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SCREENING OF THERMOPHILIC BACTERIA PRODUCING LIPASE ENZYME IN GEOTHERMAL AREAS OF SIPOHOLON-TARUTUNG, NORTH SUMATRA, INDONESIA

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ABSTRACT

Thermophilic microorganisms can synthesize stable molecules in hot conditions, including enzyme molecules. Thermophilic bacteria develop at high temperatures, grow in hot springs, desert lands, and spas. This study aimed to obtain and isolate thermophilic bacteria that produce lipase enzyme and to know the macroscopic and microscopic thermophilic bacteria that have lipolytic potential. Hot water samples were obtained from the Sipoholon-Tarutung Hot Springs, North Sumatra, Indonesia and then recorded the physical-chemical form and bacterial characterization of these hot springs. Eleven bacteria were selected based on differences in morphological characteristics, seven of which could produce lipase enzymes and carry out macroscopic and microscopic characterizations. BTPL-01 is the isolate which has the highest protease activity because the proteolytic index value is 0.85.

KEYWORDS: Bacteria, Enzymes, Hot Springs, Isolation, Screening, Thermophilic.

1. INTRODUCTION

Thermophilic bacteria produce thermostable enzymes that are very important in industrial and biotechnological processes such as molecular biology techniques for research and diagnostic uses (enzymes that process DNA and RNA) and the ability of enzymes to convert flour, food, waste management, paper making and synthesis of substances organic matter.[1] The main factor affecting the life of microorganisms is temperature. Some bacteria can live at low temperatures and cannot survive at high temperatures and also bacteria that can survive at high temperatures but they cannot live at low temperatures.[2] Thermophile bacteria can be isolated from various sources, including hot springs both on land and at sea, land that is

always exposed to sunlight, fermented materials such as compost and hot water installations.[3]

Based on the optimum growth temperature, microorganisms are generally distinguished from psychrophilic, psychotropic, mesophilic, thermophilic, and hyperthermophilic microorganisms. Psychrophilic bacteria live in a temperature range of 0-20°C and psychotropic bacteria can grow at a temperature of 0-35°C. Mesophyll bacteria can grow at a temperature of 20-45°C and thermophilic bacteria grow at a temperature of 45-65°C. Hyperthermophilic bacteria live at temperatures above 90°C and maximal at 100°C, but in some bacteria, they can live at temperatures of 80-113°C.[4]

Enzymes are unstable in organic solvents and can be denatured or lost in catalytic activity. However, lipase can be stable and remain active in an organic solvent without the addition of stabilizing compounds. The type of substrate from lipase is also sometimes insoluble or is slightly soluble in water media. Therefore, in a phenomenon like this, an organic solvent or organic-water solution is used as a reaction medium. Because lipase still has its catalytic ability in an organic solvent, making lipase is widely applied in the field of biotechnology.[5]

For industrial needs generally isolated from various types of microorganisms. Microorganisms can produce enzymes in varying amounts and types, the production time is faster and easier to control. Production and trade of enzymes by hydrolytic enzyme groups such as amylase, protease, catalase and lipase.[6] One type of enzyme that has an important and unparalleled role in the growth of biotechnology is the lipase enzyme. This enzyme has special properties that can break the ester bonds in fat and glycerol. Also, lipase can catalyze organic reactions both in aqueous media and in non-water media.[7] Lipase enzymes play a role in the separation of fatty acids and the dissolution of oil stains in industrial equipment so that oil can be dissolved in water. Some of the reactions catalyzed by lipase enzymes include hydrolysis, alcoholysis, esterification, and interesterification reactions.[8]

Indonesia has natural resources that support various types of bacteria originating from hot springs sources. One of them is the Sipoholon-Tarutung geothermal region located in North Tapanuli Regency, North Sumatra province. In the form of hot spring with temperatures between 40^0C -80^0C, with temperatures around 30^0C, acidity (pH) is neutral displacement around 6.70.[9] Based on this, a study on Isolation and Screening of thermophilic bacteria producing lipase enzymes from the Geothermal region of Sipoholon-Tarutung, North

Sumatra. Thermophilic microorganisms are the most dominant group of microorganisms from other extremophilic microorganisms.

MATERIAL AND METHODS

1.1 Sample Collection

This study used a survey method and *purposive sampling* technique for sampling from Sipoholon-Tarutung hot springs around which there are several vegetations such as rocks that have been overgrown with moss and weeds. Hot springs were taken in pond IV with three extraction points and used a sterile bottle of 100 ml at 10 cm below the surface of the water between 50-60^{0}C, then the samples were stored in the container and taken immediately to the laboratory before 24 hours.

1.2 Isolation and Screening for Protease Producing Thermophilic Bacteria

Isolation of thermophiles bacteria using the *pour plate* method on *nutrient agar* medium (20 g/L) taken 1 ml from hot water samples, for each sampling point and incubated at 50^{0}C for 24 hours. Different isolates can be identified by observing the morphology of the colonies, thermophilic bacteria are planted into the selective media. The presence of lipase activity is characterized by the formation of a clear zone around the bacteria on selective media, and then the lypolitic index is calculated.

2.3 Characterization of Thermophilic Bacteria

Characterization of thermophilic bacteria was done through macroscopic observation to the colony edge, colony shape, colony color, elevation, and surface. Microscopic observation of the shape of bacterial cells was performed by Gram staining. Potential isolates were characterized by different biochemical methods such as the catalase test.

3. RESULTS AND DISCUSSION

3.1 Isolation and Screening of Thermophilic Bacteria Producing Lipase Enzym

From the results of the research that has been done, 97 isolates of thermophilic bacteria were obtained from the Sipoholon-Tarutung hot springs source, eleven thermophilic bacterial isolates were acquired based on their difference in morphology (Table 1). The total count of bacteria after 24 hours incubation is also indicated.

Table 1: The results of isolation of thermophilic bacteria and Lipolytic Index from the Sipoholon-Tarutung Hot springs source were incubated at 50^0C.

Isolate	Temperature	pH	Number of colony bacteria	Isolate code	Diameter (mm)		Lipolytic Index
					Colony	Clear zone	
Pond IV point I	55^0C	6.9	34	BTPL-01	6,00	11,13	0,85
				BTPL-02	0	0	0
				BTPL-03	6,00	8,35	0,39
				BTPL-04	6,00	8,93	0,48
Pond IV point 2	53^0C	6.9	35	BTPL-05	0	0	0
				BTPL-06	6,00	8,06	0,34
				BTPL-07	6,00	7,57	0,26
Pond IV point 3	57^0C	6.9	28	BTPL-08	0	0	0
				BTPL-09	6,00	9,44	0,57
				BTPL-10	0	0	0
				BTPL-11	6,00	7,35	0,22

The results of screening on all thermophilic bacteria obtained seven isolates of thermophilic bacteria that have the potential to eliminate the lipase enzyme. While the other four isolates did not indicate lipolytic properties with a marked absence of a clear zone around the colony. Table 2 shows the proteolytic index value of each isolate producing the lipase enzyme. Each isolate had a different IL value, lipolytic index isolates ranged from 0.22 to 0.85. BTPL-01 isolates have the highest index value, while BTPL-11 isolates have the lowest index value (Fig. 1).

An important factor that must be considered in isolating bacteria is the media component used to grow these bacteria. The media must contain nutrients for bacterial growth. In addition to nutrition, other things to consider are environmental factors, such as acidity and temperature. Extreme pH conditions can damage the structure of bacterial cell walls and damage the metabolic system.[10] The presence of thermophilic bacteria in these hot springs is caused by environmental conditions supported by biotic and abiotic factors. Biotic conditions in hot springs where there are grasses, moss and other organic sources found around the hot springs so that they can be used as energy sources. Abiotic factors also influence the presence of thermophilic bacteria such as alkaline pH caused by high mineral content which causes high diversity of microorganisms.[11]

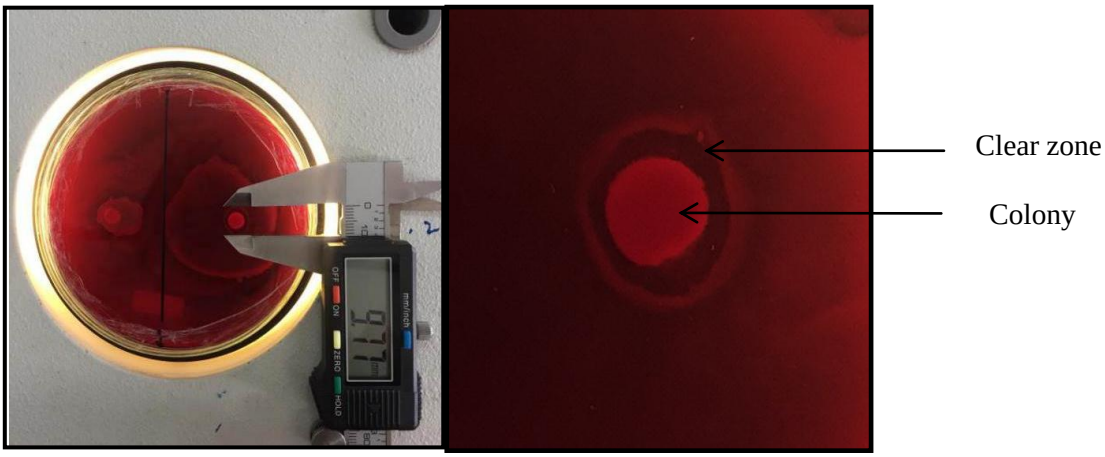

Figure 1: BTPL-09 bacterial isolates with clear zones.

Lipolytic enzymes are at the center of attention because of their potential in the application of the lipid biotechnology industry. Currently, the lipase enzymes used in the detergent industry, lipids and biodiesel are mostly derived from microbes, especially fungi and bacteria.[12] If around the colonies there are clear zone regions ≥ 2 cm, then it can be said that these bacteria include good and potential bacteria to produce an enzyme.[13]

There is not always a good correlation between the clear zones around the colonies on solid media and the ability of these organisms. The causative factors include differences in the types of microorganisms, the growth rate of each isolate in the solid medium, the amount of inoculum given to the medium and type of enzyme produced.[14] The group of lipolytic bacteria produces lipase, an enzyme that catalyzes the hydrolysis of fat into fatty acids and glycerol. Lipolytic bacteria can cause food containing fat to become rancid. Many bacteria that are aerobic and active proteolytic are also lipolytic.[15]

Bacteria that are not able to produce lipase enzymes can be caused by two things, namely cells that consume fatty acids and the absence of lipase activity produced by bacteria so that no reaction occurs between specific mediums with free fatty acids formed from the process fat hydrolysis.[16]

3.2 Characterization Macroscopic, Microscopic and Biochemical Test Thermophilic Bacteria

The characterization of thermophilic bacteria was carried out on all isolates obtained which produced the lipase enzyme. The results of the macroscopic characterization of the colony and cell microscopy from isolates of lipase producing thermophilic bacteria can be seen in

Table 2. The results of macroscopic characterization obtained for all isolates generally have almost the same morphological forms of colonies as yellowish and creamy colonies, irregular, flat elevation and have the edge of the colony is Entire, slippery, irregular and notched. All isolates showed different physiological properties. Microscopic analysis showed all six isolates were Gram-negative and Catalase test positive.

Table 2: Characterization of Thermophilic Bacteria.

Isolate code	Characterization of Colony					Gram	Catalase test
	Colour	Shape	Elevation	Edge	Form		
BTPL-01	Yellow	Circular	Convex	Serrate	Bacil	Positive	Positive
BTPL-03	Yellow	Sircular	Convex	Serrate	Bacil	Positive	Positive
BTPL-04	Yellow	Sircular	Flat	Entire	Bacil	Positive	Positive
BTPL-06	Yellow	Rhizoid	Flat	Serrate	Bacil	Positive	Positive
BTPL-07	Yellow	Irregular	Flat	Serrate	Bacil	Positive	Positive
BTPL-09	Yellow	Irregular	Flat	Serrate	Bacil	Positive	Positive
BTPL-11	Yellow	Sirkuler	Flat	Serrate	Bacil	Positive	Positive

Macroscopic characterization was carried out on seven thermophilic bacterial isolates with lipase enzyme activity that had been obtained with morphological characteristics namely yellow BTPL-01 isolates, circular shapes, flat elevated serrate edges, yellow BTPL-03 isolates, circular shapes, seerrate edges with convex elevation, yellow BTPL-04, circular shape, entire edge with flat elevation, yellow BTPL-06, circular shape, flat elevated serrate edges, yellow BTPL-07, irregular shape, serrate edges with flat elevation, BTPL -09 yellow, irregular shape, serrate edges with flat elevation, and yellow BTPL-11, circular shape, serrate edges with flat elevation.

CONCLUSION

Thermophilic bacteria obtained by producing lipase enzymes as many as seven isolates were found in Sipoholon-Tarutung hot springs with BTPL-01 isolate having the highest and potential proteolytic index and seven lipolytic isolates having different macroscopic and microscopic characteristics

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