophilic bacteria; Bora Hot Springs

Abstract

Thermophilic bacteria can survive at high temperature, in which hot spring is one of its habitats. Indonesia has many hot springs with potential as a habitat for thermophilic bacteria. The purpose of this study was to obtain isolates thermophilic bacteria from Bora hotspring located in Central Sulawesi. This study applied a descriptive-observational study design, characteristics of bacterial properties identified using conventional methods according to the Bergey's Manual of Determinative Bacteriology. The study was conducted in 3 stages. The first stage was bacteria cultivation on the appropriate media, followed by stage of isolated and the last stage by identified characteristics of thermophilic bacteria which included microscopic and macroscopic morphology, Physiological and biochemistry test. The results of the isolates indicted 4 representative isolated of thermophilic bacteria from Bora Hot Spring namely TM022, TM023, TM024, TM026. The bacteria isolates obtained were bacillus, coccus and Gram negative and positive, while the physiological test of all isolates were able to grow and showed changes in the medium. This study is useful in providing characteristic of indigenous thermophilic bacteria isolates that produces thermostable enzymes.

How to Cite

Ifandi, S., & Alwi, M. (2018). Isolation of Thermophilic Bacteria from Bora Hot Springs in Central Sulawesi. *Biosaintifika: Journal of Biology & Biology Education*, 10(2), 291-297.

✉ Correspondence Author:
Siman, Sekaran, Lamongan Regency, East Java 62261
E-mail: slamet.ifandi90@gmail.com

p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Indonesia is a country with much geothermal activity and high biodiversity. Indonesia also has the largest geothermal resources in the world, with as many as 252 locations spread over 26 Provinces. The large number of hydrothermal areas, waste composting, petroleum drilling wells and natural gas shows potentials that can be empowered and applied in the development of science. Even studies in the last two decades revealed that 99% of the bacteria present in the environment have not been yet explored for biotechnology applications (Panda *et al.*, 2013; Wijayati *et al.*, 2014).

Thermophilic bacteria are microorganisms that live in extreme condition up to a temperature of 121°C. Groups of thermophilic microorganisms also produce the most enzymes compared to animals and plants.

This enzyme is widely used because of its intrinsic-thermostability and its resistance to changes in physical and chemical factors. Currently, there are only 2 types of thermophilic bacteria enzyme most widely used as a biocatalyst which are derived from the group *Thermusaquaticus* and *alkaliphiles* is sellulase 103 (Khalil, 2011). In addition, protease enzymes derived from thermophilic bacteria are also widely used for bio-industrial food, pharmaceutical and various biological applications because of its stable properties such as are resistant to various solvents and acidic - alkaline pH (Mohammad *et al,* 2017).

Research regarding thermophilic bacteria is getting wider attention. Some local thermophilic bacteria isolates have been successfully isolated from a number of places. Isolated microorganisms in Indonesia including thermophilic bacteria from Pacet Hot Spring (8 genus including *Thermus*sp, *Acetogenium*sp, *Bacillus*sp, *Thermotrix*sp, *Thermodesulfo bacterium* sp, *Thermomicobrium*sp, *Pseudomonas*sp and *Sulfobacillus*sp) (Asnawi, 2006). A study conducted by Maria and Surya, (2012) succeeded in identifying 2 genera namely *Bacillus*sp and *Vibrio* sp.

A study related to thermophilic bacteria from Bora Hot Springs has previously been conducted by Khoridah, (2015) and Hardi *et al.* (2016), which successfully isolated B1211 bacteria which has potential use for the production of chitinolytic and chitinases enzymes. The highest activity of chitinase enzyme from B1211 bacteria isolate, is 0.75 U/mL obtained with colloidal chitin 1% concentration and temperature 60°C.

Isolation, characterization and identification are the first steps of exploration of indigenous microorganisms conducted in one region (Mahmudah *et al,* 2016). This study used conventional exploration method through macroscopic observation, microscopic, physiology test and biochemical test.

The following study determines genus identification and optimization of bacterial growth. Determination of genus of bacterial isolate in this study was in accordance with *Bergey's Manual of Determinative Bacteriology* with microscopic observation through several tests such as morphological test. Some of these bacterial identification include colony morphology (size, shape, color) and macroscopic morphology as well as breeding.

The geothermal springs located in the Bora Sigi Biromaru have an average temperature of 45-70°C. Currently the area is used as tourist destination by surrounding resident Knowing the role of thermophilic microorganisms as a thermostable enzyme producer is necessary to explore the potential of thermophilic bacteria by by isolating, characterizing and identifying. Study on the isolation, characterization and identification of thermophilic bacteria had never been done and was considered as novelty in this study. The study provides benefits by giving information on the thermophilic bacteria diversity of thermostable enzyme producers from the hot springs.

METHODS

Bora is a highland area. Almost 75% of the area has a dry and rocky soil. Bora village is geographically located at an altitude of 1700 meters above sea level, with temperature ranging from 27-30°C. Bora Village is one of local tourist destination due to its beautiful scenery and a hot spring that attract locals residents as far as Palu Municipality. Based on the result of measurement of environmental parameters, the average temperature of the hot spring is around 50°C, with pH reaches 6.8 - 8.5 and salinity level about 2.2 ppm (Bora Village Profile, 2011).

This study was conducted in Basic Biology Laboratory and Biotech Laboratory, Faculty of Mathematics, University of Tadulako, Palu. The tools used during study including microscope, autoclave, oven, incubator, tube rack, laminar air flow, test tube, erlenmeyer, petri dish, hot plate, measuring cup, digital balance, pipette, ose needle and loop, sample bottle, cover glass, and an identification key book "Bergey's Manual of Determinative Bacteriology "(Madigan *et al.*, 2003) Brock Biology of Microorganism.

This study applied descriptive observatio-

nal method; all data obtained according to the phenotype of representative thermophilic bacterial isolates during macroscopic and microscopic observations and were used for conventional identification by using Bergey's Manual of Determinative Bacteriology identification key book. The data collected in this study consisted of primary data and secondary data.

Sampling and media preparation

Hot water samples were taken using sterilized sample bottles. Praparation of Organic Gaus Agar (OGA) medium. The composition consisted of medium agar 3gr, D-glucose 2.5 gr, 1.25 gr of NaCl, Peptone 1.25 gr, Triptone 3.75 gr, 250 ml Aquades with pH 6.8-7.1. Preparation of Thermus Medium Modified (TMM) medium. Composition consists of MgSO47H2O 0.0025 gr, KNO3 0.0250 gr, (NH4) 2 SO4 0.0875 gr, NaCl 0.0250 gr, Yeast extract 0.0250 gr, Peptone 0.0250 gr, Triptone 3.75 gr, medium Agar 3 gr, Pati 0.0750 gr, Aquadest 250 ml, pH 6.8-7.1. All the ingredients were put into 250 ml glasses and 100 ml aquades were added, then heated and sterilized into the autoclave for 15 minutes at 121 ° C.

Isolation and Selection of Thermophilic Bacteria

Isolation of bacteria was performed using spread method. The water sample contained in the bottle was homogenized, then taken 1 ml using pipette into petri dish containing selective medium *Organic Gaus Agar* (OGA) and *Thermus Medium Modified* (TMM). The medium allowed to be transported at the temperature of 50 ° C for 48 hours. Therefore, it would resulted to representative bacteria. The growing bacteria were inoculated back into sterile petridish containing OGA and TMM mediums using quadrant method, then incubated at 50 ° C for 24-48 hours.

Characterization of Thermophilic Bacteria

Characterization of thermophilic bacteria was done through macroscopic observation to the colony formed, colony edge, elevation, texture and pigmentation. Microscopic observation to the shape of bacterial cells was performed by Gram staining.

Physiological tests were performed by growing isolates based on observed growth of various media, temperature, pH and NaCl. Carbohydrate fermentation biochemical test. Isolate bacteria taken 1 ose and put into medium carbohydrate broth of glucose, sucrose, lactose. Media that contained isolate, incubated for 2 days. Any changes in color were observed. Red indicates the absence of acid while yellow indicates the presence of acid. The formation of gas and color changes in the medium was caused by the addition of PP (Phenolphthalein) indicator. The fermentation activity could be observed with acid and gas production during the growth phase.

RESULTS AND DISCUSSION

All isolates of thermophilic bacteria obtained were the result of selection of microbial colonies grown on selective OGA (*Organic Gaus Agar*) and TMM (*Thermus Medium Modified*) medium. Acquired isolates were identified based on macroscopic, microscopic, physiological and biochemical descriptions. The isolation found 4 isolates of thermophilic bacteria from representative Bora Hot Spring is TM022, TM023, TM024 and TM026.

Characterization Macroscopic and Microscopic Thermophilic Bacterial

Bacterial characterization was performed on four isolates of thermophilic bacteria is TM022, TM023, TM024, TM026 isolates which had different colony form.

Differences in the macroscopic characteristics of thermophilic bacteria were due to the effect of the medium used so as to exhibit different phenotypic characteristics (Sianturi, 2008). The bacterial phenotype characteristics are not static so it is possible that the same bacteria may exhibit different morphological characteristics (Ochman *et al.*, 2005).

In colony color observation, all isolates had the same basic white color (figure 1). This result was similar to the study conducted by Dirnawan *et al.*, (2000) on the exploration of thermophilic bacteria from Mount Pancar, which also showed coloration dominated by white. A study conducted by Fitri and Yasmin (2011) also showed that most of the bacterial colonies were round-shaped, the color of the base colonies was beage – white. The colony shape and the size also varied greatly depending on the species.

Differences in the morphologic appearance of microorganism colonies on the media were one of the efforts of bacteria to survive in conditions of stress or toxic environment. At the beginning of growth, the bacteria used the carbon source present in the medium, so the colonies grew slighly. When the carbon source in the medium began to decrease, the bacteria tried to break the carbon bond, so it would be easy to use. Such activity usually caused bacterial colonies to become more rough and somewhat wrinkled

Table 1. Macroscopic characterization of representative thermophilic bacterial isolates from Bora Hot Spring

Observation Macroscopic	Isolate Code			
	TM022	TM023	TM024	TM026
Shape of the Colony	Circular	Circular	Circular	Circular
Surface of the Colony	Convex	Flat	Flat	Flat
Texture of the Colony	Shiny	Shiny	Opaque	Opaque
Color of the Colony	White	White Transparent	Beage	White
Edge of the Colony	Iiregular	Irregular	Irregular	Irregular

Table 2. Microscopic characterization of representative thermophilic bacterial isolates from Bora Hot Spring

Microscopic Observation	Isolate Code			
	TM022	TM023	TM024	TM026
Size of Cell (µm)	0.5 µm	1.0 µm	1.0 µm	0.5 µm
Gram	Negative	Positive	Positive	Positive
Shape	Diplobasil	Coccus	Stapilococcus	Streptococcus
Magnification	1000 x	1000 x	1000 x	1000 x

(Alwi, 2007).

The microscopic observations of thermophilic bacterial isolates were generally Gram-positive, coccus shaped, whereas the TM022 isolate was Gram-negative and bacillus shaped (Table 2).

The Study carried out by Akanbhi*et al.*, (2010) on the identification of thermophilic bacteria from aerobic sources generally resulted the bacteria obtained were Gram positive. Gram positive bacteria have cell walls with low lipid concentrations, which will dissolve by 96% aklohol and form small pores, so the main dye (crystal violet) is difficult to remove (Jumawita *et al.*, 2014). In addition Gram positive bacterial groups generally have aerobic and facultative anaerobic properties and have the ability to live on physiological conditions such as temperature, pH , and and salt concentration.

The discovery of Gram negative and positive isolates in Bora Hot Spring can be linked to environmental conditions. According to Mahmudah *et al.*, (2016) Gram-negative bacteria require relatively simpler nutrients compared to Gram-positive bacteria. This means the ability of this group of bacteria to grow in a wider environment than Gram positive bacteria

Physiological Test

The diversity of thermophilic bacteria found in Bora Hot Spring is influenced by the environmental conditions that support bacterial life such as biotic and abiotic factors. As the environment has high temperatures, hot water becomes one environment for microorganisms. The temperature of the environment suitable for microba varies and some are resistant to high temperature. Microbial sustainability at high temperatures is caused because thermophilic bacteria have different protein structures compared to mesophyll microbes so that they are able to survive at extreme temperatures. The existence of biotic components is capable of supporting the growth of thermophilic microorganisms, such as deciduous leaves, branches, grains, pollen, and insecticides found around hot springs as organic substances that can be utilized by living microorganisms in hot springs (Dirnawan *et al.*, 2000).

One of the abiotic factors that affect bacterial life is temperature and pH. Microbes that inhabit aquatic environments have characteristics that vary in regards to physiochemical parameters including temperature, salinity, pH and nutrient load (Pitri *et al.*, 2015; El Gayar *et al.*, 2017). Groups of extreme microorganisms generally thrive in extreme ecosystems. The environment is strongly influenced by very high or low pH, high temperature, high salinity, high pressure and various these combinations (Aanniz *et al.*, 2015).

All bacterial isolates grew on 50oC (Table 3). Madigan *et al.*, (2000) states that microorganisms that grow optimally at temperatures above 45^{o}C are thermophilic bacteria, whereas according to Rudiger *et al.*, (1994), that the optimum growth temperature of thermophilic microorganisms can be distinguished in general

that psychrophils grow at a temperature of about 3-20°C, mesophile 13-45°C, thermophile 45-65°C, extreme thermophilic temperature 65-85°C and hypertermofil at temperatures above 85°C.

Observation of pH parameter of all isolate of thermophilic bacteria indicated that the optimum pH was in range of 6-8.5. Brock (1997), explains that thermophilic bacteria can survive in extreme conditions with an optimum pH of about 6-8.5 and 4.5 - 8.7 pH in hot alkaline water (Pitri *et al.*, 2015). Sari (2012) states that an alkaline hot spring has a high mineral content that allows thermophilic microorganisms to survive and can affect the growth and diversity of thermophilic microorganisms.

Regions that have a degree of acidity (pH), gas and mineral composition, various oxidation/reduction and nutrients can lead to the presence of genetic diversity and metabolism of every living microorganism in it (Pitri *et al.*, 2015).

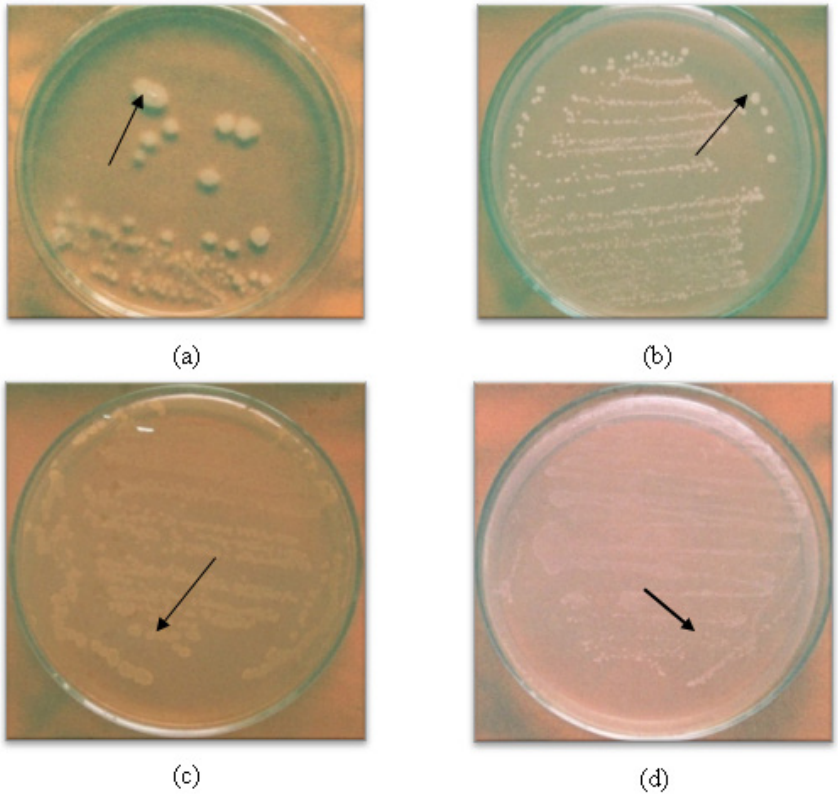

Figure 1. Macroscopic characterization of representative thermophilic bacteria from Bora Hot Spring A : Morphologic Observation of Macroscopic Isolate TM022 B : Morphologic Observation of Macroscopic Isolate TM023 C : Morphologic Observation of Macroscopic Isolate TM024 D : Morphologic Observation of Macroscopic Isolate TM026

Table 3. Observation of Physiological tests representative thermophilic bacterial isolates from Bora Hot Springs

Isolate	Observations								
	Temperature (°C)			pH			NaCl		
	(30)	(50)	(0)	(5)	(6.8)	(7-8.6)	(2%)	(6%)	(10%)
TM022	-	+	-	-	+	+	+	-	-
TM023	-	+	-	-	+	+	+	-	-
TM024	-	+	-	-	+	+	+	-	-
TM026	-	+	-	-	+	+	+	-	-

Note : *(+) Able to grew, (-) Unable to grew*

Testing of NaCl concentration showed that all isolates were able to grow at 2% NaCl concentration, whereas at 6-10% NaCl concentration were not able to grow. These results were consistent with the study of Syafriani *et al.* (2013) that bacterial isolates were capable of growing and developing at 3% NaCl content compared 5% concentration, while the 7% NaCl concentration, bacteria cannot live. At 3% NaCl concentration the color of the media turned to cloudy, while the 7% NaCl concentration the media looked clear. According to Prasetyo (2006), the more cloudy the media the higher the optical density (OD) and the more population increase of cells in the media.

Biochemical Test

In the carbohydrate test of glucose, lactose and sucrose, it was found that all isolates of thermophilic bacteria were able to ferment carbohydrates which indicated by change of medium color from green to yellow (Table 4).

Table 4. Observation of biochemical tests of representative thermophilic bacterial isolates from Bora Hot Springs

Isolate	Observatoins		
	Glucose	Lactose	Maltose
TM022	+	+	+
TM023	+	+	+
TM024	+	+	+
TM026	+	+	+

Note : (+) Glucose: medium color turns yellow with no gas; (+) Lactose: the medium remains green; (+) Maltosa: medium color turns yellow with no gas

The result of fermentation test of glucose, lactose and maltose carbohydrates all isolates showed positive result (Table 4). This was indicated by the color change of fermentation medium of glucose and maltose from green to yellow, while lactose test indicated no color change in during incubation for 24 hours.

According to Harley (2002), the yellow color of the media was caused by the phenol solution that forms acid in the carbohydrate media. The resulting lactose fermentation did not change color, because bacteria are able to ferment lactose and cause variation to medium color.

The color change was caused by isolates capability to use glucose, lactose and maltose as the carbon source. In addition, carbohydrate fermentation could occur aerobically on the agar surface and the anaerobically at the base of the medium. On the surface of the medium, glucose catabolism was done through the Embden-Meyerhof path resulted in pyruvic acid which degraded completely in cycle citric acid to CO_2, H_2O, and energy (Haryani *et al.*, 2012).

CONCLUSION

Based on the result of study, there were 4 isolate thermophilic bacteria, TM022, TM023, TM024, TM026. Characterization of thermophilic bacterial isolates macroscopically showed different phenotypic properties, whereas microscopic observation showed bacillus and coccus shaped bacterial cells as well as Gram positive and negative bacteria. Observation of physiological test of all isolates grew at 50°C, pH 6.8-8.6 and 2% NaCl concentration.

This study contributes to the development of conservation strategies and provides information on the characteristic diversity of indigenous thermophilic bacteria as a potentialthermostable enzyme producer from Bora Hot Spring. The novelty of this research is the determination of characterization and identification of thermophilic bacteria isolate based on macroscopic observation, microscopic, physiological test, biochemical test and optimization of its growth in various media.

ACKNOWLEDGEMENT

The authors would like to thank the Head of Basic Biology and Biotechnology Laboratory, Department of Biology, Faculty of Science University of Tadulako, which has provided facilities for the processing of samples up to completion.

REFERENCES

Aanniz, T., Ouadghiri, M., Melloul, M., Swings, J., Elfahime, E., Ibijbijen, J., Ismaili, M., Amar, M. (2015). Thermophilic bacteria in Moroccan hot springs, salt marshes and desert soils. *Brazilian Journal of Microbiology*. 46(2) 443-453.

Akanbi, T. O., Kamaruzaman, A. L., Abu Bakar, F., Sheikh Abdul Hamid, N., Radu, S., Abdul Manap, M. Y. and Saari, N. (2010). Highly Thermostable Extracelluler Lipase Productcing Bacillus Starin Isolated Froma Malaysian Hot spring and Identifiedusing16S rRNA gene sequencing. *International Food Reserch Journal*. 17,45-53.

Alwi, M. (2007). *Introduction to General Biology*.University of Tadulako. Palu.

Asnawi, H. (2006). Thermophilic Bacterial Diversity at Padusan Pacet Hot Spring Tourism Park, Mojokerto Regency, East Java.Biologi Major, Faculty of Mathematics and Science, UM.

Devi, S., Agustien, A &Periadnadi. 2013. The screening of thermo-amylolyticbacteria from Sungai Medang Hot Spring, Kerinci, Jambi. *Journal of Biology.* University Andalas.77-82 (ISSN : 2303-2162).

Dirnawan, H., Suwanto,A.,& Purwadira,T. (2000).Exploration of Extracellular-Hydrolytic Enzyme Producers Thermophilic Bacteria from Mount Pancar Hot Spring. Vol 7. (2)52-55.

El-Gayar, K.E., Al Abboud, M.A., and M. Essa, A.M. (2017).Characterization of Thermophilic Bacteria Isolatedfrom two Hot Springs in Jazan, Saudi Arabia. *Journal of Pure And Applied Microbiology.* Vol. 11(2), p. proof.

Fitri, L. and Yasmin, Y. 2011. Isolation and Observation of Morphology of Chitinolytic Bacteria Colony.*JurnalIlmiahPendidikanBiologi*. Volume 3, Nomor2 : 20-25

Harley, J.P. and L.M. Prescott. 2002. Laboratory Exercises in Microbiology. 1st ed. The McGraw-Hill Companies, USA.

Hardi, J., Jusman, Razak, A.R., Silva. (2016). Production and Test of Chitinase Enzyme Activity of Thermophilic B1211 Bacterial Isolate from of Bora Hot Spring. KOVALEN: *Journal of Chemical Research. 2(3):67-72* .ISSN: 2477.

Haryani, Y., Chainulfiffah, dan Rustiana. Fermentasi karbohidrat oleh isolat *salmonella* spp. dari jajanan pinggir jalan. *Jurna Ind.Che. Acta*. Vol. 3 (1) : ISSN 2085-0050.

Jumawita.,Agustien, A., dan Djong Hon Tjong. Characterization of Amilo-Thermophilic Obligat Bacteria from Semurup Hot Spring.2014. *Jurnal Biologi Andalas*. 3(3) : 249-253 (ISSN : 2303-2162)

Khalil, A. (2011). Isolation and characterization of three thermophilic bacterial strains (lipase, cellulose and amylase producers) from hot springs in Saudi Arabia. *African Journal of Biotechnology*. Vol. 10(44).

Khoridah, E.N. 2015. Production of Chitinase Enzyme from Thermophilic Bacteria Isolate of Bora Hot Spring. *Skripsi.* Palu: University of Tadulako.

Madigan,M.T.,J.M.,Martinko,J., Parker. (2003). *Brock Biology of microorganisms*, Pearson Edition, Inc, London.

Madigan,M.T., and B.L., Marrs. (1997). *Extremophiles,* Sci, American, April. 82-87.

Mahmudah, R., Baharuddin, M., Sappewali. (2016). Identification of thermophilic bacterial isolates from Lejja Hot Spring, Soppeng Regency. Al-Kimia Journal: Vol.4 No. 1.

Maria dan Surya. (2012).“Isolation and Identification of Thermophilic Bacteria from Songgoriti Hot Spring after Two Days of Incubation.*Journal of Technique Pomits.*Vol. 1(1).

Mohammad, B.T., Al Daghistani, H. I., Jaouani A., Latif, S.A and Kennes,C. Isolation and Characterization of Thermophilic Bacteria from Jordanian Hot Springs: *Bacillus licheniformis* and *Thermomonas hydrothermalis* Isolates as Potential Producers of Thermostable Enzymes. 2017. Hindawi : *International Journal of Microbiology.* Volume 2017.

Ochman, H.,Lerat,G.,and Daubin,V. (2005). Examining bacterial species under the spector of genetransfer and exchange, PNAS.102.1. 6595-6599.

Panda, M.K., Sahu, M.H., and Tayung, K. (2013). Isolation and characterization of a thermophilic *Bacillus* sp.with protease activity isolated from hot spring of Tarabalo, Odisha, India. *TUMS : Iranian Juornal Microbiol*. 5(2): 159–165.

Pitri, R.E., Agustien, A., dan Febria, F.A. (2016). Isolation and Characterization of Thermophilic Amylus Bacteria from Medang River Hot Spring. Journal of Biology, Andalas University (J. Bio. UA.) 4(2). 119-122.

Prasetyo, H. 2006. Kandungan Selenium Total dalam BakteriTermofilikTerseleksidariSumber Air Panas.[Skripsi]. Institut Pertanian Bogor. Bogor.

Rudiger, A., Suna A., Antranikian*, (*1994). Carbohydrases handbookof Enzymes Catalysisin Organic Sipthesis. Drauz and Waldmann(Ed.).,Wenheim, Germany.

Sianturi,D.C.,(2008). Bacterial Isolation and Test of Rough Thermophile Activity from the Sebirubiru Hot Spring, North Sumatra.[Thesis]. University of North Sumatra..

Sari, U. M. (2012). Screening and Characterization of Thermophilic Cellulolytic Bacteria fromMedang River Hot Spring, Kerinci Regency, Jambi. [Skripsi]. Padang. Andalas University.

Yanti, N.A., Ahmad, S.W., Ambardini, S., Muhiddin, N.H., Sulaiman, L.I. (2017). Screening of Acetic Acid Bacteria from Pineapple Waste for Bacterial Cellulose Production using Sago Liquid Waste. *Biosaintifika: Journal of Biology & Biology Education*. Vol 9 (3) 387-393.

Wahyuna, D., Agustien, A and Periadnadi. 2012. Isolation and characterization of thermoproteolytic bacteria from hot springs at Sungai Medang, Sungai Penuh, Jambi. JurnalBiologiAndalas. 1(2) : 93-98

Wijayati, N., Astutiningsih, C., Mulyati, S.(2014). Transformasi α-Pinena dengan Bakteri *Pseudomonas aeruginosa* ATCC 2592 *Transformation* α*-Pinena by Bacteri Pseudomonas aeruginosa ATCC 25923*. *Biosaintifika: Journal of Biology & Biology Education* 6 (1) (2014).