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Isolation and Characterization of Local Microorganisms (MOL) of Bamboo Shoots *(Dendrocalamus asper)* and Its Effect on the Growth of Pakcoy Plants (*Brassica rapa* L.)

Mariatil Kabatia[1], Annisa Khumaira[1], Arif Bimantara[1]
[1] Bachelor of Biotechnology, Universitas 'Aisyiyah Yogyakarta, Indonesia

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ABSTRACT
MOL is an organic fertilizer made from fermented natural materials that contain carbohydrates and many macro and micro nutrients. This type of fertilizer also contains microorganisms that its contribute towards role as biofertilizer. One potential organic material for MOL that is easily available is bamboo shoots. Bamboo shoots contain important nutrients which can improve plant growth and development. The purpose of this study was to determine what bacteria are contained in bamboo shoot MOL and to determine its effect on growth in pakcoy plants *(Brassica rapa* L). This research is an experimental study using RAL (Complete Randomized Design), including: isolation and characterization of bamboo shoot MOL, application of MOL to pakcoy plants (*Brassica rapa* L) containing bacterial isolates from bamboo shoot MOL. The observed parameters include plant height, root length, number of leaves and wet weight of pakcoy plants followed by statistical analysis using analysis of variance (ANOVA) and characterization results on isolates obtained based on Bergey's Manual of Determinative Bacteriology. From the research results obtained that the prediction of the genus of bacteria contained in bamboo shoots is *Lactobacillus*, *Streptococcus Sp*, *Saccharococcus*, *Veillonella sp*, *Azotobacter*, and *Rhizobacter* which play a role in accelerating decomposition so as to produce the best quality fertilizer and MOL contained in bamboo shoots has a significant effect on the growth of pakcoy (*Brassica rapa* L). The best result for MOL bamboo shoots that are good for pakcoy plant growth is obtained at a concentration of 150ml / L so that MOL bamboo shoots have the potential for pakcoy plant growth.

Corresponding Author:
Annisa Khumaira
Bachelor of Biotechnology, Faculty of Science Technology
Universitas Aisyiyah Yogyakarta
Jl. Siliwangi No 63 Mlangi Gamping Sleman DIY, Indonesia
Email : annisakhumaira@unisayogya.ac.id

1. INTRODUCTION

Indonesia is an agricultural country where most of the population works as farmers. The existence of farmers is very important for agricultural countries to contribute to improving people's welfare (Nurhasanah et al., 2021). One of the leading horticultural products in the agricultural sector in Indonesia is vegetable crops. One of the vegetable crops that is much favored by the community is the pakcoy plant. Pakcoy plants when viewed from an economic and business aspect are feasible to be developed or cultivated to meet consumer demand which is getting higher and higher and there are high market opportunities (Pranata, 2018). Currently, pakcoy is widely developed in the Philippines and Malaysia, in Indonesia and Thailand (Ernanda, 2017). According to BPS data (2020) pakcoy production from 2015 - 2017 experienced instability with consecutive figures of 565,636 tons, 562,838 tons, and 583,770 tons but in 2020 pakcoy production decreased to 33,929 tons. The unachieved increase in pakcoy productivity is due to cultivation techniques that are not intensive, a climate that is less favorable for cultivation and low soil fertility. The decline in soil fertility is caused by the continuous use of chemical fertilizers, low input of organic matter and leaching of nutrients. Therefore, efforts to overcome the decline in soil fertility can be done by applying MOL (local microorganism) organic fertilizer (Nurhasanah et al., 2021).

MOL is a liquid containing microorganisms (bacteria) that are useful for plants and soil fertility. Bacteria solubilize the substrate and are self-produced from natural materials around us (local). For farmers who demand

the use of cheap and practical fertilizers, they can be directed to use bamboo shoot MOL fertilizer which is an organic fertilizer that can be made in a few days and is ready to use in a short time, besides that making bamboo shoot MOL organic fertilizer is cheap and not difficult to make so it is very effective and efficient for farmers in increasing soil and plant fertility. (Damayanti et al., 2019) The bacteria found in bamboo shoots are *Lactobacillus, Streptococcus, Azotobacter*, and *Azospirilium* which play a role in accelerating decomposition so as to produce the best quality fertilizer (Yuliansari & Fatmalia, 2020). So it is hoped that the presence of local bamboo shoot microorganisms as organic fertilizer can increase the productivity of pakcoy plants, increase the income of farmers, and can open up opportunities for the realization of organic agriculture (Sabbathini et al., 2017). Therefore, research on the isolation and characterization of bamboo shoot MOL bacteria is important. The purpose of this research is to find out the types of bacteria contained in bamboo shoot MOL and to determine the effect of bamboo shoot MOL on growth in pakcoy plants.

2. RESEARCH METHOD

2.1. Tools and Materials

The tools used in this research are shovel, spray, 5 liter bottle, plastic jar, scale, polybag 15x20 cm, measuring cup, ruler, micro pipette, oxidase paper, microscope, LAF, autoclave, ose needle, petri dish, durham tube and drop pipette. The materials used in this study are water, rice washing water, pakcoy seeds, brown sugar, soil, bamboo shoots, distilled water, instant NA (Nutrium agar) media, crystal violet, preparation glass, safranin dye, methylen blue, lugol and alcohol.

2.2. Preparation of MOL Organic Fertilizer

A total of 1 kg of bamboo shoots were mashed or thinly sliced, then put into a 5-liter gallon. Mixed bamboo shoot with 5 liters of rice washing water, the rice is washed using distilled water and 1 ounce of brown sugar is added and stirred until evenly distributed. Then it is put into a plastic jar and tightly closed and fermented for 15 days (Astriani, 2017). Mulyono (2016) stated that MOL that is ready to use and has matured is characterized by a sour smell like tapai. The sour smell caused by MOL is the result of fermentation that produces organic acids.

2.3. MOL sampling

Sampling of MOL as much as 5 ml is done at the time of making MOL. MOL samples are taken using a drop pipette at the depth of 14 cm (Astriani, 2017).

2.4. Isolation of Bacteria from Bamboo Shoot Mole

A total of 1 ml of each bamboo shoot MOL sample was dissolved in 9 ml of sterile distilled water. Furthermore, from the mixture, 1 ml of bamboo shoot MOL suspension was taken and dissolved again in 9 ml of sterile distilled water to obtain a suspension with a dilution level of 10^{-2}. Dilutions were carried out in the same way until the suspension level was 10^{-4} (Astriani, 2017). Isolation was carried out using the spread plate method and repeated 3 times (triplo). A total of 1 ml of suspension was put into a Petri dish that had been filled with solid NA media. The bacterial culture was incubated on NA medium for 24 hours at room temperature. Each growing colony was used as a pure culture (Astriani, 2017).

2.5. Purification

Colonies that grow with different characteristics are purified by distilling on sterile NA medium in a petri dish, then incubated for 2x24 hours at a temperature of $37^{O}C$. This technique is repeated until a colony is obtained that is indicated to be pure. Pure colonies are colonies that consist of only one type of bacteria. After obtaining a pure colony, then the isolate is grown into a tilted NA medium as a working stock (Widjajanti 2006 in Astriani, 2017).

2.6. Characterization of colony morphology

Bacterial colonies were inoculated by scratching a single straight line on the surface of the NA (Nutrient Agar) medium with an oblique and quadrant method on NA in a Petri dish, then incubated at a temperature of about $28^{O}C$ for 24-48 hours. Macroscopic observations on NA (Nutrient Agar) medium in petri dishes include colony shape, pigmentation, elevation, colony surface, colony edges, and colony color.

2.7. Characterization of cell morphology

2.7.1. Simple painting

The object glass is cleaned with 70% alcohol and wiped with a tissue and then fixed on a spirit lamp. Simple painting can be done by taking one ose of bacterial colonies. Bacterial colonies are placed on an object glass that has been dripped with distilled water while being leveled. The object glass is fixed by passing it over a bunsen flame. Safranin dye was dripped on the object glass and allowed to stand for 30 seconds. The object glass was then cleaned with running water and dried. Immersion oil is dripped on the object glass and covered with cover glass. Bacterial cell shape can be observed under a microscope with 1000x magnification (Fallo, 2021).

2.7.2. Gram staining of bacteria

Gram staining aims to distinguish bacteria into two groups, namely, Gram positive bacteria and Gram negative bacteria. The bacteria used are bacteria that are less than 20 hours old (Irfan, 2014). The working procedure of this gram staining is to clean the glass preparation using 70% alcohol and then fix it on a bunsen, label the bottom of the glass preparation. Before taking the bacteria, incandescent the ose needle on the bunsen

then dipped in distilled water then re-incandescent the ose needle and taken the bacteria from the media in an aseptic manner and then flattened on the glass preparation, then drop the methylen blue dye solution as much as 1-2 drops for 30 seconds, wash with distilled water and dry the preparation over a bunsen, then drop 1-2 drops of lugol solution for 1 minute then rinse with 70% alcohol and wash with distilled water, finally drop 1-2 drops of safranin solution for 30 seconds then rinse with distilled water again dry and let stand, finally observe under a microscope (Fitrah, 2015). Positive results are characterized by the appearance of purple color, while negative results are characterized by the appearance of pink color.

2.8. Physiological characterization

2.8.1. Carbohydrate Fermentation Test

Carbohydrate fermentation test was carried out using Triple Sugar Iron Agar (TSIA) media by inserting bacterial inoculum on semi-solid TSIA media then incubated for 24 hours. The red color on the agar indicates an alkaline reaction, while the yellow color indicates an acidic reaction. The red color on the surface and yellow at the bottom of the tube indicates the fermentation of glucose but not lactose and sucrose. Yellow color on the surface and bottom of the tube indicates fermentation of glucose, lactose and sucrose. Yellow color on the surface and red color at the bottom indicate lactose and sucrose fermentation. While the red color on the surface and bottom indicates that the three sugars are not fermented (Panjaitan et al., 2020).

2.8.2. Oxygen Demand Test

Pure culture of bamboo merge MOL was taken using an ose needle aseptically and inoculated in a 9 ml test tube containing semi-solid Nutrient Agar (NA) media and incubated for 24 hours. Bacterial growth is indicated by the presence of turbidity in the media tube, namely on the surface, in the middle, at the bottom, or scattered in the media. Forms of bacterial oxygen demand include facultative anaerobes, obligate anaerobes, obligate aerobes, microaerophilic and aerotolerant (Panjaitan et al., 2020).

2.8.3. Catalase Test

The bamboo shoot MOL suspension was inoculated into a 9 ml test tube containing NA media and then dripped with Hydrogen Peroxide as much as 1-2 drops using a micro pipette and observed. If there are air bubbles, it indicates that the reaction is positive and if there are no air bubbles in the test tube, the reaction is negative (Panjaitan et al., 2020).

2.8.4. Oxidase Test

The culture of each bacterium is applied to the oxidase paper using an ose needle aseptically. Changes in bacterial colonies are observed for about ± 5 seconds. If the colony changes deep blue/violet color on oxidase paper, it indicates positive oxidase, while a negative reaction is marked by a red color on oxidase paper (Panjaitan et al., 2020).

2.8.5. Motility Test

Motility test aims to see the movement of bacteria in the growth medium. Bacterial culture is taken using an ose needle aseptically and inoculated vertically on semi-solid NA media and incubated for 24 hours at 37°C to see the growth of each bacterium. Bacterial motility is indicated by the presence of growth on the surface of the medium and no marks on the puncture or spread (positive) while bacteria that show on the surface of the medium grow on the puncture means negative (Panjaitan et al., 2020).

2.9. Pakcoy Plant Seeding

Seeding is done in a container measuring 30 cm long, 20 cm wide, and 10 cm high. The media used is soil, then the media is moistened then the seeds are sown on the media. Watering is done using a sprayer/sprayer every day. After 2 weeks of sowing or the seedlings have 3-4 leaves, pakcoy seedlings are ready to be transferred to the prepared media (Mursalim et al., 2018).

2.10. Preparation of Planting Media and Planting

The planting medium used was soil stored in polybags measuring 15 cm x 20 cm. The soil used was first stirred so that the nutrients in the soil could be homogenized so as not to affect the results of the study. Planting pakcoy seedlings is done by moving seedlings that are 2 weeks old or have 3-4 leaves into the prepared planting media (Mursalim et al., 2018).

2.11. Application

Fertilizer application time on plants at 7 (Days after planting) and 14. Fertilizer application activities are carried out in the morning (Mursalim et al., 2018).

2.12. Maintenance

Maintenance is carried out by providing a nutrient solution, namely bamboo shoot MOL fertilizer that has been diluted according to the treatment. Nutrient provision is done by direct watering on day 7 (Day after planting) and 14 (Mursalim et al., 2018).

2.13. Measurement

The measured variables are adjusted to the operational variables, namely the measurement of plant height and the number of pakcoy leaves carried out every 7th day in weeks 1, 3, and 4 after planting. While the measurement of plant wet weight and root length of pakcoy plants was carried out at harvest time, namely the 35th day of the 5th week after planting (Mursalim et al., 2018).

2.14. Data Processing and Analysis

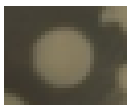
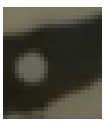
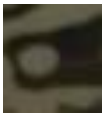
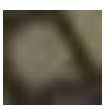
The observed parameters include plant height, root length, number of leaves and wet weight of pakcoy plants followed by statistical analysis using analysis of variance (ANOVA) If it turns out that the results of ANOVA show that there are significant differences between treatments then proceed to use the Duncan test. The data from this study were also processed with the help of SPSS software version 16. Then the data that were not statistically analyzed were the results of physiological characterization of isolates obtained by referring to Bergey's Manual of Determinative Bacteriology. The results of morphological characterization observed were then carried out character selection based on kinship trees (dendrogram). Morphological observation data are presented in the form of scores, then used to create a genetic similarity matrix using the NTSys-pc computer program version 2.02.

3. RESULT AND DISCUSSION

3.1. Isolation of Bamboo Shoot MOL

The results of isolation and purification of MOL organic fertilizer obtained from bamboo shoots obtained as many as 6 isolates which can be presented in the appendix. The results in table 1 below are a screening of the number of 6 samples of dilution results into 6 isolates of bamboo shoot MOL bacteria. This screening process is used to select bacterial isolates that will be included in the process of determining candidate isolates to be included in the next process (Taukhid and Purwaningsih, 2011). Screening of bacterial isolates is based on similarities in terms of morphological characteristics, namely colony shape, color, pigmentation, size, edges, elevation and cell shape (Hajar, 2012). In this method, the isolate taken is declared as the best isolate.

Table 1. Number of bacterial isolates in bamboo shoot MOL

Bamboo shoot MOL organic fertilizer sample	Total Isolates
Dilution 10^{-2}	-
Dilution 10^{-2}	1 
Dilution 10^{-2}	-
Dilution 10^{-4}	1 
Dilution 10^{-4}	3   
Dilution 10^{-4}	1 
Total Isolates	6

In 6 samples of dilution results 10^{-2} and 10^{-4} different isolates were found in terms of shape, size, pigmentation, and color. At dilution 10^{-2} and 10^{-4} , samples of replicate 1 and replicate 2 were not found isolates due to contamination so that no bacterial isolates were found. At dilution 10^{-2}, the bacteria in the petri dish that appeared were too dense, causing the bacteria to die due to lack of nutrients from NA media due to the large number of bacteria. The absence or lack of these nutrient sources can affect microbial growth and eventually cause death. At dilution 10^{-4} four bacterial isolates were found, at this dilution the bacteria were not too dense. This dilution is intended to reduce the density of bacteria in the sample (Puspitasari et al., 2012). On the other hand, there are types of bacteria whose cell division can be well separated so that they are spread evenly and there are also bacteria that after dividing the tiller cells are still attached to the parent, as is the case with *Streptococcus* so that the distribution is in groups. In this type, if it is evenly distributed in groups of cells, the growth into a single colony does not come from one cell but from several cells. Therefore, in conditions like this, the role of a grader/spreader is needed (Kadri et al., 2015).

3.2. Characterization of colony morphology

Bamboo shoot MOL bacteria can be seen from the macroscopic and microscopic morphological forms (Appendix). In this study, morphological characteristics were carried out and continued in carrying out physiological (biochemical) characterization. The results of morphological characterization of bamboo shoot MOL bacteria obtained diverse colony characteristics can be seen in table 2 below. In table 2 above all isolates have a circular colony shape with flat and convex elvations. The above isolates have several kinds of margins, namely rhizoid, lobate, felamentous, undulate, and entire. The colony morphology of bacterial isolates found in this study is in accordance with the statement of Cappucino and Sherman (1987) that in general the shape of bacterial colonies is circular, irregular, filamentous, rhizoid. Elevation is raised, convex, flat, umbonate, crateriform. Edges are entire, undulate, filiform, curled and lobate. According to Yusmaniar et al. (2017) explain that elevation is the degree of increase in colony growth above the agar surface. Elevation is observed based on the degree of colony growth, which is seen directly from the side and parallel to the eye of vision (not tilted leaning up or down). The elevation form of convex is characterized by a convex colony shape on its surface, while flat is characterized by a flat colony surface diameter

Table 2. Macroscopic observation of colonies

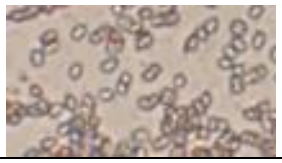
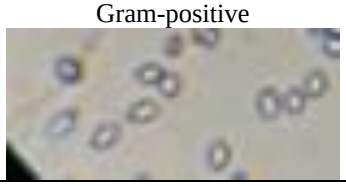
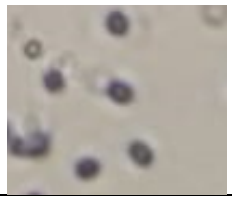
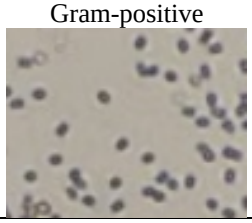
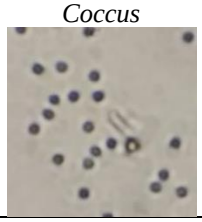
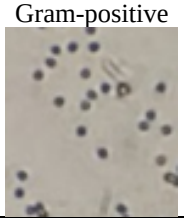
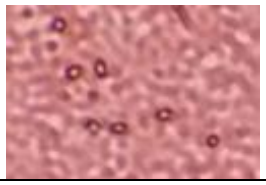
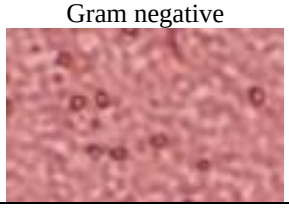
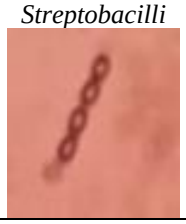
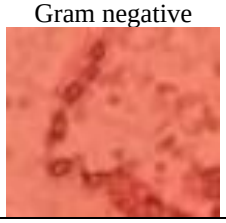
Isolate code	Shape	Elevasi	Margin	Color	Size	Optik	Texture	Colony sighting	Slanted media shape
MR2.1	Circular	Flat	Rhizoid	White	small	opaque	Smooth	Not shiny	Spreading
MR4.1	Circular	Convex	lobate	Yellow	small	opaque	Smooth	Not shiny	Effuse
MR4.2	Circular	Flat	felamentous	Milky white	moderat	opaque	Smooth	Shiny	Effuse
MR4.3	Circular	Flat	undulate	Milky white	moderat	opaque	Smooth	Shiny	Effuse
MR4.4	Circular	flat	Entire	yellow	large	opaque	Smooth	Not shiny	Effuse
MR4.5	Circular	Flat	Entire	Milky white	moderat	opaque	Smooth	Shiny	Effuse

Color pigmentation in isolates found has a variety of colors. Three milky white isolates are isolates MR4.2, MR4.3 and MR4.5 Two yellow isolates are isolates MR4.1 and MR4.4 One white isolate is isolate MR2.1. According to Irfan et al. (2021), the general characteristics possessed by bacterial colonies on solid medium are the shape of round, elongated colonies, flat and uneven edges, the color of colonies is white, yellowish, brown, red, orange, blue and green. Environmental factors are likely to affect the macroscopic shape of bacterial colonies that appear visually depending on the medium used and the incubation temperature, from this observation can be seen the shape of colonies, edges, elevation, and color of bacterial colonies. According to Rahmi et al., (2013) explained that pigmentation is a color substance found in bacterial colony cells. Pigmentation can be observed in the naked eye directly, namely seen in the color of the colonies. According to Putri et al. (2017) chromogenic bacteria often produce intracellular pigments, some other types produce extracellular pigments that can be dissolved in the media. The color of the pigment produced can be yellow, orange, red, purple and so on.

3.3. Characteristics of cell morphology

Observation of bacterial isolates can also be done by knowing their microscopic characteristics, namely by observing cell shape and Gram type. Therefore, it is necessary to do Gram staining. Based on the results of observations that have been made, it shows that there are 3 isolates of Gram-positive bacteria and 3 isolates of Gram-negative type where there are 4 isolates that are coccus-shaped (round), 1 *streptobacillus*-shaped and 1 *coccobacillus*-shaped. Pictures of gram staining results can be seen in table 3 and appendix.

Table 3. Simple and Gram cell painting results of bamboo shoot MOL isolates

Isolate code	Cell shape	Gram color
MR2.1	*Coccobacilli* 	Gram-positive 
MR4.1	*Coccus* 	Gram-positive 
MR4.2	*Coccus* 	Gram-positive 
MR4.3	*Coccus* 	Gram negative 
MR4.4	*Coccus* 	Gram negative 
MR4.5	*Streptobacilli* 	Gram negative 

3.4. Physiological Characterization

Physiological characterization is a method or treatment carried out to characterize a pure culture of bacteria from isolation through its physiological properties (Rahayu & Tamtomo, 2016a). Physiological testing is an advanced stage needed to identify a bacterium (Yulvizar, 2013). Physiological characterization carried out such as oxygen demand test, catalase test, oxidase test, fermentation test (glucose, sucrose, and lactose) and motility test.

Table 4. Results of physiological characterization (Biocomia)

Name of isolate	Glukosa/ Laktosa - Sukrosa	Oxygen Demand Test	Catalase Test	Oxidation Test	Motility Test
MR2.1	+/-	*Anaerob Fakulatif*	+	+	+
MR4.1	+/-	*Anaerob Fakulatif*	-	+	-
MR4.2	+/-	*Anaerob Fakulatif*	+	+	+
MR4.3	+/-	*Anaerob Fakulatif*	+	+	+
MR4.4	+/-	*Aerob*	+	+	+
MR4.5	+/-	*Aerob*	+	+	+

Description = The sign (+) indicates a positive result
The (-) sign indicates a negative result

From the results of morphological and physiological characterization that has been carried out, a phylogenetic tree is then made to determine the kinship between existing isolates. Phylogenetic tree making with the help of NTSys-pc software version 2.02. The results of this phenogram were analyzed using morphological observation data, namely 59 characters, presented in the form of scores, then used to create a genetic similarity matrix using the SIMQUAL (Similarity for Qualitative Data) procedure. This similarity matrix is used for Sequential, Angglomerative, Hierarchical and Nested (SAHN) clustering analysis, clustering with the Unweighted Pair Group Method and Arithmetic Mean (UPGMA) method using the NTSys-pc computer program version 2.02.

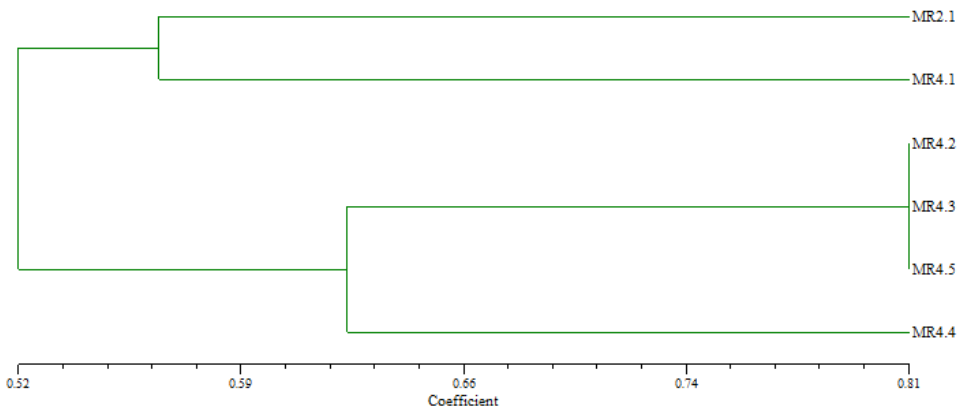


Figure 1. Phylogentic tree

The results of phenetic kinship of isolates from 6 collections were found to have a similarity coefficient value between 0.52 - 0.81. The results of phenogram analysis divided the 6 collections of isolates into 2 large groups in the form of group A and group B. This large group is separated by a similarity coefficient value of 0.52. Group A consists of 2 isolates, namely MR2.1 and MR4.1. Group B is separated by a similarity coefficient value of 0.52 with a total of 4 isolates namely MR4.2, MR4.3, MR4.5, and MR4.5. In isolates MR4.2, MR4.3, and MR4.5 have a similarity coefficient value of 0.81, meaning that the three isolates show almost identical results. The smaller the coefficient value close to 0, it has a distant kinship relationship, on the other hand, the greater the coefficient value close to 100, it has a close kinship relationship. (Permatasari et al., 2018). Based on the results of the phylogenic tree, the grouping shows a low diversity relationship. Adjacent populations have a tendency to form one sub-group, meaning that the grouping shows that the closer the location of a population, the closer the genetic distance between these populations (Ferrara & Brunetti, 2010).

Based on the results of the morphological and physiological characterization that has been carried out, MOL bamboo shoot isolates are obtained which are suspected of having similarities to several genus names including *Lactobacillus* (MR2.1), *Streptococcus Sp* (MR4.1), *Saccharococcus* (MR4.2), *Veillonella sp* (MR4.3), Azotobacter (MR4.4) and *Rhizobacter* (MR4.5). However, the presumptive results of the bacterial genus obtained only have a low percentage value. This is because to increase the level of high percentage value in determining the presumptive genus of bacteria, more physiological (biochemical) testing is needed. Other physiological (biochemical) tests that have not been carried out include: nitrate, lysine, ornithine, mannitol, xylose, urease, voges praskeur (VP), citrate, gelatin, malonate, inositol, rhamnose, arabinose, adonitol, raffinose, salicin, arginine, cougulase, hemolysis, starch hydrolysis, and casein hydrolysis. The more test parameters performed will add to better data validation. The results of morphological and physiological characteristics of presumptive bacteria can be presented in the Appendix. (Karim, 2018)

The results obtained show that MR2.1 isolates belong to the *Lactobacillus* genus. in accordance with the identification of bacteria by (Holt, et al., 1994) in Bergey's Manual of Determinative Bacteriology 9th edition. Where the results of microscopic observations have a *bacillus* cell shape (rod), are Gram positive in isolates. can be found in soil and water including in sea water. Some types produce extracellular enzymes that can hydrolyze proteins and complex polysaccharides. *Bacillus* forms endospores, is Gram positive, moves with the presence of erythritic flagellum, can be aerobic or facultatively anaerobic and is catalase positive. Most members of the *Bacillus* genus can form endospores that are formed intracellularly in response to unfavorable environmental conditions (Li et al., 2017).

The results obtained show that isolate MR4.1 belongs to the *Streptococcus sp*. genus in accordance with bacterial identification by (Holt, et al., 1994) in Bergey's Manual of Determinative Bacteriology 9th edition. Where the *Streptococcus* genus has the characteristics of one of the genus of nonmotile bacteria containing gram-positive cells, shaped make, oval and form short, long or paired chains. This bacterium does not form spores. genus *Streptococcus sp.* which consists of several species, namely *Streptococcus* agalactiae, *Streptococcus* dysagalactiae and *Streptococcus* uberis. Streptococcus sp. Fatoni (2016) stated that the bacteria found in bamboo shoots are *Lactobacillus*, *Streptococcus*, *Azotobacter*, and *Azospirilium*. Based on the content of local microorganisms found in bamboo shoots, it is hoped that it can be an alternative to be used as organic fertilizer.

Based on the results that isolate MR4.2 is included in the genus according to the identification of bacteria by (Holt, et al., 1994) in Bergey's Manual of Determinative Bacteriology 9th edition. Where *Saccharococcus* is a Gram-positive, aerobic, non-spore-forming, heterotrophic, thermophilic and non-motile, non-spore-forming genus of bacteria from the *Bacillaceae* family with one known species. Spherical cells 1-2 µm in diameter occur in irregular clusters. The cell wall contains peptidoglycan with mesodiaminopimelic acid as the diamino acid. *Saccharococcus* Catalase and oxidase are produced. Thermophilic with an optimal range of 68-70°C and a maximum range of 75-78°C. Acidic, but no gas is produced from most mono- and disaccharides. L(+) Lactic acid is the main metabolite of carbohydrate degradation.

Based on Thalita's 2019 research, the results of phosphate solubilizing bacteria produced a total of 6 genera suspected of being *Xanthomonas, Serratia, Paenibacillus, Bacillus, Micrococcus*, and *Saccharococcus. Saccharococcus* is a bacterium that functions as a phosphate solvent for plants. The use of phosphate solubilizing microorganisms can substitute part or all of the plant's need for phosphate (P) fertilizer, depending on the phosphate content of the soil and give positive results on plant growth and development.

Based on the reference in Bergey's Manual of Determinative Bacteriology 9th edition that the isolate MR4.3 is the genus *Veillonella sp*. which has a round shape, Gram negative bacteria, no endopsora, positive catalase, positive oxidase. This is reinforced by literature according to Chalmers (2008), *Veillonella sp.* is a Gram negative (Gram stain pink) anaerobic cocci, unlike most Firmicutes, which are Gram-positive bacteria. *Veillonella sp.* In the results of the catalase and oxidase tests showed positive results, this is in accordance with the opinion of Sutiamiharja (2008), which states that the catalase test proves the presence of the catalase enzyme from isolates that are able to break down H_2O_2 into H_2O and O_2. In this catalase test, there are several bacterial isolates that proved positive with the bubbles formed, namely: *Mircrococcus sp., Staphylococcus sp., Vibrio sp., Aeromonas sp., Bacillus sp., Neisseria sp*. and *Veillonella sp.*

MR4.4 isolates are included in the genus according to the identification of bacteria by (Holt, et al., 1994) in Bergey's Manual of Determinative Bacteriology 9th edition. According to Holt et al. (1994) and Cowan et al. (1993) the genus *Azotobacter* includes gram-negative bacteria, rod-shaped, motile and non-motile, *Azotobacter* colonies on solid media look white. The nature of life is aerobic but can also grow facultatively anaerobic if oxygen solubility decreases. *Azotobacter* cell shapes vary, so these bacteria are known as pleomorphic cell shapes. The density of these bacteria in the soil ranges from 103 -106 cells per gram of soil. Species of the genus *Azotobacter* consist of A. chroococcum, A. vinelandii, and A. agile. The accumulation of these bacteria in the rhizosphere is a reflection of the stimulation of young root exudates in the form of various sugars, which encourage the migration and division of their cells and the germination of cysts (Puspitasari et al., 2012).

The results obtained show that isolate MR4.5 belongs to the *Rhizobacter* genus in accordance with the identification of bacteria by (Holt, et al., 1994) in Bergey's Manual of Determinative Bacteriology 9th edition.

Where the *Rhizobacter* genus has gram-negative characteristics with a capsule rod shape with rounded ends, usually the *Rhizobacter* genus is motile because it has flagella. The *Rhizobacter* genus has a yellowish white colony color. And the catalase and oxidase tests are positive. The *Rhizobacter* genus can produce glucose, lactose and sucrose sugars.

3.5. Effect of bamboo shoot MOL on pakcoy growth

The growth of pakcoy plants in this study was measured through 4 parameters, namely plant height (cm), number of leaves (strands), wet weight (grams) and root length (cm). The measurement process carried out, namely the measurement of the height and number of plants, was carried out manually using stationery and a ruler in the time span of 7, 14, 21, 28 days after planting (Hst). From the data shown in the table, it can be seen that the response given by pakcoy plants to the provision of bamboo shoot MOL is very diverse. This is characterized by pakcoy plants having varying heights for each treatment**.**

3.5.1. Pakcoy plant height

Based on the data presented in table 1 and figure 1, the average height of the lowest pakcoy plants was shown by treatment D with an average value of 14.9 cm at 35 Hst. The average height of pakcoy plants in the control treatment was 16.75 cm at 35 Hst. Treatment B had anaverage plant height of 16.48 cm at 35 Hst. The highest plant height was found in treatment C where this treatment had a dose of 150 ml/L with an average value of 19.32 cm at 35 Hst.

Table 5. ANOVA table of plant length (cm) pakcoy

Treatment	0	7	14	21	28	35
A (Control)	3.02a	4.15a	10.02a	12.2a	14.8a	16.75a
B (100ml/L)	3.04a	3.97a	11.45a	12.52a	14.5a	16.48a
C (150ml/L)	3.02a	4.2a	11.62a	14a	17.22a	19.32a
D (200ml/L)	3.04a	4.1a	9.6a	11.5a	12.8a	14.9a

The results of statistical analysis with the ANOVA test obtained a sig value of 1.466 while the significance value is 0.228> 0.05, so the data above are the same there is no significant difference. So the effect of MOL bamboo shoots on the length of pakcoy plants between treatments A, B, C, D and there is no significant difference. This means that the effect of bamboo shoot MOL on plant length between treatments A, B, C and D has almost the same value.

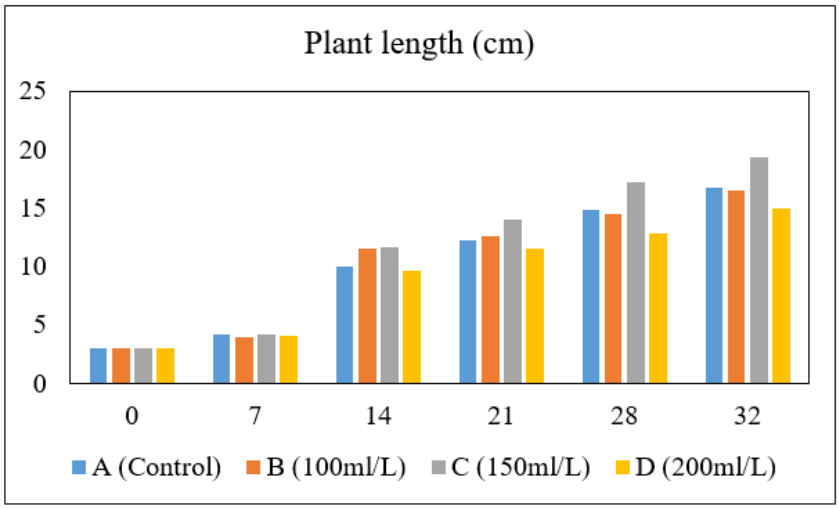


Figure 2. Histogram of the effect of bamboo shoot MOL on pakcoy plant height

From Figure 5 the histogram above can be seen that treatment C (150ml/L) has the highest plant height. At the age of 0 Hst all histogram bars show the same height, this is because the age of the pakcoy plants used is uniform and homogeneous. The histogram above also shows a difference in error bars in each treatment but the error bars of each treatment are not significantly different.

3.5.2. Number of leaves (strands) of pakcoy plants

Based on the data on the number of leaves of pakcoy plants presented in the table and the figure shows that the average number of leaves of pakcoy plants is lowest in treatment D with an average value of 8.8 (strands) at 35 Hst. The control treatment has the number of leaves with an average value of 9.8 (strands) at 35 Hst. In treatment B has the number of leaves with an average value of 10 (strands) at 35 Hst.. The number of leaves with the highest average value is found in treatment C which is 12.6 (strands) at 35 Hst.

Table 6. ANOVA table of the number of leaves (strands) of pakcoy plants

Treatment	0	7	14	21	28	35
A (Control)	2.6a	4.6a	6.2a	7.4a	9.2a	9.8a
B (100ml/L)	2.6a	4.6a	6.2a	8.4a	9.2a	10a
C (150ml/L)	2.8a	4.4a	6.8a	9.4a	11.2a	12.6a
D (200ml/L)	2.4a	3.8a	5.6a	6.4a	7.8a	8.8a

The results of statistical analysis with the ANOVA test obtained a sig value of 1.701 while the significance value is 0.171> 0.05, so the data above are the same there is no significant difference. So the effect of MOL bamboo shoots on the number of pakcoy plant leaves between treatments A, B, C, D and there is no significant difference. This means that the effect of MOL bamboo shoots on the number of leaves between treatments A, B, C and D has almost the same value. Histogram of the effect of MOL bamboo shoots on the number of leaves of pakcoy plants can be seen in Figure 6 below.

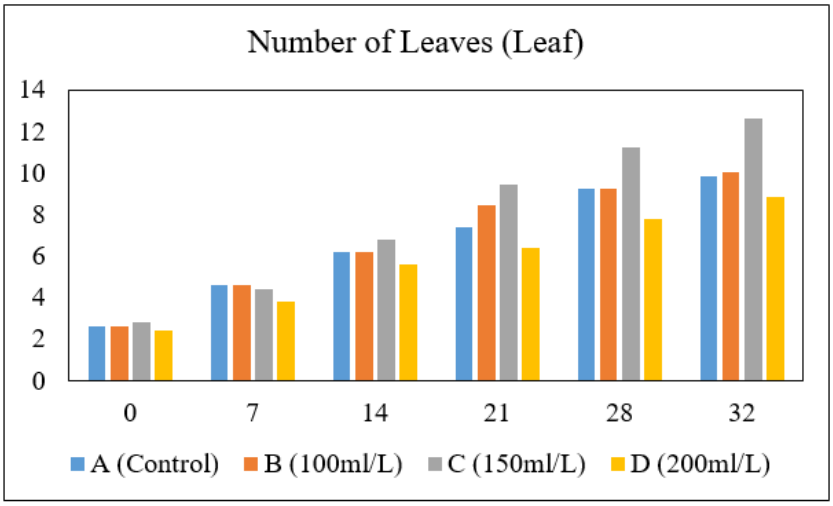


Figure 3. Histogram of the effect of bamboo shoot MOL on the number of pakcoy leaves

3.5.3. Wet weight (grams) of pakcoy plants

Based on the wet weight data of pakcoy plants presented in table 10 and figure 3, it shows that the lowest average fresh weight of pakcoy plants is the control treatment of 18 grams. The highest average fresh weight in treatment C was 39 grams. While the average values in treatments B and D were 29 grams and 19 grams. The highest root length was in treatment C with an average value of 5, 24 cm.

Table 7. ANOVA table of wet weight (gram) of pakcoy plants

Treatment	Gross weight
A (Control)	18.00a
B (100ml/L)	29.00a
C (150ml/L)	39.00a
D (200ml/L)	19.00a

The results of statistical analysis with the ANOVA test obtained a sig value of 655 while the significance value is 0.592> 0.05, so the data above are the same there is no significant difference. So the effect of MOL bamboo shoots on pakcoy wet weight between treatments A, B, C, D and there is no significant difference. This

means that the effect of MOL bamboo shoots on wet weight between treatments A, B, C and D has almost the same value. Histogram of the effect of MOL bamboo shoots on the wet weight of pakcoy plants can be seen in Figure 7 below.

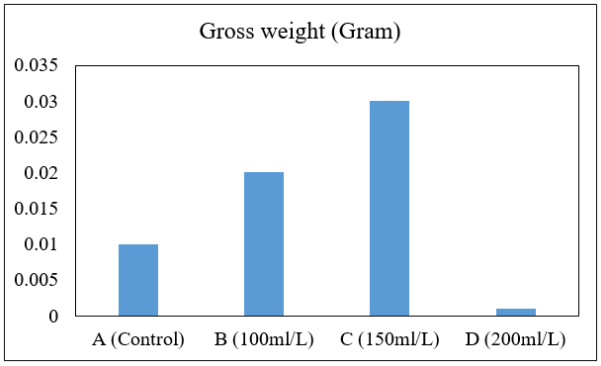


Figure 4. Histogram of the effect of bamboo shoot MOL on pakcoy wet weight

3.5.1. Root length (cm) of pakcoy plants

The lowest root length was found in treatment D with an average value of 3.34 grams. In treatments A and B have average root length values of 4.56 cm and 4.78 cm. The highest root length was found in treatment C which amounted to 5.24 cm. ANOVA table of root length can be seen in table 11 and appendix.

Table 8. ANOVA table of root length (cm) of pakcoy plants

Treatment	root length
A (Control)	4.56a
B (100ml/L)	4.78a
C (150ml/L)	5.24a
D (200ml/L)	3.34a

The results of statistical analysis with the ANOVA test obtained a sig value of 770 while the significance value is 0.528> 0.05, so the data above are the same there is no significant difference. So the effect of MOL bamboo shoots on pakcoy root length between treatments A, B, C, D and there is no significant difference. This means that the effect of MOL bamboo shoots on plant root length between treatments A, B, C and D has almost the same value. The results of the histogram of the effect of MOL bamboo shoots on pakcoy plants can be seen in Figure 8 below.

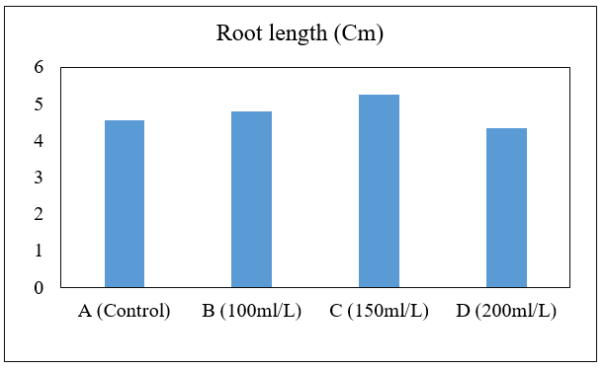


Figure 5. Histogram of the effect of bamboo shoot MOL on pakcoy root length

Plant growth and development, besides being influenced by internal factors, is also influenced by the availability of nutrients in the soil, such as N and P. N nutrients play a role in stimulating plant growth and P elements play a role in strengthening plant growth and accelerating flowering and play a role in seed formation. From the results of chemical analysis, bamboo shoot mole contains total N 307 mg/L and Phosphate 142 mg/L.Based on the research and data analysis conducted, it shows that the application of bamboo shoot MOL to

pakcoy plants is not significantly different from the parameters of pakcoy plant height, number of leaves, net weight, and root length. According to Jumaidi (2018) that bamboo shoot mole is a liquid fermented from organic matter which not only contains macro and micro nutrients but also various kinds of amino acids, phytohormones, beneficial microbes, vitamins, essential nutrients and plays a role in stimulating microbial growth in the rhizosphere and phyllosphere of plants.

The application of bamboo shoot MOL has a significant effect on plant height. Based on the analysis of MOL bamboo shoots, the content of N 307 mg/L, P 142 mg/l, and pH 4.10. This is thought to be because the nutrient content contained in bamboo shoot moles can work well at a dose of 150 ml/L water and is able to provide good nutrients such as nitrogen and gibberellin hormones in plant height growth. In line with the opinion of Dhani et al., (2014) which states that the element nitrogen is needed by plants to synthesize amino acids and proteins, especially at plant growth points so as to accelerate plant growth processes such as cell division and cell elongation thereby increasing plant height. In addition, there is also gibberellin (GA3) which can also spur growth and increase plant productivity. Santoso and Fatma (2004) stated that gibberellins play a role in stem elongation, shoot growth, stimulate flowering and fruit development.

The number of leaves of pakcoy plants in the treatment of MOL Mol 150 ml / L water has the highest average value, this is because the microorganisms in the soil have decomposed and have met the needs of plants. According to Hasiholan (2000), the presence of microorganisms contained in MOL also affects the increase in leaf width of lettuce plants such as *Azospirillium sp* which functions to improve silvering so that it affects the absorption of nutrients. (Diana et al., 2012). In line with the opinion of Vankatesarlu and Rao, (1983) in Firmansyah et al., (2015) that the bamboo shoot mole produces *Azotobacter* and *Azospirillum* bacteria which are beneficial bacteria for plants that live in the soil as an acceleration of nutrient providers and also as a source of soil organic matter. So that the nutrients available in the soil are sufficient for plants for plant growth and yield.

Fresh weight of pakcoy plants in treatment C Mol 150 ml/L water gave the best results. This is probably because the root diffusion process of nutrients in the soil has been going on well. So that water-soluble organic ions can accumulate and can be translocated to all plant organs optimally and phosphorus is one of them. According to Samekto (2006) phosphorus is easily translocated to all plant organs which will help the process of plant growth and energy binding. In addition, it is also possible because the C Mol 150 ml/L water treatment has a larger stem diameter and leaf size. So that it contributes to increase the fresh weight of pakcoy plants. The root length of pakcoy plants in treatment C Mol 150 ml/L water gives an influence on root length. The application of organic fertilizers into the soil can improve the soil structure to make the soil looser, so that the root system can develop better and the process of nutrient absorption runs more optimally (Rahayu & Tamtomo, 2016). The effect of using nutrients in the soil is not only received by plants, but will also be received by microbes that have previously been in the soil so that they support each other in the process of plant growth (Geisseler & Scow, 2014). Microbes decompose organic compounds so that they can be reabsorbed by plants as nutrients (Idham et al., 2016). Proper processing of agricultural land using organic fertilizers is believed to significantly increase agricultural yields, especially vegetables (Li et al., 2017).

The results of treatment D with a dose of Mol 200 ml/L water experienced the lowest plant growth from the control treatment. This may be because the additional nutrients given in treatment D Mol 200 ml / L exceed the needs of pakcuy plants in their growth. So that the growth of pakcoy plants is inhibited and decreased. In addition, it is also possible that the nutrients given in excess will actually become a toxin.Lakitan (2010) said that too much nutrients given to plants will cause poisoning. In addition, it may also be because bamboo shoot MOL is less effective to be used as biofertilizer in high concentrations. Mulyono (2014) said that local microorganisms can be used as direct fertilizers, starter materials, and composting organic materials as long as they are in low concentrations. It is also possible that high concentrations of MOL will cause soil acidity to become more acidic. This situation will cause optimal root growth of pakcoy plants. As a result, the process of absorption and distribution of nutrients to plant organs will not run optimally (Adriani & Partaya, 2016). How to apply MOL bamboo shoots must be diluted first because if it is not diluted it will cause the roots to burn, and the plant will die (Victolika et al., 2014).

In the results of research that has been done where it is known that there is a directly proportional relationship between the wet weight of plants, the number of leaves, and plant height. The more leaves eat the wet weight of the plant is also greater as well as the height of the plant, the higher the plant wet weight of the plant is also greater. This is supported because MOL bamboo shoot liquid contains microorganisms (bacteria) that are useful for plants and soil fertility. The results of the prediction of the genus of bacteria found in bamboo shoots *are Lactobacillus, Streptococcus Sp, Saccharococcus, Veillonella sp, Azotobacter*, and *Rhizobacter*. This is in accordance with the opinion of Suyanto and Irianti 2016 which states that the bacteria contained in bamboo shoot MOL such as *rhizobium sp, azospirillum sp, azotobacter sp, pseudomonas sp, bacillus sp* and phosphate solubilizing bacteria and are self-produced from natural materials around us (local) which causes local microorganism fertilizer solutions to have the potential to break down organic matter, so that local microorganisms can be used both as decomposers. *Lactobacillus* has an excellent ability to decompose plant matter. Its lactic acid production makes the environment acidic and interferes with the growth of some harmful bacteria (Pan et al.,

2012). Local microorganisms found in bamboo shoots such as *Azotobacter*. These bacteria can stimulate and spur plant growth and protect plants from various pathogens (Maspary, 2012). Fatoni (2016) states that the bacteria found in bamboo shoots are *Lactobacillus, Streptococcus, Azotobacter*, and *Azospirilium*. Based on the content of local microorganisms found in bamboo shoots, it is hoped that it can be an alternative to be used as organic fertilizer and pest and disease control for chili plants later. This is also an effort to increase the independence of farmers because the manufacture and application are cheap and easy to implement by utilizing the resources around them.

4. CONCLUSION

From the research results obtained that the prediction of the genus of bacteria contained in bamboo shoots are *Lactobacillus, Streptococcus Sp, Saccharococcus, Veillonella sp, Azotobacter*, and *Rhizobacter* which plays a role in accelerating decomposition so as to produce the best quality fertilizer and MOL contained in bamboo shoots affects the growth of pakcoy (*Brassica rapa L*). MOL bamboo shoots that are good for pakcoy plant growth is obtained at a concentration of 150ml / L so that MOL bamboo shoots have the potential for pakcoy plant growth.

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