ISOLATION AND IDENTIFICATION OF PROFENOFOS PESTICIDE-TOLERANT BACTERIA FROM AGRICULTURAL LAND IN SLEMAN REGENCY

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ABSTRACT

The intensive application of organophosphate pesticides containing profenofos as the active compound may lead to soil contamination and the emergence of pesticide-tolerant bacterial strains with potential for bioremediation. Comprehensive identification and characterization of these bacteria are crucial to support sustainable agricultural and environmental management practices. The aim of the study is to analyze the characteristics and identify indigenous bacteria tolerant to profenofos pesticide in Sleman agricultural land, DIY. This study is qualitative research using an exploratory-descriptive. Isolation was carried out by dilution to a level of 10-7 followed by a spread plate on Mineral Salt Medium (MSM) which was given with 100 ppm profenofos pesticide. Bacterial isolates were characterized and then identified using profile matching with reference genera. Based on the results of the study, it is found that 10 bacterial isolates tolerant to organophosphate pesticide with the active ingredient profenofos that have a similarity index of ≥70%. Based on the reference bacteria, 5 different genera are obtained, namely Paracoccus (D3, D8), Micrococcus (D5, D6, D9), Enterococcus (D2, D10), Arthrobacter (D1, D4), and Neisseria (D7). The bacterial isolates obtained have different characteristics. The characteristics of these bacteria are gram positive and negative bacteria with round, irregular, and rhizoid shapes. Their margins are lobate, curled, entire, undulate, and ciliate. Their elevations are umbonate, raised, and flat. The color of the bacteria is white to light orange, with various physiological test results.

Keyword: *Bacteria, Characterization, Identification, Pesticide, Tolerance*

Introduction

Pesticides are a mixture of several chemicals that function as pest control (Suahartono,2014). Pesticides are used by farmers to maintain the quality of their agricultural products from plant pests (Zelila, 2016). Pesticides that are widely used by farmers, one of which is the organophosphate group. Organophosphate pesticides such as prophenophosphates are broad-spectrum contact and gastric toxins. Profenofos is an insecticide that is useful for controlling pests such as *Lepidoptera* and mites (National Center for Biotechnology Information, 2023).

The application of profenofos pesticides on plants causes fewer pests, but there are negative impacts caused. Pesticides have an impact on the environment because they are a source of pollutants that are very harmful to the environment both in the soil and in the waters (Tayade *et al.*, 2016). Environmental pollution causes disruption of the life system of soil organisms because Pesticides not only kill plant pests, but also useful organisms in the soil (Wijaya, 2014). Pesticides applied to the environment affect the diversity of soil bacteria and soil nutrient content is affected by the application of pesticides to the environment (Asri *et al.*, 2018).

Extreme environments due to pesticide contamination in bacterial habitats require it to adapt. Bacteria are basically organisms that depend on the environment, so bacteria are able to survive if there are substances or compounds in their habitat that can help their life cycle (Nurbaeti, 2014). Organic pollutants can be useful as a source of carbon and energy in the bacterial growth process (Fidiastuti *et.*

al., 2019). Most of the organophosphate pesticides present in the environment are degraded by bacteria as a source of carbon and/or phosphorus nutrients (Singh & Walker, 2006). Bacterial cells can directly benefit from the mineralization of pollutants intact due to the presence of functional enzymes (Lumbanraja, 2014). The enzymes produced by the bacteria will catalyze the breakdown of the substrate into a simpler product (Al-Hawash *et al.,* 2018).

Native bacteria are bacteria that are isolated from the environment in which they live. Native bacteria have a wide range of benefits for humans and the environment, so they have the potential to be bioremediation (Ikerismawati, 2019). The use of bacteria to remove and degrade toxic compounds from sewage-contaminated soil is an efficient technique and can clean up the polluted environment (Tabit & Naggar, 2013). Seeing the potential for indigenous bacteria to degrade pesticide pollutants, it is necessary to isolate and identify indigenous bacteria in soil exposed to organophosphate pesticides made from prophenophosphate. This needs to be done as a first step in order to support bioremediation technology.

Methods

Material

The ingredients used in this study include aquades, absolute alcohol, aluminum foil, 70% alcohol, bacteriological agar, glucose, galactose, HCl, H2O2 3%, iodine, gauze, cotton, umbrella paper, label paper, filter paper, KOH, crystal violet, lactose, malachite green, Minimal Salt Medium (MSM), Nutrient Agar (NA), Nutrient Broth (NB), Nutrient Gelatin (NG), NaOH, wrab plastic, Curacron brand organophosphate pesticide, soil samples, Sulphide Indole Motility (SIM), Simmon Citrate Agar (SCA), sucrose, safranin, spiritus, Strach Agar (SA).

Equipment

The tools used in this study include autoclaves, bunsen, cetok, drygalski, erlenmeyer, beaker glasses, measuring cups, hot plates, glass objects, rubber bands, plastic bags, matches, cuvettes, cameras, measuring flasks, LAF, masks, magnetic stirrer, microscopes, micropipettes, ovens, ose kolong, ose needles, petridish, drip pipettes, latex gloves, spectrophotometry, soil testers, Durham tubes, test tubes, eppendorf tubes, pipette tips, filter syringes, analytical scales, vortexes and shakers.

Working Procedure

Abiotic Data Collection

The physical properties of the soil taken i.e. pH and soil moisture are observed with *a soiltester* at each observation point. Soil chemical properties such as organic matter, C-organic, total N, P, and K content were carried out at the Laboratory of the Faculty of Geography UGM. Meanwhile, testing the content of organophosphate pesticides was done in the laboratory.

Soil Sampling

Soil sampling was carried out on agricultural land in Sleman Regency, DIY. Soil samples were taken with three points by paying attention to the area of agricultural land using a cetok, then homogenized in plastic and stored in containers Falcon Eppendorf. The samples were taken to the Microbiology Laboratory of FMIPA UNY for isolation research and bacterial identification.

Pesticide Tolerant Bacteria Isolation Prophenonos

The soil samples that have been taken are then weighed using an analytical balance of 10 grams and dissolved in 100 ml of sterile aqueducts containing 0.85% physiological NaCl and homogenized using a shaker for 1 hour at a speed of 150 rpm. Bacterial isolation is carried out by means of spread plates. In the spread plate method , starting from 1 ml, the suspension is put into 9 ml of aqueducts in a test tube and then homogenized. Tiered dilution is carried out after homogenization to 10-7 by means of 1 ml of dilution 10-1 added to a test tube containing 9 ml of aquades so that a dilution of 10-2 is obtained, and so on until a dilution of 10-7 is obtained. Cultures from 10-5 to 10-7 dilution were inoculated on MSM media so that those given 100 ppm pesticides were then incubated at a temperature of 37oC for 1-2 days

with the cup position upside down. Bacterial isolates were selected by paying attention to the characteristics (color, shape, and size of the colonies) that differ from each other (Li *et. al.,* 2019). The selected isolate is then purified (purified) and transferred to a new growth medium of Nutrient Agar (NA) and incubated for 24 hours. One colony was transferred to the new NA media over and over again until a pure culture was obtained.

Phenotypic Characterization of Prophenonotic Pesticide-Tolerant Bacteria

Morphological Characteristics

Macroscopic morphological observations were carried out using Nutrient Agar (NA) plate and Nutrient Agar (NA) slant media. Small pettidles are inoculated using the point method using a needle ose. Growing bacterial colonies are observed in shape, color, edges, diameter, and elevation. Colony observation in the NA slant was carried out by growing bacteria in the NA slant media and observing the morphology of the colony. Meanwhile, microscopic morphology is carried out gram painting to see the shape of the cell and the gram (positive/negative) properties of bacteria and endospore painting to determine the presence of bacterial endospores.

Gram painting is carried out with bacteria that are 16 – 18 hours old placed into a glass of an object that has been dripped with aqueducts while leveling to make an isolate spread and then fixed (passed several times) on the Bunsen fire. The painting consists of crystal violet (gram A), lugol iodine solution (gram B), absolute alcohol (gram C) and safranin (gram D). The results of gram painting were observed under a microscope with purple gram-positive bacteria and red gram-negative bacteria (Brown & Smith, 2015).

Endospore painting is carried out with bacteria that are 48 hours old by making a bacterial application and fixing it on a bunsen fire. The painting consists of a solution of malachite green and safranin. Heating is carried out at the time of dripping malachite green, which is covered with filter paper so that dye enters the endospores. On observation, it can be seen that the endospores will be bluish-green and round or oval in shape, while the bacterial cells will be red (Brown & Smith, 2015).

Physiological Characteristics

Cattalase Test

The test was carried out by applying bacterial isolates (without sterile aqueducts) to the glass of the object asepsis. Bacterial isolate application dripped with 3% H_2O_2%. A positive (+) reaction is shown when bubbles form in the isolate dripped with 3% H_20_2, while the absence of bubbles shows a negative result (-) (Putri *et al.,* 2012).

Motility Test

The method of testing is that the bacterial isolates are inoculated in an upright NA medium using a needle ose. Bacterial isolates grown in NA media were incubated for 24–48 hours at a temperature of 28oC. If the isolates only grow along the puncture mark, it indicates non-motile while if it grows widening around the incision, it shows motile (Harti, 2015).

Carbohydrate Fermentation Test

The carbohydrate test which included a solution containing lactose, glucose, maltose, galactose and sucrose with *a phenol red* indicator was put into a test tube of 5 ml NB filled with durham tube. A total of 1 ounce of bacterial isolate is added to each sugar. Incubation for 24 hours at 37°C. If the color of the media changes to yellow, it means that the bacteria are positive (+) forming acids from fermentation, if the media remains red, it shows a negative result (-) (Rostinawati & Lestari,2017).

Citrate Test

The method of citrate test is to use a needle by inserting it at the end of the media and then scratching it zig-zag. The next step is to incubate for 24-48 hours at a temperature of 28 ^oC. If the media changes color to blue, it shows a positive (+) reaction, while if the media remains green, it shows a negative reaction (-) (Syauqi, 2017).

Gelatin Hydrolysis Test

Bacterial cultures were inoculated on gelatin media aseptically and then incubated for 2 days. The test tube is then inserted into the refrigerator for 25 minutes. Observations included the occurrence or absence of gelatin dilution compared to controls (Ega *et al.,* 2017).

Oxygen Requirement Test

The bacteria were taken using an ose loop and then put into a test tube containing NB media and incubated for 3 days at a temperature of 37°C. A positive reaction can be seen by the appearance of bacterial growth forming a cloudy color on the media. The cloudy color on the surface of the media indicates that the bacteria are aerobic, the cloudy color on the entire media indicates that the bacteria are facultative, while the cloudy color on the base of the media indicates that the bacteria are anaerobic. Negative results can be indicated by the absence of discoloration in the media (Febriani, 2021).

H2S Test

The test was conducted using SIM media to make it upright by isolating bacteria by inoculating using a stab (stab down) and the culture was incubated for 2 x 24 hours at a temperature of 37 °C. The change of the media to blackness showed positive results, because there was a reaction of Fe to black FeS due to the formation of metal sulfide (Azizah, 2016).

Amylum Starch Hydroxy Test

Bacterial isolates were inoculated by being lined using ose loop on the Starch Agar (SA) *plate* media and inoculated at 37oC for 2 days. The media is then dripped with an iodine solution. Positive testing is characterized by the formation of clear zones around the bacterial growth area after being given a few drops of lugol iodine solution (Wahyuni *et al.,* 2014).

Salinity Effect Test

The pH test was carried out using NB media. Three test tubes containing NB media with salinity levels of 0%, 0.1%, 10%, and 20% were inoculated using ose *loop* and incubated for 24 hours at room temperature in *a shaker* of 150 ppm. Bacterial growth can be observed by looking at its turbidity using spectrophotometry (Islamiah & Rahmawati, 2017).

pH Effect Test

Test tubes containing NB media with pH 5, 7 and 9 were inoculated using an ose *loop* and incubated for 24 hours at room temperature in a *shaker* of 150 ppm. Growing bacteria can be observed by looking at their turbidity using spectrophotometry (Islamiah & Rahmawati, 2017).

Identification of Prophenophosphate Pesticide-Tolerant Bacteria

Bacterial identification was carried out by looking for reference bacteria from Bergey's Manual of Determinative Bacteriology. Descriptive-exploratory data processing on bacteria is carried out by way of profile matching of reference bacteria. Data in the form of bacterial characteristics results and reference bacterial characteristic data were analyzed and made a dendrogram using the Multi-Variate Statistical Package (MVSP) 3.1 applications with a Cluster Analysis test. The similarity index of each bacterium was used a Simple Matching Coeficient (SSM) coefficient and grouping was carried out using the Unwight Pair Group Method (UPGMA) algorithm.

Results and Discussion

Environmental Characteristics of Soil Samples

The diversity and abundance of bacteria is influenced by the abiotic factors of the soil from the bacterial farmland is isolated, so that there is a dynamic interaction between the two. These environmental conditions consist of chemical factors, physical factors and the presence of

organophosphate pesticides with the active ingredient prophenophosphate in the soil. Environmental caractéristiques data are used to describe the environmental conditions when bacteria are discovered.

The bacteria were isolated from agricultural land using the pesticide Profenofos, namely the Curacron brand, but based on the test results, no pesticide residues were found. This can be caused by several factors such as bacterial activity, climate and edaphic. Edaphic factors such as pH and soil moisture affect the process of bacterial degradation to pesticides and affect the growth of soil bacteria, which is related to enzyme activity. The activity of these enzymes will work to affect the process of decomposition of organophosphate pesticide compounds in the soil. The presence of soil organic matter affects the diversity and abundance of bacteria which it uses as a source of nutrients.

Climate factors also affect the presence of pesticide residues in the soil. Soil sampling is carried out when it is raining heavily so that soil washing can occur considering that the location of agricultural land has a high enough slope so that there is a flow of water. This farmland also has a location close to the river so that it is possible for the pesticide to flow into the river.

Insulation and Purification of Profenofos Pesticide Tolerant Bacteria

The number of bacteria tolerant to prophenophosphate pesticides obtained was 10 isolates. Bacteria are obtained through the Spread Plate on the media Mineral Salt Medium (MSM) agar to be given 100 ppm of prophenotypic pesticides. The addition of profenofos pesticides to the media is carried out for the screening of tolerant bacteria where organophosphate pesticides with the active ingredient prophenophosphate are toxic pesticides that can affect bacterial growth.

Extreme environments such as the addition of pesticides to MSM media as much as 100 ppm require bacteria to adapt. Bacteria that are able to grow in a medium to which the pesticide is added profenofos 100 ppm are bacteria that are able to use pesticide active compounds as a source of carbon (Lumantouw, 2013). The use of minimally carbon media is evidence that bacteria are able to utilize the carbon and phosphorus contained in organophosphate pesticides by mineralization. Bacteria utilize nutrients from pesticides to carry out activities to fulfill their lives.

Bacterial colonies that have been obtained from the isolation process are then purified using NA Plate by the Quadrant Streak which is done repeatedly. After the pure bacteria are then transferred to the NA media Slant. This purification aims to obtain pure isolates of each representative of the bacterial group with the same colony morphological characteristics (Marista *et al.*, 2013). The results of isolation and purification of profenofos pesticide-tolerant bacteria can be seen in Figure 1a is the result of isolation using the Spread Plate and b) is a process of purification of prophenotic pesticide-tolerant bacterial isolates.

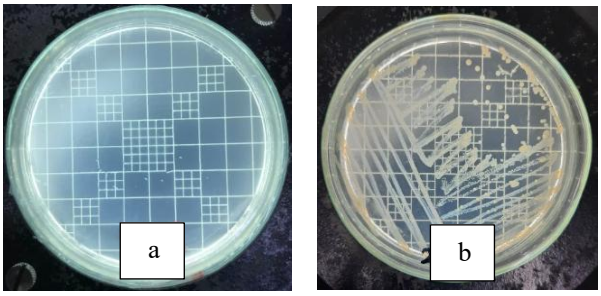

Figure 1. Results of the isolation and purification process of profenophose pesticide-tolerant bacteria

Characteristics of Pesticide Tolerant Bacteria Prophenophosphates

The characterization of the microscopic morphology of profenofos pesticide-tolerant bacterial isolates was carried out in two ways, namely gram painting and endospore painting. Observations of gram staining and endospore staining were observed using a microscope with a magnification of 400x10. The results of gram staining were obtained that 4 isolates were gram-positive marked by purple bacterial cells, while 7 isolates were gram-negative marked by red bacterial cells. The cause of the difference in gram staining is likely to occur due to differences in the composition and structure of the bacterial cell wall (Beooks *et al.*, 2017). The peptidoglycan layer of the cell wall material in gram-positive bacteria is more than that of gram-negative bacteria, which is 50%, while gram-negative bacteria only consist of 5-

10% (Putri *et al.,* 2017). The results of the gram painting can be seen in Figure 2a is a gram-positive bacterium and b is a gram-negative bacterium.

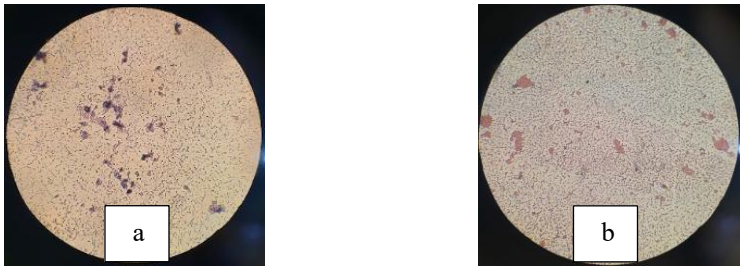

Figure 2. Microscopic Morphological Characteristics of Prophenonos Pesticide-Tolerant Bacteria

Table 1. Results of Microscopic Characteristics of Profenofos Pesticide-Tolerant Bacteria

Yes	Isolation Code	Properties of Grams	Cell Shape	Cell Arrangement	Endospore
1.	D1	Positive	Coccus	Monococcus	Negative
2.	D2	Positive	Coccus	Monococcus	Negative
3.	D3	Negative	Coccus	Monococcus	Negative
4.	D4	Positive	Coccus	Diplococcus	Negative
5.	D5	Positive	Coccus	Monococcus	Positive
6.	D6	Positive	Coccus	Monococcus	Positive
7.	D7	Negative	Coccus	Monococcus	Negative
8.	D8	Negative	Coccus	Monococcus	Negative
9.	D9	Positive	Coccus	Monococcus	Negative
10.	D10	Positive	Coccus	Monococcus	Negative

The second microscopic identification carried out was endospore staining. The results of endospore staining, namely isolates coded D5 and D6, showed that there were green endospores when observed due to dye Malachite Green. Its vegetative cells have a red color due to the dye safranin. Generally, bacterial endospores are not easy to dye with dye, but once dyed, it will be difficult to remove. This is the basis of the endospore painting method using dyes Malachite Green. Heating in endospore painting functions so that dyes can penetrate into the endospores.

Based on the results of the macroscopic morphological characterization of bacterial colonies, namely four isolates (D3, D7, D8 and D10) have a shape Irregular. D4 isolate has a shape rhizoid, while isolates D1, D2, D5, D6 and D9 have the shape of round D1, D2, D4, D7, D10 isolates have a margin of bacterial colonies São Paulo features notched edges. Bacterial isolates D5 and D6 have bacterial margins Undulate that is, the wavy edge. D3 isolates have a margin curled i.e. curled up, while the D9 isolate, which is the ciliate which is a thread-like edge. On observation, it can be seen that two bacteria have elevation Umbonate namely D1 and D2 isolates which are characterized by convex shapes in the more prominent parts. Isolates D3, D4, D5, D8, D9 and D10 have elevation raised which is characterized by real thinness visible but flat on the entire surface. Isolates D6 and D7 have elevation flat which is characterized by a height that is difficult to measure, noticeably flat with the media. The color of the bacterial isolate tolerated by the pesticide profenofos in the observation varied, namely yellowish-white, yellow, light orange and white. The size of all bacterial isolates falls into the large category.

The second macroscopic morphological characteristic is bacterial growth in the Nutrient Agar (NA) slant media. Nutrient Agar (NA) media is a solid medium that is commonly used to grow bacteria in the laboratory. Bacterial growth in the NA slant is useful for determining the morphology of colonies in the tubular media. Bacterial isolates D1, D3, D5, D6, D8, D9 and D10 have a filiform growth type characterized by growth along an even inoculum container. D2 isolates have an echonulate growth type characterized by growth along the inoculated or jagged container. D4 isolate has a beaded growth type

characterized by pearl-like growth of granular grains along the inoculation container and D7 isolate has a spreading growth type characterized by an even growth of several mm around the line of the inoculation container.

Table 2. Results of Colony Characteristics on *Nutrient Agar* (NA) *Plate Media*

Isolation Code	Characteristics of colonies in Nutrient Agar plate media					Colony Color
	Configuration	Margin	Elevation	Diameter	Size	
D1	Round	Lobate	Umbonate	26 mm	Big	Yellowish white
D2	Round	Lobate	Umbonate	26 mm	Big	Yellowish white
D3	Irregular	Curled	Raised	15 mm	Big	Light orange
D4	Rhizoid	Lobate	Raised	22 mm	Big	Yellowish white
D5	Irregular	Undulate	Raised	26 mm	Big	Yellow
D6	Round	Undulate	Flat	24 mm	Big	Yellowish white
D7	Irregular	Lobate	Flat	34 mm	Big	White
D8	Irregular	Entire	Raised	18 mm	Big	Light orange
D9	Round	Ciliate	Raised	35 mm	Big	Yellow
D10	Irregular	Lobate	Raised	24 mm	Big	Yellow

Table 3. Growth of Bacterial Isolate in Nutrient Agar (NA) Media slant

Yes	Isolation Code	Growth Type
1.	D1	*Filiform*
2.	D2	*Echonulate*
3.	D3	*Filiform*
4.	D4	*Beaded*
5.	D5	*Filiform*
6.	D6	*Filiform*
7.	D7	*Spreading*
8.	D8	*Filiform*
9.	D9	*Filiform*
10.	D10	*Filiform*

Physiological testing is carried out to determine the properties of bacteria tolerant to the pesticide prophenophosphates in using various types of carbon sources and other chemical compounds to analyze their metabolic ability. The results of the motility test showed that the pesticide tolerant bacteria were positive or motile (moving) characterized by diffuse growths on puncture marks in the NA media. This indicates that prophenophos-tolerant bacteria have a motor device.

Prophenophos pesticide-tolerant bacteria have the enzyme catalase that can catalyze H_2O_2 into water (H_2O) and oxygen (O_2). The catalase test had a positive value, which was characterized by the presence of water bubbles or bubbles after the bacterial isolate was dripped with hydrogen peroxide (H_2O_2) (Putri & Kurnia, 2018). This means bacteria have catalase enzymes that can catalyze H_2O_2 into water (H_2O) and oxygen (O_2).

The results of the H_2S production test on all bacterial isolates showed negative results which means the bacteria are unable to break down cystine so they cannot produce H_2S. The isolate does not produce

black-colored deposits. The results show that there are no changes to the SIM media. In the citrate needs test, it was found that D3, D4, D10 isolates had negative results marked by blue fixed color while the other 7 isolates, namely D1, D2, D5, D6, D7, D8 and D9 had positive results marked by a change in media color from blue to green. The change in color of the medium to blue is caused because bacteria utilize citrate as the only source of carbon so that the pH becomes alkaline and the color changes.

Table 4. Results of Physiological Characteristics (Biochemistry)

No	Physiological Characteristics	Isolation Code									
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
1.	Motility Test	+	+	+	+	+	+	+	+	+	+
2.	Cattalase Test	+	+	+	+	+	+	+	+	+	+
3.	H_2S Test	-	-	-	-	-	-	-	-	-	-
4.	Citrate Test	+	+	-	-	+	+	+	+	+	-
5.	Gelatin Hydroxy Test	-	-	-	-	+	+	-	-	+	-
6.	Hydroxy Starch Test	-	-	-	+	-	-	-	-	-	-
7.	Glucose Fermentation Test	+a/+g	+a/+g	+a/-g	+a/-g	-a/-g	-a/-g	+a/-g	-a/-g	-a/-g	+a/+g
8.	Sucrose Fermentation Test	+a/+g	+a/+g	+a/-g	+a/-g	-a/-g	-a/-g	+a/-g	-a/-g	-a/-g	+a/-g
9.	Maltose Fermentation Test	+a/+g	+a/+g	+a/-g	+a/-g	-a/-g	-a/-g	+a/-g	-a/-g	-a/-g	+a/+g
10.	Lactose Fermentation Test	+a/-g	+a/-g	+a/-g	+a/-g	-a/-g	-a/-g	+a/-g	-a/-g	-a/-g	+a/-g
11.	Galactose Fermentation Test	+a/+g	+a/+g	+a/-g	+a/-g	-a/-g	-a/-g	+a/-g	-a/-g	-a/-g	+a/+g
12.	pH 5 Growth Test	-	+	+	+	-	+	-	+	+	+
13.	pH 7 Growth Test	+	+	+	+	+	+	+	+	+	+
14.	pH 9 Growth Test	+	+	+	+	+	+	+	+	+	+
15.	0% Salinity Growth Test	+	+	+	+	+	+	+	+	+	+
16.	0.1% Salinity Growth Test	+	+	+	+	+	+	+	+	+	+
17.	10% Salinity Growth Test	+	+	+	+	+	+	+	+	+	+
18.	20% Salinity Growth Test	-	+	+	+	-	-	+	+	+	+

Caption : + (Positive); - (Negative); +a/+g (Acid-positive and gas-producing); +a/-g (Acid-positive and does not produce gas) ;-a/+g (Acid-negative and does not produce gas)

Bacterial isolates that have the gelatinase enzyme can hydroxy gelatin, namely D1, D2, D3, D4, D7, D8 and D10 marked surface of melted media. D5, D6 and D9 isolates have negative results characterized by solidifying media after being placed in the refrigerator. Hydroxy starch testing was carried out to see for the presence of the enzyme amylase which is marked by the appearance of clear zones after being dripped with iodine solution. The results of the observation showed that isolates D5, D6 and D9 had the ability to hydrolyze starch, while isolates D1, D2, D3, D4, D7, D8 and D10 did not have the ability to

hydrolyze starch characterized by no clear zone after being dripped with iodine solution (Putri *et. al,* 2012).

The pesticide-tolerant bacteria found were tested for glucose, maltose, lactose, and galactose fermentation, which was evidenced by the change in the color of the medium from red to yellow. This indicates that the six isolates are able to lower the pH of the medium to acid, which is a sign of the fermentation process. D5, D6, D8 and D9 isolates are isolates that are not able to utilize glucose, maltose, sucrose, lactose and galactose as their food sources.

The purpose of testing the effect of pH is to determine the ability of bacteria to live in an acidic pH of 5, neutral which is 7 and alkaline which is 9. D2, D3, D4, D6, D8, D9 and D10 isolates are able to grow in media that has a pH of 5, while D1, D5 and D7 are not able to grow in a medium with a pH of 5. All bacterial isolates are capable of growing in pH 7 and 8 media. pH plays an important role in the availability of nutrients for soil bacteria and also plays a role in the enzyme workability produced by bacteria (Irfan,2014).

Testing the effect of salinity to determine the ability of bacteria to survive in different salinity conditions. The salinity levels used are 0%, 0.1%, 10% and 20%. The increase in salinity value in the bacterial growth medium has a negative impact on the diversity and abundance of bacteria (Yulma *et. al.,* 2019). The salinity levels are 0% and 0.1%, i.e. all bacterial isolates are able to survive. At 10% levels, bacterial isolates other than D3 are able to survive and at 20% levels, i.e. bacterial isolates D1, D5, and D6 are able to survive. Salt has hygroscopic properties so it is able to absorb water in bacterial cells, so bacterial metabolism is disrupted due to lack of fluid and causes bacteria to die (Yulma *et. al.,* 2019).

Identification of Prophenophosphate Pesticide-Tolerant Bacteria

The results of the data analysis were in the form of a similarity matrix that showed a comparison of the similarity of the characteristics of the bacteria that had been isolated with the reference bacteria used. The kinship relationship between isolates is shown by the construction of a dendrogram where the greater the similarity value of the isolate, the closer the kinship relationship. Character testing in this study was carried out for phenotic numerical classification in the form of microscopic morphology, macroscopic morphology, and physiological character. The genus found is São Paulo São Paulo, Stuart O'Neill, Stuart O'Neill, Arthrobacter and Neisseria that have similarities ≥70%.

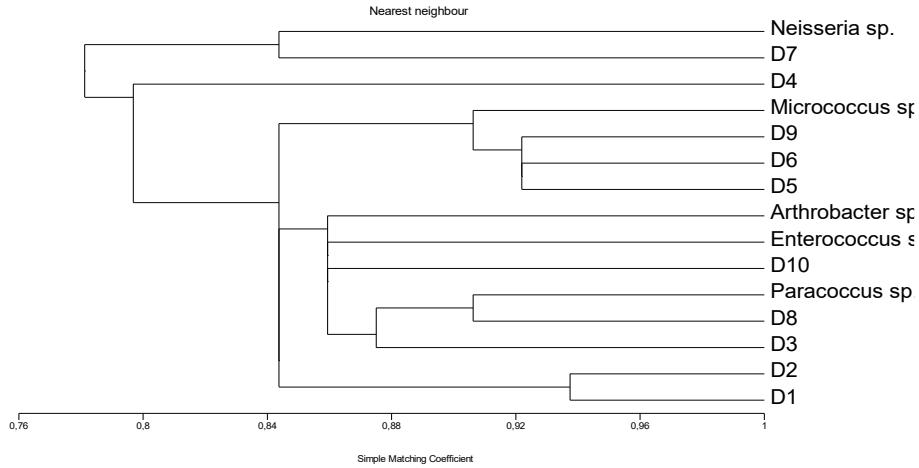

Figure 3. Dendrogram of Bacterial Strains Phenotic Numerical Classification

These five genera are prophenophosphate pesticide-tolerant bacteria which are able to grow and utilize the nutritional content in pesticides for their metabolism. It has also been reported that these bacteria are able to grow and degrade other types of organophosphates, but there is little information about the types of bacteria that grow on the pesticide prophenos. Bacteria Stuart O'Neill and Neisseria can survive in pesticide-contaminated conditions by adapting to utilize nutrient sources from pesticides by degradation (Sadashiv, 2015). Bacteria Stuart O'Neill and Neisseria found tolerant to

chlorpyrifos and mancozeb pesticides isolated from agricultural land in concentrations of 10 ppm (Azizah, 2016).

In addition to these two bacteria, there are bacteria Arthrobacter and Stuart O'Neill that are able to live in an environment containing chlorpyrifos pesticides. Chlorpyrifos pesticides are capable of being degraded by Arthrobacter in high concentrations (Mali *et. al.,* 2022). Bacteria *Stuart O'Neill* able to survive and decompose the pesticides chlorpyrifos, lambda-cyhalothrin, deltamethrin, spinosad and lufenuron (Gomes *et al*., 2020). Therefore Stuart O'Neill, Neisseria, Stuart O'Neill and São Paulo São Paulo can be identified as a profenofos pesticide-tolerant bacteria where chlorpyrifos is also an organophosphate pesticide.

In addition to chlorpyrifos, monocrotophos is also an organophosphate pesticide. Bacteria São Paulo São Paulo found from sludge which has the ability to survive and degrade monocrotophos and hebicides of the amine group quickly (Subhas, 2003). Monokrotophos and chloripyrifos are pesticides of the organophosphate group, just like prophenophosphates which have carbon and phosphate as well as ester bonds so that bacteria can be used as a source of nutrients. Bacteria can degrade organophosphate pesticides because it is possible to have a hydrolysis enzyme called phosphatase that converts organic phosphorus into inorganic phosphorus and esterase which is useful for breaking ester bonds into acids and alcohols (Rahmansyah & Sulistinah, 2009 ; Yulipriyanto, 2010). The enzymes produced by the bacteria will catalyze the breakdown of the substrate into a simpler product (Al-Hawash *et al.,* 2018).

In this study, bacterial isolates isolated on agricultural land in Sleman Regency were able to grow on MSM media that had been given 100 ppm profenofos pesticide. Pesticides added to mineral media can decrease the number of isolates tested because pesticides are toxic. Such toxic properties can inhibit metabolism and cause death (Utami *et. al.,* 2013). Bacteria that are able to grow in the medium to which pesticides are added are bacteria that are able to use pesticide active compounds as a source of carbon (Wahyuni *et. al.,* 2013). Bacteria Pesticide tolerant is a bacterium that has the ability to grow in the selection medium with pesticides added. Degradation is one of the activities of bacteria to survive or tolerate pesticides (Lumantouw *et. al.,* 2013).

The addition of organophosphate pesticides with the active ingredient profenofos in MSM media is carried out for the screening of tolerant bacteria where pesticides are toxic pesticides that can affect bacterial growth. Media Mineral Salt Medium (MSM) is a medium that has a minimum carbon source. The composition of MSM consists of mineral salts that are essential for bacteria. Mineral Salt Medium (MSM) can be used in the field of bioremediation due to the absence of a carbon source so that it forces bacteria to utilize other carbons such as carbon from pesticides (Prabharkaran, 2014). Tolerant bacteria are bacteria that have the power to survive in a certain environment in which there are substances that help in their life (Nurbaeti, 2014).

Bacteria do their activities need nutrients as a source of energy. Some of the essential nutrients that bacteria need are carbon, nitrogen and phosphorus (Wulan *et. al.,* 2012). Synthesis and cell growth and enzyme activity that bacteria produce to degrade pollutants require nutrients. The essential elements that bacteria need to meet nutrient availability are carbon (in the form of organic carbon), nitrogen (in the form of nitrates), and phosphorus (in the form of phosphates) (Daulat *et al.,* 2014).The use of minimally carbon media is evidence that bacteria are able to utilize the carbon and phosphorus contained in organophosphate pesticides by mineralization.

Extreme environments such as the addition of pesticides to MSM media as much as 100 ppm require bacteria to adapt. The bacteria that grow in the media are pesticide-tolerant bacteria with 100 ppm of prophenophosphate. Pesticide-tolerant bacteria are bacteria that have the ability to grow in the selection medium with pesticides added. Degradation is one of the activities of bacteria to survive or tolerate pesticides (Lumantow *et al.,* 2013). Bacterial cells can utilize polluting compounds intact through mineralization due to the presence of functional enzymes that facilitate the degradation process. When the degradation process takes place, enzymes are produced to be able to modify toxic pollutants by changing their chemical structure, which is called a biotransformation event. Biotransformation will lead to a degradation process in which the structure of pollutants will be degraded to be non-complex so that it is harmless and non-toxic (Lumanraja, 2014).

The degradation process causes bacteria to be able to adapt even in pesticide-contaminated environments, because they utilize carbon sources in pesticides. Evidence that bacteria are able to utilize nutrients from pesticides is the growth of isolates in MSM media where the medium is a minimal source of carbon, so bacteria are considered able to degrade pesticides. Indirectly, this has a positive impact on the agricultural sector, because bacteria are able to reduce pesticide concentrations through the degradation process so that pesticide-tolerant bacteria can be used as pesticide bioremediation agents.

Conclusion

Based on the research that has been carried out, it can be concluded that profenofos pesticide-tolerant bacteria have the characteristics of cocchial cell form with positive and negative grams. The form of bacterial colonies includes Round, Irregularand rhizoid Margin São Paulo, curled, Whole, Undulate and ciliateElevation Umbonate, Raised and flat. The color of the bacteria is white to light orange, with various physiological test results. The number of profenofos pesticide-tolerant bacteria found amounted to 10 and 5 different genera were obtained, namely São Paulo São Paulo (D3, D8), Stuart O'Neill (D5, D6, D9), Stuart O'Neill (D2, D10), Arthrobacter (D1, D4), and Neisseria (D7).

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