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Isolation And Identification Of Thermophilic Bacteria From Hot Spring In Kerinci – Jambi

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Abstract – This research aims to identify the genus of thermophilic bacteria found in the hot spring source of Abu Kerinci Jambi River Hot Spring Village, which can be used for practical material and advanced research on the cultural stock obtained using explorative descriptive methods. The research data were obtained from isolation results using nutrient agar media that was incubated at a temperature of 50°C for 24 to 48 hours, macroscopic observations, microscopic and biochemical tests, as well as genus identification using Bergey's Manual of Determinative Bacteriology. The results of the study showed that there were 6 isolates of thermophilic bacteria that were successfully isolated, and after the identification of the 6 isolates of the bacteria, we obtained 5 isolates (S1, S3, S4, S5, and S6) from the genus Pseudomonas and 1 isolate (S2) from the genus Vibrio.

Key Words – Isolation, Identification, Thermophilic, *Pseudomonas*, *Vibrio.*

1. Introduction

Indonesia is known as an archipelago with a tropical climate and many volcanic areas that have high volcanic activity, one of which is in Jambi Province, especially in the Kerinci Regency area. The existence of volcanoes causes the emergence of hot springs, this can occur because magma that does not reach the surface in the freezing process releases heat (Geost, 2019). One of the hot springs is located in the Kerinci Regency area, namely the hot springs of Sungai Abu Hot Spring Village, which is 11 KM from Sungai Penuh City. After measuring the temperature and pH at several different points, the hot water temperature of Sungai Abu Hot Spring Village was found to be around 55º-60°C and pH 7.8-8.0. Through these temperature measurements, microorganisms that have the potential to live and grow are thermophilic bacteria.

Research on thermophilic bacteria found in several hot springs been carried out such as (Muharni, 2009) has found bacteria of the genus Bacillus obtained from the hot water of Lake Ranau South Sumatra which has a temperature of 37.3˚-63.7°C. In addition (Kurniawan, 2017) has also found *Bacillus sp.* bacteria from Semurup hot springs in Kerinci Regency, Jambi which has a temperature of 60°-80°C with a pH of 7. (Asnawi, 2006) has successfully isolated several types of thermophilic bacteria from Pacet hot water, East Java, namely *Bacillus sp., Thermus sp., Acetogenium sp.,* and *Pseudomonas* sp.

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Thermophilic bacteria are microorganisms that can survive in extreme environments such as environments with high temperatures of 45°-80°C. In many cases, besides being able to adapt, extreme environmental conditions are also utilized by thermophilic bacteria to produce (Mahmudah et al., 2016). Thermophilic bacteria obtained from hot springs are usually researched by isolating, characterizing, and testing their enzymatic potential, this is because thermophilic bacteria can produce thermostable enzymes or heat-resistant enzymes that can be used in industry, waste treatment, mineral weathering, or for biotechnology studies (Tuntun & Huda, 2014).

2. Materials and methods

2.1 *Preparation of Tools and Materials*

The tools used in this research are 100 ml glass bottles, thermal containers, thermometers, digital pH meters, digital scales, bunsen burner, micropipettes, test tubes, test tube racks, Petri dishes, erlenmeyers, ose needles, incubators, microscopes, glass objects, Durham tubes, electric stoves, autoclaves, wire gauze, refrigerators, and cameras. The materials used were samples of thermophilic bacteria, methylated spirits, matches, tissue, cotton, label paper, distilled water, Nutrient Agar (NA), strach agar, SIM agar, Simmons citrate agar, trypticase soy agar, MR-VP broth, nutrient gelatin, Brom timol blue lactose broth, Brom timol blue dextrose broth, Brom timol blue sucrose broth, 96% alcohol, 0.85% NaCl solution, crystal violet, safranin, iodine, barrit reagent a, barrit reagent b, erlich reagent, hydrogen peroxide, methyl red, aluminum foil, and malachite green.

2.2 *Sample Collecting*

Sampling begins with preparing all the tools that have been sterilized, and then determining the sampling point. Furthermore, the bottle that will be used to carry the sample to the laboratory is rinsed with sample water to be taken 3 times. Then dip the bottle carefully at a depth of about 10 cm below the water surface. After the bottle is filled with sample water, put it in a thermal container, and then take it directly to the laboratory for identification (National Standardization Agency, 2008).

3. Results and discussion

3.1 *Isolation and Colony Count Observation of Thermophilic Bacteria*

The pour plate method was used to isolate the bacteria on the NA medium. To make NA media dissolve 20 g/2% v/v of synthetic NA media in 1000 mL of distilled water. For 24 to 48 hours, cultures are incubated at 50°C. The number of colonies of thermophilic bacteria grown on NA media as much as 1 ml sample obtained 6 isolates which are then coded with S1, S2, S3, S4, S5, and S6. Data from the observation of the number of bacteria can be seen in Figure 1.

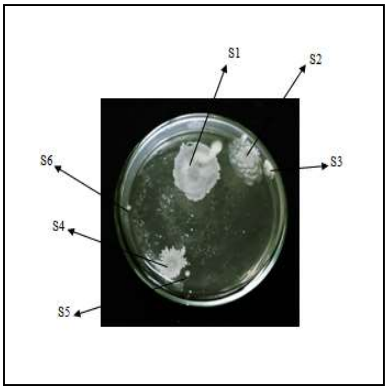

Figure 1. Bacterial colonies on NA media

3.2 *Morphological Observations*

Morphological observations are obtained as follows:

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Table 1. Morphological observation

Isolate	Morphological Characteristics of Bacterial Colonies			
	Colony Shape	**Elevation**	**Margin**	**Colors**
S1	*Irregular*	*Convex*	*Irregular*	**White**
S2	*Irregular*	*Convex*	*Lobate*	**White**
S3	*Irregular*	*Convex*	*Lobate*	**White**
S4	*Round*	*Convex*	*Rhizoid*	**White**
S5	*Round*	*Convex*	*Smooth*	**White**
S6	*Round*	*Convex*	*Smooth*	**White**

3.3 *Gram Staining*

Gram staining observations can be seen in Figure 2.

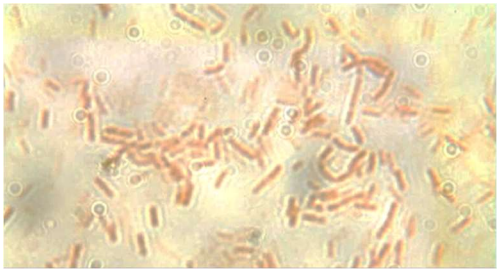

Figure 2. Gram-negative

3.4 *Biochemical Test*

Biochemical tests obtained the following data:

Table 2: Biochemical Test Data

Biochemical Test			Isolates					
			S1	**S2**	**S3**	**S4**	**S5**	**S6**
Amylum Hydrolysis			+	+	-	-	-	-
Gelatin Hydrolysis			+	+	-	-	-	-
Carbohydrate Fermentation	**Dextrose**	**Acid**	-	+	-	-	-	-
		Gas	-	+	-	-	-	-
	Lactose	**Acid**	-	-	-	-	-	-
		Gas	-	-	-	-	-	-
	Sucrose	**Acid**	-	+	-	-	-	-
		Gas	-	+	-	-	-	-

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Indole Production	-	-	-	-	-	-
Catalase Test	+	+	-	+	+	-
Methyl Red (MR) Test	+	+	+	+	+	+
Voges Proskauer Test	-	-	-	-	-	-
Citrate Utilization Test	-	-	-	-	-	-
Hydrogen Sulfide (H2S) Test	-	-	-	-	-	-

The following results of the biochemical test reactions:

Positive results in the amylum hydrolysis test can be seen in Figure 4.

S1 (+) S2 (+)

Figure 4. Amylum hydrolysis

Positive results on the gelatin hydrolysis test can be seen in Figure 5.

S1 (+) S2 (+)

Figure 5. Gelatin hydrolysis

Positive results in the dextrose broth carbohydrate fermentation test can be seen in Figure 6.

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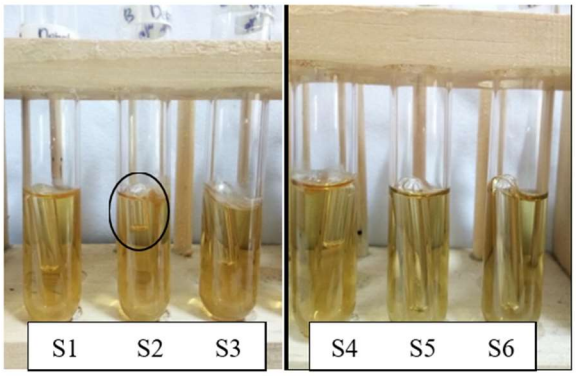

Figure 6. Carbohydrate fermentation (Dextrose)

Description:

Isolate reacted positively (+) acid and gas: shown in the black circle is S2.

Positive results on the sucrose broth carbohydrate fermentation test can be seen in Figure 7.

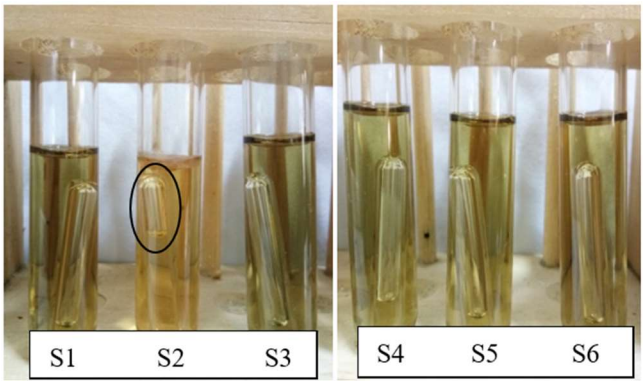

Figure 7. Carbohydrate fermentation (Sucrose)

Description:

Isolate reacts positively (+) acid and gas: shown in the black circle is S2

Positive results on the catalase test can be seen in Figure 8.

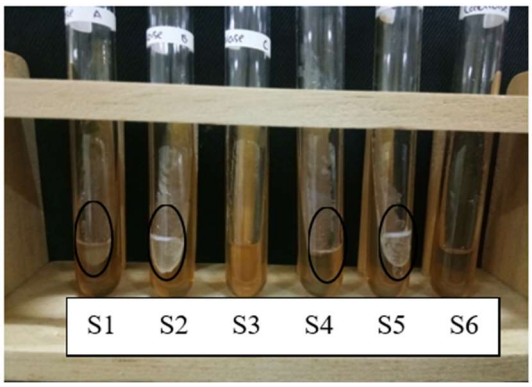

Figure 8. Catalase test

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Description:

Isolates reacted positively (+): shown in the circle that is S1, S2, S4, and S5

The positive results of all isolates in the methyl red (MR) test can be seen in Figure 9.

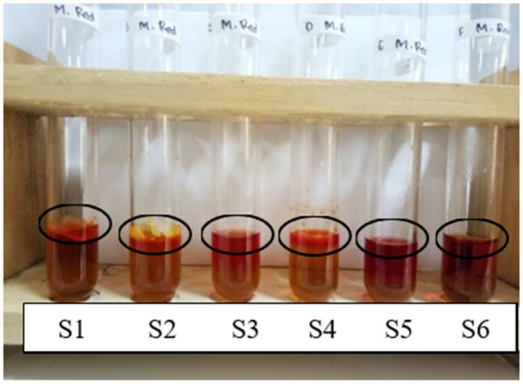

Figure 9. Methyl red (MR) test

Description:

All isolates reacted positively (+) shown in black circles

Hot water samples in this study were taken from the hot springs of Sungai Abu Hot Springs Village, Kerinci Jambi with a location point of 2º2'43 "S 101º26'21 "E. This hot spring has a temperature ranging from 55º-65ºC with a pH of 7.8-8.1. In this study, the incubation temperature used was 50ºC, this is following research (Irena, 2010), (Tuntun & Huda, 2014) and (Kurniawan, 2017) which used a temperature of 50ºC to incubate thermophilic bacteria.

Isolation of thermophilic bacteria in this study used NA (Nutrient Agar) media which fulfills elements such as beef extract, peptone, and agar that bacteria need to grow and multiply. After isolation and morphological observations, as can be seen in Figure 1 and Table 1, further observations were made on gram staining and spore staining to see the classification of thermophilic bacterial isolates obtained and to determine whether the bacterial isolates were able to form spores.

The results obtained are all isolates of thermophilic bacteria including gram-negative bacteria and in spore staining only isolates with codes S2 and S3 can form spores. Gram staining aims to classify bacteria into 2 large groups, namely gram-positive bacteria and gram-negative bacteria. Gram-positive bacteria will be purple and gram-negative bacteria will be red. Gram-positive bacteria can retain crystal violet dye despite being washed with alcohol while gram-negative bacteria will lose crystal violet dye after being washed with alcohol so that the color that appears is counter, namely safranin which is red (Pelczar & Chan, 2008).

3.5 *Identification through biochemical test results*

The next identification is to see the reaction in the biochemical test, namely to see the ability of bacteria to hydrolyze amylum and gelatin and the results of 5 isolates of thermophilic bacteria found that isolates with codes S1 and S2 can hydrolyze amylum (Figure 4) and gelatin (Figure 5) while the other 3 isolates are not. This is characterized by the formation of a clear zone on the starch agar medium and the state of the media that does not freeze in gelatin hydrolysis. According to (Cappuccino & Sherman, 2014) this amylum hydrolysis test uses starch agar to show the hydrolytic activity of exoenzymes. This medium is composed of nutrient agar with additional starch which acts as a polysaccharide substrate. Bacteria grown in this medium will decompose the substances in the medium and if the bacteria can produce the enzyme amylase, the amylum will be decomposed. This activity can be tested using iodine dripped on the surface of the media that has been grown by bacteria. If the amylum has been hydrolyzed it will form a clear zone surrounding the bacterial colony and if the amylum is not hydrolyzed then the media is only purple-black.

Meanwhile, gelatin is a protein produced from the hydrolysis of collagen which is a large component of connective tissue and tendons in humans and animals. Below a temperature of 25ºC gelatin will solidify and above 25ºC gelatin will liquefy. Melting of gelatin can also occur if there are bacteria capable of producing proteolytic extracellular enzymes, namely gelatinase, which can hydrolyze proteins into amino acids, and even at low temperatures (4ºC) gelatin will not solidify (Cappuccino & Sherman, 2014).

Furthermore, thermophilic bacterial isolates on carbohydrate fermentation using 3 media, namely dextrose broth, lactose broth, and sucrose broth in a test tube containing a Durham tube. Based on the results obtained from 6 isolates, only 1 isolate, S2, can ferment carbohydrates in dextrose broth medium (Figure 6) and sucrose broth (Figure 7). The occurrence of carbohydrate fermentation is indicated by changing the color of the media to cloudy yellow and there are bubbles in the Durham tube.

The catalase test (Figure 8) performed on 6 isolates of thermophilic bacteria resulted in 4 isolates namely S1, S2, S4, and S5 positively reacting to hydrogen peroxide, while the other 2 isolates namely S3 and S6 showed no reaction to hydrogen peroxide. Furthermore, the results of the methyl red test (Figure 9) on 6 isolates of thermophilic bacteria showed positive results because incubating the bacterial culture on MRVP Broth media produced a red layer on the media when given a methyl red indicator. Meanwhile, the results of the indole production test, Voges Proskauer test, citrate utilization test and hydrogen sulfide test on 6 isolates showed negative results.

3.6 *Identification in the book Bergey's Manual of Determinative Bacteriology*

Based on the data obtained from morphological observations, gram staining, and biochemical tests, it is found that isolates with codes S1, S3, S4, and S5 are classified in the genus Pseudomonas, and isolates with code S2 are classified in the genus Vibrio. It can be seen that only S2 isolates can ferment carbohydrates while the other 4 isolates are not. According to (Buchanan & Gibbons, 1974) Pseudomonas is a group of bacteria whose metabolism is not fermentative, some include facultative chemolithotrophs, can use hydrogen or carbon monoxide as an energy source, oxygen molecules as electron acceptors and some can use nitrate as an alternative electron acceptor. Catalase positive, some species produce acid oxidatively from alcohols and aldose sugars, especially at high concentrations. Many species accumulate poly-hydroxybutyrate as an intracellular carbon reserve.

In addition, the results of other chemical tests are also supported by research conducted by Mahmudah et al (2016) who identified thermophilic bacterial isolates from Lejja hot springs, Soppeng Regency and research (Runtuboi et al., 2018) which isolated and identified thermophilic bacteria from hot springs in Moso Muara Tami District Jaya Pura City Papua Province.

4. *Conclusions And Suggestions*

Based on the research that has been done on the isolation and identification of thermophilic bacteria originating from the hot springs of Sungai Abu Kerinci Hot Springs Village, Jambi, 6 isolates of thermophilic bacteria originating from the genus Pseudomonas were found, namely isolates S1, S3, S4, S5, and S6 and the Vibrio genus from isolate S2. For further research, it is recommended that identification be carried out up to the species level (molecular) so that specific types of thermophilic bacteria-producing thermostable enzymes are obtained.

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