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Activity Test of Catalase Enzyme in Rhizospheric Soil Bacteria

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Abstract *– This research was conducted at the Microbiology Laboratory of UIN Walisongo Semarang to identify and analyze the catalase enzyme in rhizosphere bacteria around the Laboratory of Campus 2 of UIN Walisongo Semarang. This research was carried out in several stages, namely isolation of rhizosphere bacteria, characterization of rhizosphere bacteria, gram staining of rhizosphere bacteria, and catalase test of rhizosphere bacteria. The data analysis technique used is the descriptive data analysis technique. The results showed that 9 isolates of rhizosphere bacteria produced catalase enzyme in the presence of gas bubbles when reacted with* H_2O_2.

Keywords: *Catalase enzymes, Rhizosphere bacteria, Laboratory of Campus 2 of UIN Walisongo Semarang*

INTRODUCTION

The rhizosphere is a dynamic ecological structure in which the structure of microbial communities is formed by the presence of exudates derived from plant roots. The exudate nutrients secreted by plants such as sugars, secondary metabolites, minerals, and carbohydrates serve as carbon and nutrient sources of soil-borne microbes and, depending on the quality and quantity of these exudates, will affect the degree of biological activity and microbial communities that will be prevalent or dominant in the rhizosphere. Therefore, the rhizosphere is the highest point of resource exchange within the biosphere (Rivas, et al., 2022).

Around the roots of the soil, some bacteria greatly affect plant growth. Its existence will trigger plant growth, so it is said to be PGPR (Plant Growth Promoting Rhizobacteria) (Sari, 2014). In the nutrient cycle, plant growth, soil formation processes, and biological control of rhizosphere microbial root pathogens play an important role. According to Ulum (2018), in non-rhizosphere soils, the population of microorganisms is less than microorganisms in the rhizosphere.

The enzyme catalase in bacteria functions as a decomposer of hydrogen peroxide into water and oxygen. The enzyme catalase is a hemoprotein consisting of four heme groups. Heme serves to react with peroxide compounds. Hydrogen peroxide will cause death in bacteria that cannot lyse the toxic content of H_2O. The enzyme catalase plays a role in the process of cell lysis (Pulungan and Diana, 2018). Need to know the enzyme catalase is significantly beneficial to soil and plants. One of the benefits of the enzyme catalase for plants is proven by the overall report that the enzyme is located in the peroxisome which plays an important role in plant growth, development, and stress response is also associated with fruit ripening (Wang, et al., 2019). The results of research by Kaushal, et al (2018) show that the enzyme catalase can be an indicator of bioremidiation, especially the remediation of oil-polluted soil. The enzyme catalase also plays a role in purifying water polluted with textile waste by removing hydrogen peroxide contained in the water.

The action of enzymes is very specific. The enzyme catalase is produced by aerobic obligate bacteria that act as catalysts in the lysis of hydrogen peroxide to produce oxygen. The catalase test is carried out to distinguish catalase-negative bacteria and catalase-positive bacteria characterized by bacteria that produce oxygen gas bubbles (Kusnadi et al, 2012).

The laboratory located at Campus 2 UIN Walisongo Semarang has a variety of plants

that thrive around it. This prompted researchers to test the activity of the enzyme catalase in several soil samples taken from the roots of several plants, such as ketapang, mango, and guava. This study is also important to analyze several soil samples in which there are microorganisms, especially rhizosphere bacteria that have the ability to produce the enzyme catalase.

MATERIALS AND METHODS

This research was carried out at the Microbiology Laboratory of Walisongo State Islamic University Semarang from September to October 2022.

1. Research Subject

Rhizosphere soil bacterial culture obtained around Campus 2 Laboratory UIN Walisongo Semarang.

2. Tools and Materials

This study used tools in the form of beakers, petri dishes, latex gloves, Laminar-air flow (LAF), test tubes, test tube racks, round ose needles, straight-end ose needles, spray bottles, object glass, hot plate stirrer, bunsen burners, 500 ml Erlenmeyer, autoclaves, spatulas, volume pipettes, analytical balances, and microscopes.

As well as the materials used consist of soil samples from (location), equates, agar nutrient media (NA), cotton, 70% alcohol, violet crystal paint, iodine, alcohol, immersion oil, safranin, and H_2O_2 solution.

3. Research Methods and Design

The research method and design used refer to the research of Pambudi, et al (2016) as follows.

a. Manufacture of rhizosphere soil bacterial culture

The first step is the manufacture of bacterial growth media by taking nutrient powder so that as much as 5 grams to make 250 ml NA medium then put into Erlenmeyer measuring 500 ml then added equates as much as 250 ml. The solution is homogenized using a hot plate stirrer, where Erlenmeyer is tightly closed. Then, a test tube is prepared along with a cotton plug cap. After that, the medium is sterilized using an autoclave.

After that, rhizosphere soil samples were weighed as much as 5 grams with dilution up to 10-5 and inoculated on a new medium using a 4-quadrant streak plate. Next, it is incubated at room temperature for 24-48 hours until separate colonies grow in quadrants 3 or 4. Then, the test tube is made to be tilted as a place for inoculation of each different colony/isolate obtained from the streak plate petri dish to be tilted in the test tube. Next, it is coded on each isolate and incubated for 48 hours which is put into a cooler to inhibit growth.

b. Characterization of Rhizosphere Soil Bacteria

Bacteria resulting from isolation are characterized macroscopically on NA media. Macroscopic characterization of bacteria is seen in terms of shape (circular, filamentous, rhizoid, punctiform, irregular, and spindle), color, margins (entire, undulate, lobate, erose, filamentous, and curled), and elevation of the colony (flat, raised, convex, pulvinate, and umbonate).

c. Painting Gram of Bacteria

Each isolated bacteria is then smeared by adding 2 round ose water to the glass object and then adding a bacterial culture using straight ose to the water on the glass object and then flattened. Then it is dried and heated by passing glass objects on the fire 3 times.

After making the bacterial smear is complete, then add violet crystal paint to the bacterial smear until it is evenly covered which is left for 20 seconds. Then rinsed with water for 2 seconds. After that, a bacterial smear is added to iodine until it is evenly closed and left for 1 minute. Rinsed again under running water. Then, the smear is dosed with alcohol for 10-20 seconds and watered again for 2 seconds. After that, the smear is added safranin until evenly closed which is left for 20 seconds. Drizzled with

water for 2 seconds, and then dried until smear-mongering. Lastly, it is observed on a microscope with a magnification of 1000x.

d. Catalase Enzyme Testing of Rhizosphere Soil Bacteria

Bacterial culture is applied to the slide glass/object glass with ose needles aseptically. Then, on the preparation of the bacterial culture results dripped a solution of hydrogen peroxide. Preparations were observed, bacteria with positive catalase would form oxygen bubbles while negative catalase would not form oxygen bubbles.

4. Data Collection Techniques

The data collection technique used is a descriptive data collection technique. This technique is used to analyze data by describing data that has been collected from various literature.

RESULTS AND DISCUSSION

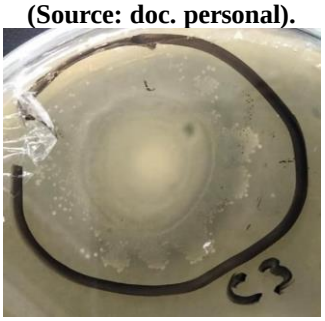
1. Isolation and Characterization of Bacteria

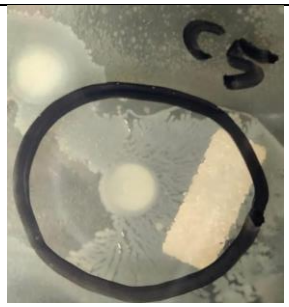
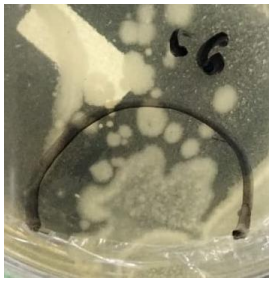
According to Alam, et al (2013), isolation is microorganisms that come from nature and are deliberately grown in an artificial medium. The principle of such isolation is the separation of bacteria from their colony.

The rhizosphere soil bacteria that were successfully isolated were as many as 9 isolates. Macroscopic characterization of bacterial morphology aims to identify that bacteria are different from one another which can be seen from several aspects. According to Zuraidah, et al (2020), the macroscopic character of bacteria can be seen from the color, edges, shape, and elevation of the colony.

The morphological characteristics of the nine isolates can be seen in Table 1.

Table 1. Morphological characteristics of rhizosphere soil bacteria.

Isolate	Figure	Isolate	Figure
C1		C2	
	Figure 1. Isolate form C1. (Source: doc. personal).		**Figure 2. Isolate form C2. (Source: doc. personal).**
C3		C4	
	Figure 3. Isolate form C3. (Source: doc. personal).		**Figure 4. Isolate form C4. (Source: doc. personal).**

Isolate	Figure	Isolate	Figure
C5	 **Figure 5. Isolate form C5. (Source: doc. personal).**	C6	 **Figure 6. Isolate form C6. (Source: doc. personal).**
C7	 **Figure 7. Isolate form C7. (Source: doc. personal).**	C8	 **Figure 8. Isolate form C8. (Source: doc. personal).**
C9	 **Figure 9. Isolate form C9. (Source: doc. personal).**		

Based on Table 1, it is known that the nine isolates have different morphological characteristics as seen in Table 2 as follows.

Table 2. Identification characteristics of rhizosphere soil bacteria.

Isolate	Form	Margin	Elevation	Colour
C1	Filamentous	Filamentous edges	Flat colony	Cream
C2	Irregular	Undulate-edges (wavy)	Umbonate colony	Cream
C3	Circular	Entire-edges	Raised colony	Cream
C4	Circular	Erose-edges	Flat colony	Cream
C5	Rhizoid	Edge	Flat colony	Cream with lobate
C6	Irregular	Grooved-edges	Umbonate colony	Cream
C7	Punctiform	Undulate-edges (wavy)	Doted colony	Cream
C8	Irregular	Grooved-edges	Flat colony	Cream-colored clouds
C9	Filamentous	Filamentous edges	Umbonate colony	Cream

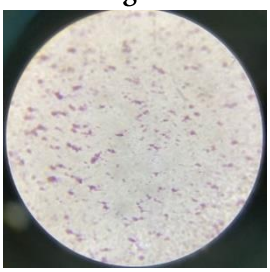
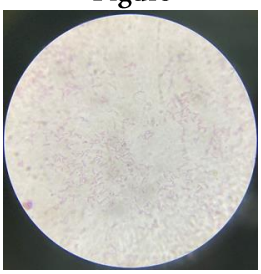
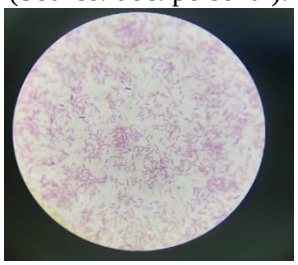
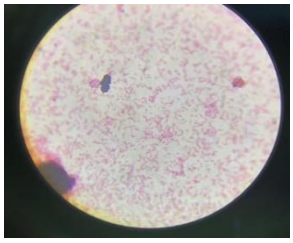
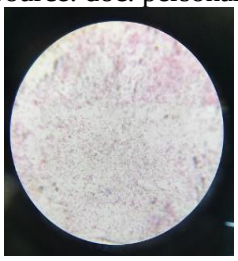
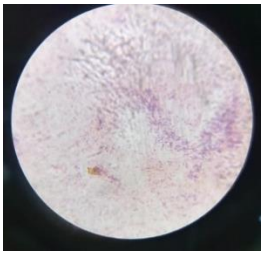
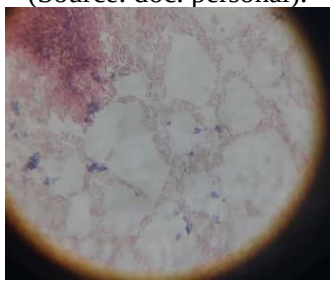
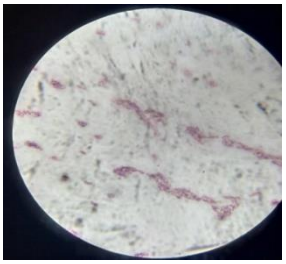
2. Gram Painting

According to Agustine (2018), gram painting is a staining technique used to identify microorganisms (bacteria). Gram painting aims to identify microbes.

The gram painting method is the administration of a solution of crystal violets, alcohol, safranin, iodine, and equates. Crystal violets are used to give microbes purple as color

Primary. The alcohol solution is used to rinse the primary solution. Safranin is used to give red to microbes as a secondary color. Iodine solution is used to strengthen color binding by bacteria. Aquades solution is used to rinse violet crystals, iodine, and safranin (Suarni, 2013 in Agustine 2018).

Table 3. Gram Painting

Isolate	Figure	Isolate	Figure
C1		C2	
	Figure 1. Painting Gram Isolate 1 (Source: doc. personal).		Figure 2. Painting Gram Isolate 2 (Source: doc. personal).
C3		C4	
	Figure 3. Painting Gram Isolate 3 (Source: doc. personal).		Figure 4. Painting Gram Isolate 4 (Source: doc. personal).
C5		C6	
	Figure 5. Painting Gram Isolate 5 (Source: doc. personal).		Figure 6. Painting Gram Isolate 6 (Source: doc. personal).
C7		C8	
	Figure 7. Painting Gram Isolate 7 (Source: doc. personal).		Figure 8. Painting Gram Isolate 8 (Source: doc. personal).

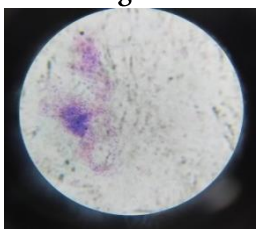
Isolate	Figure	Isolate	Figure
C9			

Figure 9. Painting Gram Isolate 9 (Source: doc. personal).

Based on the observations, it is known that each isolate after staining was seen the presence of 6 gram-negative bacteria (C1, C2, C3, C4, C5, and C7) and 3 isolates of positive bacteria (C6, C8, and C9).

According to Putri et al. (2018) in Rahmatullah, et al (2021) Microscopic identification can be done by gram staining. In gram staining, you will be able to distinguish between two types of bacteria, namely gram-positive bacteria and gram-negative bacteria. Lay (1994) in Nurhidayati, et al (2015) state that gram-positive bacteria have cell wall characteristics with thick peptidoglycan content so that violet crystal dyes are maintained even though rinsed with alcohol which will be purple. While gram-negative bacteria have cell wall characteristics with high lipid content so safranin dye is bound when rinsed with alcohol which will be colored red.

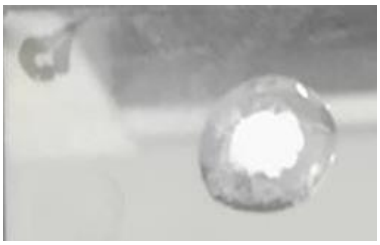
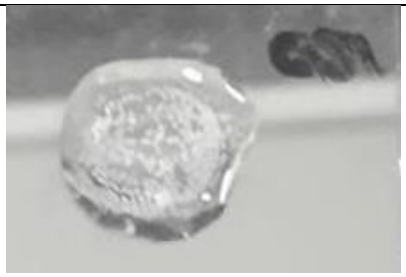
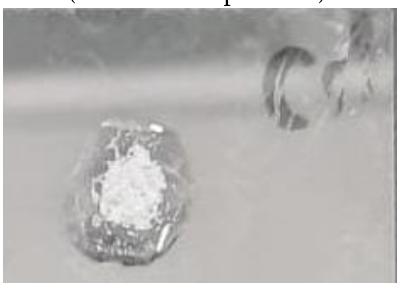
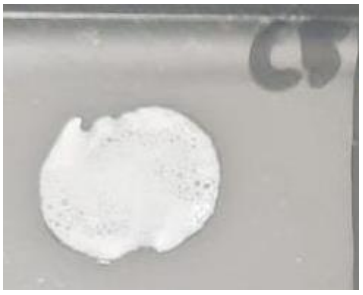
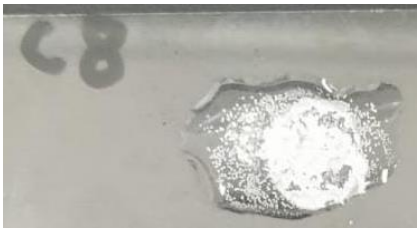
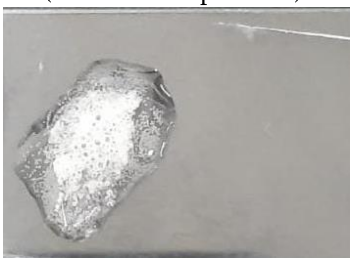
Based on Table 3 which shows the results of observations of painting the ninth gram of bacterial isolate using a microscope with a magnification of 1000x, the color and shape of bacteria are visible. C1 isolates include gram-negative bacteria (colored red) that are coccus-shaped like ellipses or spheres. Isolate C2 – C5 includes gram-negative bacteria (colored red) whose shape is the same, namely bacilli. According to Sinta (2008) in Sumanti (2014), bacillus or bacillus is shaped like a rod. C6 isolates include gram-positive (purple-colored) bacteria that are bacilli-shaped or rod-like. C7 isolates include gram-negative (red-colored) bacteria that are bacilli-shaped or rod-like. C8 isolates include gram-positive (purple-colored) bacteria in the form of streptococcus. And finally, C9 isolates include gram-positive (purple-colored) bacteria that are bacilli-shaped or rod-like.

Table 4. Identification characteristics of rhizosphere soil bacteria.

Isolate	Form	Keterangan
C1	Coccus	Gram-negative
C2	Basil	Gram-negative
C3	Basil	Gram-negative
C4	Basil	Gram-negative
C5	Coccus	Gram-negative
C6	Basil	Gram-positive
C7	Basil	Gram-negative
C8	Streptococcus	Gram-positive
C9	Basil	Gram-positive

3. Catalase Test

Table 5. Catalase Test

Isolat	Gambar	Isolat	Gambar
C1	 Figure 1. Isolate Catalase Test 1 (Source: doc. personal).	C2	 Figure 2. Isolate Catalase Test 2 (Source: doc. personal).
C3	 Figure 3. Isolate Catalase Test 3 (Source: doc. personal).	C4	 Figure 4. Isolate Catalase Test 4 (Source: doc. personal).
C5	 Figure 5. Isolate Catalase Test 5 (Source: doc. personal).	C6	 Figure 6. Isolate Catalase Test 6 (Source: doc. personal).
C7	 Figure 7. Isolate Catalase Test 7 (Source: doc. personal).	C8	 Figure 8. Isolate Catalase Test 8 (Source: doc. personal).
C9	 Figure 9. Isolate Catalase Test 9 (Source: doc. personal).		

All living things carry out metabolism, including microorganisms such as bacteria. Metabolism is a chemical reaction that occurs in cells to produce energy that plays a role in the synthesis of cell components and activities. All metabolic activities of the process are catalyzed by enzymes, one of which is the enzyme catalase. Aerobic obligate bacteria can produce the enzyme catalase which acts as an oxygen-producing catalyst from the breakdown process of hydrogen peroxide. The catalase test aims to distinguish negative and positive catalase bacteria characterized by the presence of oxygen gas bubbles (Satwika, et al, 2021).

The catalase test consists of aerobes and anaerobes, where aerobes mean that bacteria need oxygen so they produce a lot of bubbles. While bacteria that do not need oxygen are called anaerobic bacteria. The use of H_2O_2 is toxic so bacteria will produce more bubbles (Lakitan, 2011).

Based on the results of the catalase test shown in Table 1, positive results were obtained for the nine isolates. The positive result is characterized by the presence of bubbles when dripped with H_2O_2. This is to the statement of Luhova, et al (2003) in Suhartanti, et al (2010) that catalase activity can be known by the formation of O_2 gas bubbles.

When bacteria respirate, a catalase enzyme mechanism occurs that breaks down hydrogen peroxide. The defense system will be formed by bacteria when toxic H_2O_2 (Murali, 2017). The activity of the catalase enzyme is evidenced by the presence of oxygen bubbles as in studies that have been conducted.

CONCLUSIONS

The nine isolates of rhizosphere soil bacteria found around the Campus 2 Laboratory of UIN Walisongo Semarang showed the activity of the enzyme catalase which is characterized by the formation of oxygen bubbles when dripped with hydrogen peroxide.

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