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Identification of Plant Growth Promoting Rhizobacteria from Thorny Bamboo Rhizosphere with 3% KOH Gram Test and Gram Staining Test

Muhammad Yusril Hardiansyah, Yunus Musa & Abdul Mollah Jaya
Department of Agronomy, Agrotechnology Study Program, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia
*Corresponding author (Muhammad Yusril Hardiansyah, Department of Agronomy, Agrotechnology Study Program, Faculty of Agriculture, Hasanuddin University.

Abstract
Plant Growth Promoting Rhizobacteria (PGPR) is a group of beneficial bacteria that actively colonize the rhizosphere and play an important role in enhancing root growth that impacts on plant growth, yields and land fertility. PGPR is very much found in the area around plant roots or rhizosphere especially thorny bamboo rhizosphere (Bambusa blumeana). The number of microorganisms contained in PGPR spiked bamboo rhizosphere makes it difficult to know what types of bacteria are the most dominant and most active in influencing plant growth. One method that can be used to identify microorganisms is by biochemical testing processes using the 3% gram KOH test method and gram staining test. This study aims to determine the use of the 3% gram KOH test method and gram staining test in identifying Plant Growth Promoting Rhizobacteria bacteria from thorny bamboo rhizosphere (Bambusa blumeana). This test is carried out at the Biofertilizer and Potential Microbial Laboratory, Ministry of Agriculture, Faculty of Agriculture, Hasanuddin University, Makassar and takes place from October to December 2019. The method of carrying out the test includes making the media nutrient agar (NA), pouring media, planting bacteria, identifying bacteria in 2 gram reaction methods, namely the gram reaction test using 3% KOH and using a simple coloring with methylen blue and lugol. The results obtained are the 3% gram KOH test method in testing the scratch method of all samples that have slime while the scatter method has 2 samples that have slime. Then the gram staining test method on staining with methylene blue and logol produces blue for the genus Pseudomonas which means gram positive and pink or purple for the genus Bacillus which means gram positive. So it can be concluded that the 3% KOH test method and gram staining test have optimum effect in detecting microorganisms.

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Keyword
Bacteria, KOH 3%, Gram Staining, Plant Growth Promoting Rhizobacteria

Introduction

Rhizobacteria are more popular plant growth promoters called Plant Growth Promoting Rhizobacteria (PGPR) are a group of beneficial bacteria that actively colonize the rhizosphere. PGPR plays an important role in enhancing root development that impacts on plant growth, crop yields and land fertility (Wahyudi, 2009). Plants with well-developed roots will efficiently absorb nutrients so that plants are not susceptible to pathogens. In addition, increased plant growth by PGPR can occur through one or more mechanisms related to the functional character of PGPR and conditions in the rhizosphere environment (Rahni, 2012). PGPR is a biological miroroganism that can increase plant growth and yield. The bacteria contained in PGPR can be classified based on their effects on plants and the way they interact with roots, PGPR can affect plants directly and indirectly (Saharan & Nehra, 2011). The role of PGPR in increasing plant growth and production has something to do with the ability to synthesize growth substances, which produces the hormone indol acetic acid (AIA) (Thakuria et al., 2003).

Bamboo is a plant that can grow in several regions in Indonesia with a variety of functions and species. In Indonesia, there are 60 species of bamboo plants from 200 species in Southeast Asia and can be found in areas that are free from standing water, from the lowlands to the mountains. The high adaptability of bamboo makes this plant grow well in almost every type of soil (Widjaja et al. 1995). Until now, there have been many reported potential antagonistic microbial origin of bamboo rhizosphere which has antagonistic power against soil borne pathogens through antagonistic mechanisms in the form of life competition, parasitism, antibiosis and induced systemic resistance. In addition to suppressing the development of pathogens, rhizosphere microbes can also increase plant growth through various mechanisms, including through the production of growth stimulant compounds such as phytohormone. In the soil many microbes have the ability to dissolve phosphate and potassium, tether N2 and produce phytohormone. These microbes can enhance plant growth by producing phytohormone compounds indole acetic acid (IAA) as nutrients for plants (Aryantha et al. 2004; Zahir et al. 2004).

Research on the existence and diversity of bamboo rhizosphere microbes has been carried out by several researchers before. According to Sharma et al. (2010) in the rhizosphere of healthy bamboo plants found antagonistic fungi such as Aspergillus, Penicillium, Trichoderma which are able to suppress the Fusarium and Phytophthora pathogens. Research conducted by Asniah et al. (2013) showed that the inoculation of the fungus Paecilomyces sp and Chaetomium globosum from bamboo rhizosphere into the nursery soil significantly affected the decrease in the index of clubroot disease and increased wet weight of broccoli plants. Research conducted by Tu et al. (2013) in China against rhizosphere 6 bamboo species showed that the total population of fungi and bacteria and microbial activity in bamboo rhizosphere soils was very high and had a positive effect on plant growth.

The number of microorganisms contained in PGPR spiked bamboo rhizosphere makes it difficult to know what types of bacteria are the most dominant and most active in influencing plant growth. This will also have an impact on the difficulty of knowing and selecting good and bad bacteria contained in the PGPR, so that it greatly affects plants. Therefore the method that can be used to determine the type of bacteria contained is to identify microorganisms with biochemical test treatments. According to Rahayu & Gumilar (2017), Bacterial biochemistry test is a method or treatment that is carried out to identify and determine a pure bacterial culture that is isolated through its physiological properties.

Biochemical processes are closely related to cell metabolism, namely during chemical reactions carried out by cells that produce energy or that use energy for the synthesis of cell components and for cellular activities, such as movement. One method used in identifying microorganisms is gram test with 3% KOH and gram staining test with methylene blue.

The type of Gram bacteria was tested using a 3% KOH test and staining. Testing with 3% KOH only aims to determine the type of Gram, while the staining method is used to determine the type of Gram and the shape of bacterial cells. The method of testing with 3% KOH is based (Suslow et al. 1982) by mixing bacterial isolates with 3% KOH on a sterile glass preparation. Bacteria can be classified into aerobes and anaerobes. The main difference between the two is the fact that aerobic bacteria need oxygen to stay alive, while anaerobic bacteria do not depend on oxygen for metabolic processes and survival. Whereas aerobes can develop in habitats that have abundant oxygen, anaerobes can die in the presence of oxygen. These types of bacteria do have the advantage of growing areas of the body not exposed to oxygen, and they can be virulent pathogens. The difference in capacity to utilize oxygen between aerobes and anaerobes is important in the treatment of bodily infections (Lay, 1994)

Identification and determination of a pure bacterial culture obtained from the results of isolation can be done by observing the morphological characteristics of the colony, the morphology of the bacterial cell, testing its physiological and biochemical properties. In addition, identification can also be done by decomposing the pathogenicity and serology. The growth of bacteria in nature is influenced by several external factors such as susbtrat, growth, pH, temperature, and chemicals. The bacteria that appear can have the same morphology, but the nutritional requirements and ecological requirements are different. For clear observations of bacterial morphology, the body needs to be filled with color paint, this coloring is called arterial painting (Hadioetomo, 1990).

The gram method with a 3% KOH test is a good method of identifying bacteria in determining the dominant type of active bacteria that is characterized by mucus. Generally it is quite difficult and confusing to determine whether mucus is present or not even though some cultures react slowly. If the bacteria are mixed too little it will cause errors in the test and allow not react, so in this test the use of a dark color is very good. Determination of gram properties with 3% KOH has the same results with gram staining test. Tests of 3% KOH in bacteria indicate that gram (+) bacteria have thick cell walls and thin fat while gram (-) fat is thick and thin-walled cells that are in the periplasma chamber. KOH will attack this fat (lipid bilayer) and break down gram (-) cells. The breakdown of cells releases genetic material (DNA) which is an abundant substance in bacterial cells. DNA molecules are very long sticky strings (like mucus, sap or can mean sticky) which gives results like mucus when removed with an inoculum needle (Edwin, 2011).

Gram staining or gram method is an empirical method to distinguish bacterial species into two large groups namely Gram positive and Gram negative bacteria. The difference in classification between the two types of bacteria is mainly based on differences in the structure, chemical properties, and physical cell walls of the bacteria (Qiqi, 2008). Seeing and observing bacteria in a state of life is very difficult, because in addition to bacteria they are colorless also transparent and very small. In addition, living bacteria will contrast with water, where the bacterial cells are suspended. Meanwhile, to overcome this problem, a bacterial cell staining technique was developed, so that cells can be seen clearly and easily observed. Therefore this bacterial cell staining technique is one of the most important

ways in microbiological studies. The basic principle of this coloring is the existence of ionic bonds between cellular components of bacteria with active compounds from dyes called chromogens. Ion bonds occur because of the electric charge in both cellular components and in dyes. Based on the presence of this charge, it can be distinguished from acidic dyes and basic dyes. The gram staining technique can produce red and purple colors, gram-negative bacteria are characterized by red staining while the positive ones are purple (Levine, 2000).

Gram staining is a type of multilevel coloring, so there are stages in the process. Gram staining system is carried out based on the stages that have been determined in the use of dyes or bleach. Briefly, in this process the spread of bacteria on a fixed fixation sequence is subjected to a purple crystal solution, lugol liquid, alcohol solution (pale material) and safranin or some other suitable coloring agent. In this process aquades are also used in between several colors (Suriawiria, 2005). Bacteria stained by this method are divided into two groups, namely Gram Positive Bacteria and Gram Negative Bacteria. Gram-positive bacteria will retain the violet crystalline dye and will therefore appear dark purple under a microscope. The gram-negative bacteria will lose violet crystalline dyes after washing with alcohol, and when given a coloring agent that is equal to the water coloring agent fuchsin or safranin will appear red. This color difference is caused by differences in the chemical structure of the cell wall (Qiqi, 2008).

Characteristics of gram positive bacteria is a simple cell wall structure, composed of peptidoglycan without a layer of lipopolysaccharide. If given a gram naming, gram-positive bacteria will be purple. Members of many gram-positive bacteria that cause disease in humans, for example Streptococcus pneumoniae which causes pneumonia (pneumonia). Many gram-positive bacteria that produce toxins, such as Clostridium botulinum. The toxin produced by the bacteria C. botulinum is very deadly, one gram of toxin can kill more than one million people. Besides being able to cause disease and produce toxins, gram-positive bacteria can also produce beneficial ingredients. For example, antibiotics are produced by bacteria from the Actinomycetes group. Antibiotics kill other gram-positive bacteria by preventing them from forming proteins. Proteobacteria are the largest phyla in Kingdom Eubacteria. All Proteobacteria are gram-negative bacteria. but has a variety of shapes (stem, round, and spiral). Most move with flagella (Strober, 2001).

Gram-positive bacteria are bacteria that maintain methyl purple dye during the Gram staining process. This type of bacteria will be blue or purple under a microscope, while gram-negative bacteria will be red or pink. The difference in classification between the two types of bacteria is mainly based on differences in the structure of bacterial cell walls (Qiqi, 2008).

Gram-negative bacteria are bacteria that do not maintain the methyl purple dye in the Gram staining method. Gram-positive bacteria will retain a dark purple color after washing with alcohol, while gram-negative bacteria do not. In the Gram staining test, a lead dye (counterstain) is added after methyl purple, which makes all gram-negative bacteria turn red or pink. This test is useful for classifying these two types of bacteria based on differences in the structure of their cell walls. Many species of gram-negative bacteria are pathogenic, which means they are harmful to host organisms. The nature of this pathogen is generally associated with certain components of the gram negative cell wall, especially the lipopolysaccharide layer (also known as LPS or endotoxin) (Qiqi, 2008).

This study aims to determine the use of the 3% gram KOH test method and gram staining test in identifying Plant Growth Promoting Rhizobacteria bacteria from thorny bamboo rhizosphere (Bambusa blumeana).

Materials and Methods

This test was conducted at the Biofertilizer and Potential Microbial Laboratory, Ministry of Agriculture, Faculty of Agriculture, Hasanuddin University, Makassar. This test takes place from October to December 2019. Tools used were analytical scales, mortars, test tubes, test racks, petri dishes, measuring cups, erlenmeyers, ose needles, drop pipettes, micro pipettes, matches, apatulas, autoclaves, ovens, hotplate, vortex, microscope, glass preparation, laminar air flow, storage box, camera and writing stationery. The materials used are Plant Growth Promoting Rhizobacteria solution from thorny bamboo rhizosphere (Bambusa blumeana), KOH 3%, methylen blue, lugol, 70% alcohol, 96% alcohol, water, aquades, nutrient brooth (NB) 8 grams, agar 15 gram, bunsen, tissue, plastic wrap, aluminum foil paper, paper, label paper.

The steps for implementing the test are as follows:

1. Making Nutrient Agar (NA) Media

The making of NA media was done by weighing 8 grams of nutrient brooth and 20 grams of agar on the analytical balance. Furthermore, the weighed material is put into a 1000 ml size erlenmeyer and 500 ml of distilled water is then stirred until the material is dissolved. Then the erlenmeyer is heated using a hotplate while stirring and add distilled water little by little until the volume of the solution reaches 1000 ml. When the solution is homogeneous, the erlenmeyer is covered with aluminum foil and then put into an autoclave to be sterilized wet for 15 minutes.

2. Media pouring

Before starting the pouring of the media, first sterilize the laminar air flow by turning on the lights and spraying 70% alcohol to all parts of the laminar air flow and then cleaning it using a tissue. Furthermore, laminar air flow is covered with black plastic and the UV lamp is turned on for 30 minutes. After the UV sterilization process is complete, the laminar air flow blower is turned on and put all the tools and materials that will be used in the pouring media. Before inserting tools and materials into laminar air flow spraying is done first with 70% alcohol. Turn on Bunsen with matches and open the NA medium which has been sterilized wet and has been at a moderate temperature. Next, the NA media was poured over a 15-20 ml petri dish, then covered and glued with plastic wrap and labeled. The media is stored in a storage box and allowed to harden.

3. Planting Bacteria

Bacteria planting is carried out again in laminar air flow, which is done by two methods, namely the scatter method and the method of scratching. Each method is made in 5 petri dishes each. Material in the form of PGH rhizosphere bamboo solution that has been made and NA media is put into laminar air flow after spraying with 70% alcohol. The spread method is carried out by taking 1 ml of PGPR solution using a micro pipette and then spreading it over NA media and flattened using a spatula, while for the scratching method is done by taking an ose needle then sterilizing after incubation in Bunsen then dipping it in the PGPR solution and polishing it. zigzags on the surface of the NA media in a petri dish. After the bacterial planting is carried out, the cup is stored in a storage box and physical checking and observation of bacterial growth every day.

4. Bacterial Identification

Bacterial identification is carried out in 2 gram reaction methods, namely:

a. Uji a. Gram Reaction Test using 3% KOH

Testing with 3% KOH only aims to find out the type of Gram, the method of testing with 3% KOH is by mixing bacterial isolates with 3% KOH on a sterile glass preparation (Suslow et al., 1982). This bacterial identification method is done by placing 1 drop of 3% KOH on a glass preparation then the bacteria that have grown from the scatter method and the method of scraping are taken with an ose needle and rubbed in a 3% KOH solution and observed. The category of gram negative bacteria is obtained if it produces mucus (positive reaction) and the category of gram positive bacteria if it does not produce mucus (negative reaction).

b. Bacteria Gram Reaction Test using Simple Staining

Gram staining is a physiological characterization of bacteria carried out to distinguish Gram positive and Gram negative bacteria. The coloring method is carried out using methylene blue. First the glass preparation is washed using 70% alcohol and then sterilized after incubation in Bunsen. Next take an ose needle then sterilized afterglow then dipped in 96% alcohol. Take one of the aquades and put it on the glass preparation. Burning the needle back on the bunsen then aired and used to take bacteria then flattened on the glass preparation and let it dry. Drop 1-2 drops of methylene blue on the glass preparation and leave to dry. Next wash the glass preparations with distilled water and then drop 1-2 drops of lugol solution on the glass preparations and leave for 1 minute then rinse with 70% alcohol for 30 seconds then dry. After drying, put the deglass on the glass preparation then observe it under a microscope with magnification (40 / 0.65) or (160 / 0.17). If there is a change in the color of the bacteria to pink or purple, the bacteria are gram-positive and belong to the Pseudomonas group and if there is a blue discoloration, the bacteria are gram-negative and are the Bacillus group (Meynell & Meynell 1970).

Results and Discussion

Please Testing the gram reaction of PGPR bacteria from the thorny bamboo rhizosphere using 3% KOH is as follows.

Table 1. Gram Reaction Test Results Using 3% KOH on Plant Growth Promoting Rhizobacteria of Thorny Bamboo Rhizosphere with Scatter and Scratch Methods

Methods	Gram Reaction				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Spread	-	+	-	-	+
Scratch	+	+	+	+	+

Note: plus signs (+) and minus (-) are given if there is or no mucus during testing

Figure 1. Observation of bacterial gram test using 3% KOH (scratch method)

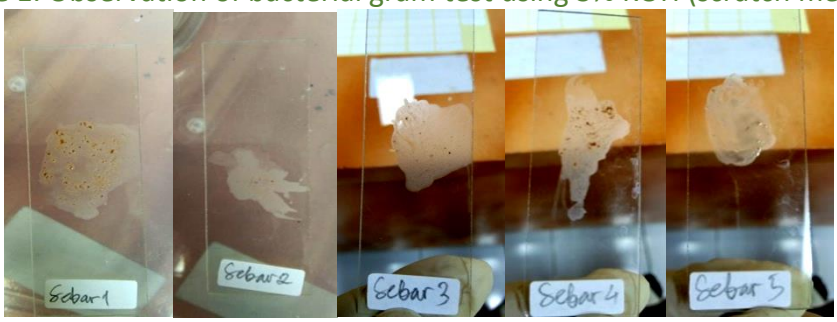

Figure 2. Observation of bacterial gram test using 3% KOH (scatter method)

The gram reaction test results (Table 1) show that the prickly bamboo rhizosphere PGPR in the scatter method and the scratch method on average produce mucus when testing the gram reaction using 3% KOH, but in the scratch method testing all have slime which means that it is entirely gram negative compared with the scatter method that only 2 samples were slimy. The formation of the mucus is due to the breakdown of bacterial cell walls due to being in high alkaline water when given 3% KOH. This is in accordance with the statement of Soekirno (2008), which states that the gram reaction can be confirmed by the solubility test of potassium hydroxide (cori). By taking one full loop of bacterial culture that is actively growing and mixed with a drop of 3% KOH solution on a clean slide glass then stirring until a flat suspension is obtained. If when the nose is removed and mucous threads are seen, then the bacteria are gram-negative, but if a watery suspension is produced and no mucous threads appear after the ose is moved repeatedly, the bacterial culture is gram-positive. This is also in line with the statement of Suslow et al. (1982), which stated that Gram negative bacteria would form mucus when tested using 3% KOH due to rupture of the bacterial cell wall due to being in a high alkaline solution (KOH 3%). While Gram-positive bacteria do not form mucus because Gram-positive cell walls have a thick layer of peptidoglycan.

Based on the results of gram bacteria test using 3% KOH, on average PGPR spiked bamboo rhizosphere bacteria have mucus which means it is gram negative, wherein gram negative bacteria consist of Enterobactericeae (Escherichia coli, Salmonella, Shigella), Pseudomonas, etc. and there are also PGH rhizosphere bamboo bacteria that do not have mucus, which means they are gram-positive, whereas gram-positive bacteria include Bacillus, Enterococcus, etc. This is in line with the statement of Podile and Kishore (2006), which states that some genus of rhizobacteria that are PGPR are Pseudomonas, Enterobacter, Bacillus, Azospirilum, Azotobacter, Burkholderia and Serratia. So it can be stated that the bacteria in PGPR rhizosphere of bamboo spines are active and play a role in the process of plant growth.

Testing the gram reaction of PGPR bacteria from the thorny bamboo rhizosphere using a simple coloring is as follows.

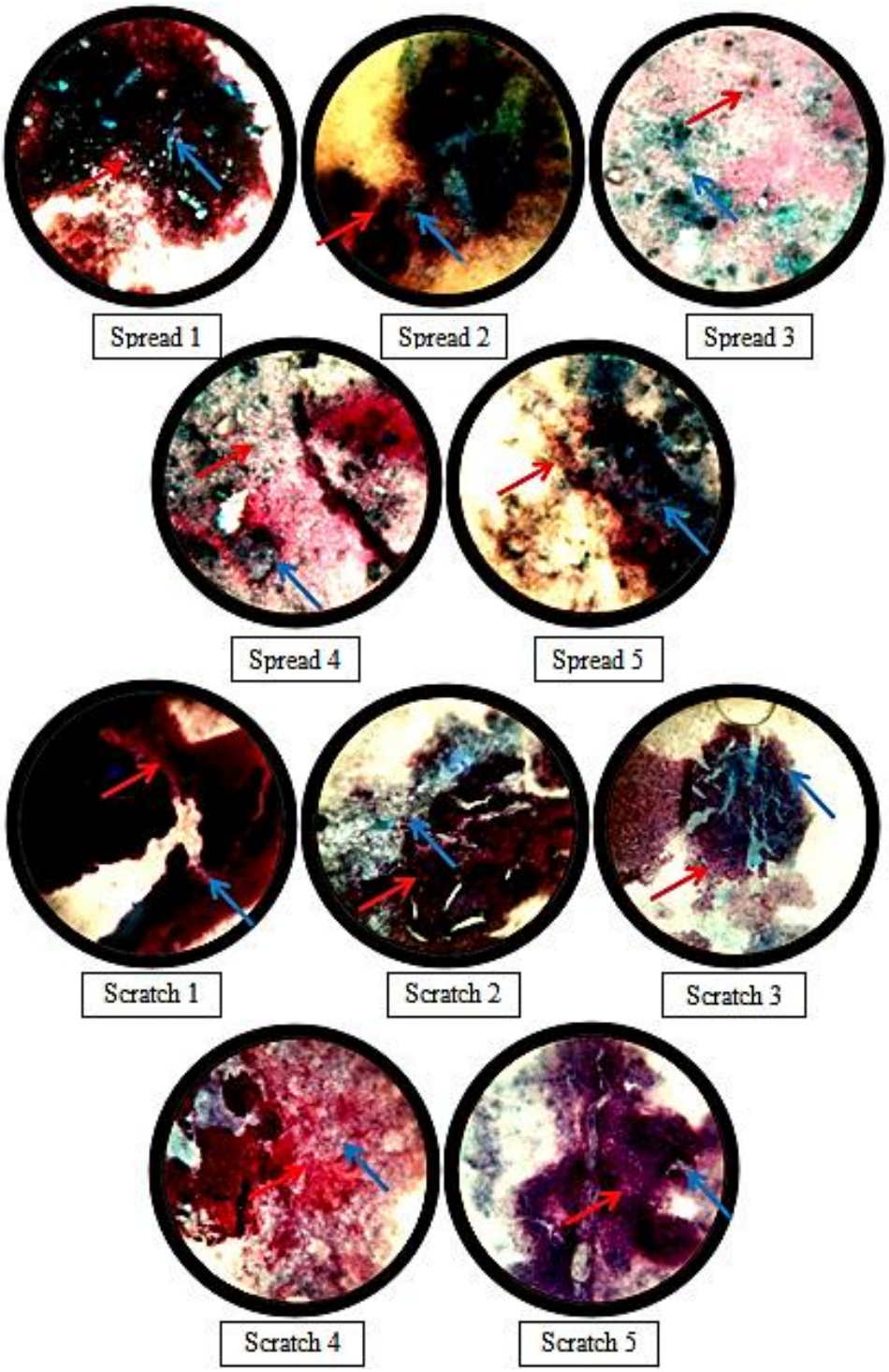

Figure 3. Microscopic observations of bacteria under a microscope with magnification
(40 / 0.65) after a simple staining method

Bacterial staining generally aims to facilitate the observation of bacterial morphology with the aid of a microscope. Bacteria are generally colorless and almost invisible because they lack contrast with the water where they might be located. Staining is needed to see bacteria very clearly both for intracellular observations and overall morphology. Simple coloring is coloring that uses a single coloring. Single dyes that are usually used in simple coloring are Methylene Blue, Basic Fuchsin, and Crystal Violet. All of these dyes can work

well on bacteria because they are alkaline and alkaline (the chromophore component is positively charged), while the bacterial cytoplasm is basophilic (likes to bases) so there is a tensile force between the chromophore component in the dye and bacterial cells, which causes the bacteria to absorb coloring well. But what is used in this test is coloring with Methylene Blue.

Microscopic observations (Figure 3) show that the Plant Growth Promoting Rhizobacteria of thorny bamboo rhizosphere on staining with methylen blue and lugol produces blue for the genus Pseudomonas and pink / purple for the genus Bacillus. This is supported by the opinion of Meynell and Meynell (1970), which states that if there is a change in the color of the bacteria to pink / purple, the bacteria are gram negative and are a group of Pseudomonas and if there is a blue color change, the bacteria are gram positive and are the Bacillus group.

In accordance with the statement of the Jambi Agricultural Training Agency (2010), which states that the content of the PGPR is dominated by Pseudomonas fluorescens and Bacillus polymixa. Benefit claims obtained are as bioprotectan, biofertilizer and as biostimulant. In line with the research of Susanti et al. (2015), which stated that several genera of bacteria were obtained from the rhizosphere of bamboo plants, including: genera Bacillus, Pseudomonas, Enterobacter.

Conclusions

Based on the research results obtained, it can be concluded that the use of the 3% gram KOH test method in identifying the Rhizobacteria Plant Growth Promoting Rhizobacteria bacteria from thorny bamboo rhizosphere (Bambusa blumeana) in the scratch method testing all samples that have slime while the scatter method has 2 samples that have slime so that the 3% KOH test method has an optimum effect in detecting microorganisms. Then the use of gram staining test method in identifying Plant Growth Promoting Rhizobacteria bacteria from thorny bamboo rhizosphere (Bambusa blumeana) on staining with methylen blue and logol produces a blue color for the genus Pseudomonas which means gram positive and pink / purple for the genus Bacillus which means gram positive so that the gram staining test method also has an optimum effect in detecting microorganisms.

References

Asniah, Widodo & Wiyono, S. 2013. Potential of Fungus Origin from Bamboo Rooting Soil as Endophytic and Biocontrol Agent of Clubroot in Broccoli. *J. Fitopatol Indones*. 1 (2): 61-68

Edwin. 2011. Microbiology Lecture Material. Banjarbaru (ID): Lambung Mangkurat University

Hadioetomo & Ratna, S. 1990. Basic Microbiology in Practice. Jakarta (ID): PT. Raja Gramedia

Jambi Agricultural Training Agency. 2010. Manufacturing Technology and Application of Plant Growth Booster Bacteria (PGPR) and Growth Regulatory Substances (ZPT). Jambi (ID): Regional Research and Development Agency

Lay, BW. 1994. Microbial Analysis in the Laboratory. Jakarta (ID): PT. Raja Grafindo.

Levine, M. 2000. An Introduction to Laboratory Technique in Bacteriology. McMillan Company, New York. Qiqi. 2008. Collection of Microbial Research Results. Jakarta (ID): Living Center Research Center

Meynell, GG. & Meynell, E. 1970. Theory and Practice in Experimental Bacteriology. 2nd Edition. London (UK): Camridge University Press.

Podile, AR. & Kishore, AK. 2006. Plant Growth Promoting Rhizobacteria. Gnanamanickam SS, editor. Plant-Associated Bacteria. Netherland: Springer.

Qiqi. 2008. Collection of Microbial Research Results. Jakarta (ID): Living Center Research Center.

Rahayu, SA. & Gumilar, MH. 2017. Test for Drinking Water Pollution in the Communities Around Bandung's Margahayu Rata with Identification of Escherichia coli. *Jurnal Sains dan Teknologi Farmasi Indonesia*. 4 (2): 50-56

Rahni, NM. 2012. Effect of PGPR Fitohormon on Growth of Corn Plants (*Zea mays*). *CEFARS. Jurnal Agribisnis dan Pengembangan Wilayah*.3 (2): 27-35.

Saharan, BS. & Nehra, V. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. *Journal of Aston*. 21(1): 1-30.

Sharma, R., Rjaka, RC. & Pandey, AC. 2010. Evidence of Antagonistic Interaction Between Rhizosphere and Mycorizhae Fungi Associated with Dendrocalamus Strictus. *J. Yeast Fungal Res.* 1 (7): 112-117.

Soekirno, 2008. Guidelines for Managing Collection and Identification of OPT (specifically for plant disease pathogens) in Horticultural Plants. Jakarta (ID): Directorate of Horticultural Crop Protection.

Strober, W. 2001. Cell Growth Monitoring. In Coligan JE, Bierer.

Suriawiria U. 2005. Microbiological Basic. Jakarta (ID): Papas Sinar Sinanti.

Susanti, WI., Widyastuti, R. & Wiyono, S. 2015. The Role of Bamboo Rhizosphere Fungi and Bacteria in Increasing Plant Growth and Desease Suppresive Soil Phenomenon. Bogor (ID): Faculty of Agriculture, Bogor Agricultural Institute.

Suslow, TV., Schroth MN. & Isaka M. 1982. Application of a Rapid Method for Gram Differentiation of Plant Pathogenic and Saprophytic Bacteria Without Staining. *Journal of Phytopathology*. 72: 917-918.

Thakuria, DNC. Talukdar, C. Goswami, S. Hazarika, RC. Boro & Khan, MR.. 2003. Characterization and Screening of Bacteria from Rhizosphere of Rice Grown in Acidic Soils of Assam*. Journal of Current Sci.* 86 (2): 978-985.

Tu, Z., Chen, L., Yu, X. & Zheng, Y. 2013. Effect of Bamboo Plantation on Rhizosphere Soil Enzyme and Microbial Activities in Coastal Ecosystem. *J. Food Agric Environ.* 11 (03): 2333-2338.

Wahyudi, AT. 2009. Rhizobacteria Plant Growth Boosters: Prospects as Biostimulator and Biocontrol Agents. Tangerang (ID): Nano Indonesia.

Widjaja, EA., Sastrapradja, S., Prawiroatmodjo, S. & Soenarko, S. 1995. Types of Bamboo. Jakarta (ID): Library.

Zahir, ZA. Khalid, A. & Arshad, M. 2004. Creening Plant Growth Promoting Rhizobacteria for Improving Growth and Yield of Wheat. *J. Appl. Microbiol*. 96: 473-480.