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Submission date: 18-Mar-2021 04:36AM (UTC-0700)
Submission ID: 1536096597
File name: GUSMIATY2121-222020PCBMB7506_1.pdf (208.3K)
Word count: 3144
Character count: 16434

Plant Cell Biotechnology and Molecular Biology 21(21&22):64-70; 2020 ISSN: 0972-2025

THE ABILITY OF RHIZOSPHERE FUNGI ISOLATE OF MAHOGANY [*Swietenia mahagoni* (L.) Jacq.] IN DISSOLVING PHOSPHATE

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Received: 03 June 2020
Accepted: 09 August 2020
Published: 18 August 2020

Original Research Article

ABSTRACT

The purpose of this study was to explore the ability of rhizosphere fungus isolates of mahogany stands in dissolving phosphate. Activities and types of microbes which can dissolve phosphate in mahogany plants are essential and needed to be known in order to obtain suitable microbial types to increase plant growth. This study was conducted for two months from March to May 2019 and analyzed in the Laboratory of Biotechnology and Tree Breeding at Faculty of Forestry Universitas Hasanuddin, Makassar. Rhizosphere microbes have important roles in nutrient cycling and during soil formation, plant growth, influencing microbial activity as well as biological control of root pathogens. Preparation of microbial culture, rejuvenation process and preparation of phosphate solubility test media were analyzed with the qualitative method by measuring the absorbance by using a UV-VIS spectrophotometer with 693 nm wavelength. The results of the rejuvenation of fungus isolates that were successfully grown were 27 isolates out of 28 isolates from genus *Rhizopus*, *Fusarium*, *Aspergillus*, *Penicillium*, *Trichoderma* and *Gliocladium*. The highest phosphate concentration was the genus Glioclodium (MB 9.2) with a 6.65 ppm concentration value, while the lowest phosphate concentration was genus Glioclodium (MB 3) with -1.47 ppm concentration value. These isolates produce phosphorus that can be used by plants.

Keywords: Rhizosphere; microorganism; fungi; phosphate.

INTRODUCTION

The condition of the soil where the tree grows influences tree growth. Part of the soil that contains microorganisms that is beneficial for plant growth is called the rhizosphere, which has a role in plant roots. According to Prayudyaningsih et al. [1], the population of microorganisms in the rhizosphere is generally more numerous and varied than in non-rhizosphere soils.

The rhizosphere is a soil layer that covers rhizoplane, which influenced by root activity and excellent habitat for microbial growth. Plant roots provide a variety of organic ingredients that generally stimulate microbial growth. The rhizosphere is used to show part of the land affected by plant roots, characterized by more microbiological activities compared to activities in soil that far from roots. The rate of metabolic activity of rhizosphere microorganisms is different from the rate of microorganism metabolism in non-rhizosphere soils [2].

Rhizosphere microbes have important roles in nutrient cycling and during soil formation, plant growth, influencing microbial activity as well as biological control of root pathogens. Microbes such as fungi can be obtained or isolated from the rhizosphere. Many microbes, especially the rhizosphere fungi, have the ability to release phosphate bound by soil components, so it is available for plants. Phosphate solvent microbes can dissolve phosphate, characterized by the formation of clear zones around colonies when grown on Pikovskaya agar [3].

Phosphate solvent microbes (P) able to help dissolve phosphate into available forms because of its ability to secrete several organic acids such as formic acid, acetate, propionate, lactate, fumarate, and succinic. These organic acids are able to bind Al and Fe to free P bound and become available [4]. The types of microbes that are often found in phosphate research are *Trichoderma* sp [5] and *Aspergillus* sp and *Penicillium* sp [6]. Research on activities and types of microbes that have the ability to dissolve phosphate in mahogany plants is essential and needed in order to obtain suitable microbial types to increase plant growth.

RESEARCH METHODOLOGY

Samples of fungus isolates used in this study obtained from a collection of 28 mahogany stand fungus isolate in the Laboratory of Biotechnology and Tree Breeding.

Microbial Culture Media

The culture media were used to grow fungi was Potato Dextrose Agar (PDA). 19.5 g of PDA, 5 g of glucose, and 10.5 g of agar weighed, after that put into Erlenmeyer and 500 ml of distilled water was added. Erlenmeyer must be covered with aluminum foil, then homogenized by using a hot plate magnetic stirrer. The media inserted into the autoclave for ± 2 hours, then the solution was poured into a petri dish and stored in Laminar Air Flow Cabinet (LAFC).

Recovery of Fungal Strains

Steps for the Recovery of fungal strains of fungi, firstly prepared tools and materials for the rejuvenation process and hands must be sterilized with alcohol. Then Petri dishes containing isolates were opened and heated. After that, one piece of fungi isolates was taken to be rejuvenated by using ose needle and put on a new sterile PDA media. The edge of the Petri dish was re-heated and then covered with plastic wrap. Fungi were observed for ± seven days.

Phosphate Solubility Test Media

The production of phosphate solvents has several steps, as follow:

Concentrated P adhesive

12 g $(NH_4)_6$ $MO_7O_{24,}$ 4 H_2O were weighed and then dissolved into 100 ml of aqua-dest in a 1 L measuring flask. Then 0.227 g $K(C_6O)C_4H_4O_{6,}$ 0.5 H_2O added with aquadest up to 1 L volume.

Concentrated P color reagents

0.52 g of ascorbic acid added to a 100 ml volumetric flask was weighed. The concentrated P reagent was pipetted as much as 50 ml and then

put into a measuring flask. Then the solution was diluted with distilled water until 100 ml.

Phosphate dissolution test

According to Pikovskaya Rao and Subba (1999), 5 pieces of mushrooms were taken using cork borer, and then the isolates were put into liquid Pikovskaya media for seven days and were shaken. Fungus suspension was placed into the tub and then centrifuged (15 minutes: 1000 rpm). Supernatant solution pipetted for 5 ml and put in a test tube. 0.5 ml of concentrated P reagents added, then shake for a few minutes and let stand for 30 minutes. After that, the absorbance was measured using a spectrophotometer with 693 nm wavelength.

RESULTS AND DISCUSSION

Rejuvenation of Rhizospehere Fungus Isolate of Mahogany (*Swietenia mahagoni*)

The results of the rejuvenation of fungi isolates that were successfully grown were 27 isolates out of 28 isolates.

Table 1 shows one isolate with MT 4.3 code cannot be used because during the rejuvenation process, other fungi often contaminate this isolate; thus, pure isolates cannot be obtained.

Table 1. Characterization of rhizosphere fungus isolates in mahogany stands at Takalar and Maros regencies

Isolate code	Color of fungus colonies	Texture	Genus (Tunggal, 2019)
MT 1.1	Brownish Yellow	Velvety	*Aspergillus*
MT 2.1	White	Soft Cotton	*Fusarium*
MT 2.3	Black on Center, White Edge	Rough Cotton	*Aspergillus*
MT 3.1	Gray on Center, White Edge	Soft Cotton	*Penicillium*
MT 3.2	Grayish Green	Velvety	*Penicillium*
MT 4.2	Yellow on Center, White Edge	Velvety	*Aspergillus*
MT 5.3	White	Velvety	*Penicillium*
MT 5.4	Black on Center, White Edge	Rough Cotton	*Aspergillus*
MT 5.5	White	Soft Cotton	*Rhizopus*
MT 6.1	Grayish Green	Velvety	*Penicillium*
MT 6.2	Grayish Black	Soft Cotton	*Aspergillus*
MT 6.3	Black on Center, White Edge	Soft Cotton	*Aspergillus*
MT 7.2	Grayish Green	Velvety	*Penicillium*
MT 8.5	Grayish Black	Soft Cotton	*Aspergillus*
MT 9.2	Brown	Velvety	*Gliocladium*
MT 10.4	Yellow on Center, White Edge	Velvety on Center, Soft Cotton Edge	*Gliocladium*
MB 1	Greenish White	Rough Cotton	*Trichoderma*
MB 2.1	Gray	Rough Cotton	*Rhizopus*
MB 3	Gray on Center, White Edge	Velvety	*Gliocladium*
MB 4.2	Greenish White	Rough Cotton	*Rhizopus*
MB 6.1	White	Soft Cotton	*Rhizopus*
MB 6.2	Greenish White	Rough Cotton	*Trichoderma*
MB 7.1	White	Rough Cotton	*Trichoderma*
MB 7.2	Greenish White	Rough Cotton	*Trichoderma*
MB 9.2	Gray	Rough Cotton	*Gliocladium*
MB 9.3	Black on Center, White Edge	Soft Cotton	*Aspergillus*
MB 10.2	White	Rough Cotton	*Rhizopus*

Note: MT (Takalar Mahogany); MB (Bengo Mahogany)

The process of rejuvenation of fungi was using the PDA (Potato Dextrose Agar) media. This medium is a frequent medium used for fungus growth because it has a low pH, which is around 4.5 to 5.6; thus, it can prevent the growth of other microorganisms (bacteria) on the media [7]. The composition of PDA media consists of natural ingredients (potatoes) and synthetic ingredients (dextrose and agar). Therefore these media are included in semi-synthetic media. Potatoes, dextrose, and agar are essential for the growth and propagation of microorganisms, since potatoes are a source of carbohydrates, vitamins, and energy, dextrose functions as sugar and energy, and agar functions to condense PDA media.

Morphological characteristics of 27 isolates of rhizosphere fungus were very diverse, as shown on the colony color of each isolate, and the texture varies from each isolate. The color of fungus isolates colonies in mahogany stands in Takalar Regency were white, yellow, brown, gray, and black. The texture of the colony was dominated by velvety texture. Some isolates had the texture of fine cotton and rough cotton. The color of fungi isolate colonies in mahogany stands at Maros Regency included white, greenish, brown, gray, and black. The textures of the colonies were dominated by rough cotton, some velvety, and fine cotton textures.

Phosphate Solubility in Mahogany Rhizosphere Fungus Isolate Test

Change in color

The results of observations after adding concentrated P was a color change; the results obtained from the fungi isolate supernatant turned blue. However, the changes that occur was varied according to the level of color density, as in Fig. 1. The process of color change took 30 minutes. The isolate in MB 3 did not appear to be bright, while the MT 10.4 isolates changed to blue. MB 9.2 changed color to dark blue. Color changes that occurred were not much different from the control. The level of color change influenced by the absorbance of each isolate. The higher the absorbance of color produced, the higher the concentration. The color change obtained indicated that the rhizosphere fungus isolates of mahogany stands were able to produce phosphate, as it can be seen from the result of phosphate concentrations that had been measured with a spectrophotometer.

The concentration of phosphate dissolution

The measurement results of the concentration level of phosphate dissolution (Table 2) obtained were able to produce phosphate with varying concentrations. Rhizosphere fungus isolates were incubated for 30 minutes.

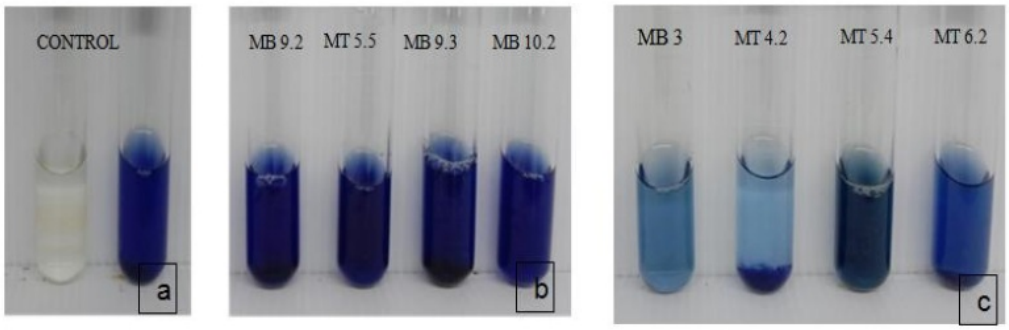

Fig. 1. Color comparison of control and phosphate concentrations, (a) Control, (b) Highest Mahogany Phosphate Concentration, (c) Lowest Mahogany Phosphate Concentration in Takalar and Maros Regencies

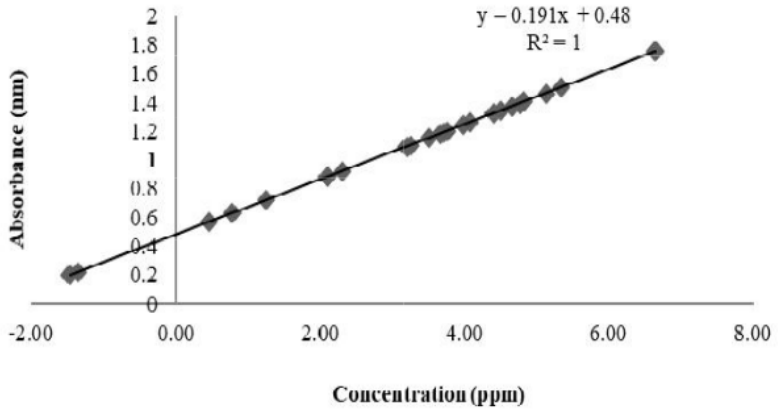

Fig. 2. Graph of the relationship between absorbance and phosphate concentration

Table 2. The measurement results of phosphate concentration dissolution of rhizosphere fungus isolates in mahogany stands with 693 nm wavelength

Isolate Code /Genus (Tunggal, 2019)	pH	Absorbance (nm)	Concentration (ppm)
MB 9.2/*Gliocladium*	6.74	1.75	6.65
MT 5.5/*Rhizopus*	6.25	1.50	5.34
MB 9.3/*Gliocladium*	4.68	1.46	5.13
MB 10.2/*Rhizopus*	6.14	1.40	4.82
MB 4.2/*Rhizopus*	6.46	1.40	4.82
MB 7.2/*Trichoderma*	6.26	1.39	4.76
MB 1/*Trichoderma*	6.79	1.37	4.66
MB 7.1/*Trichoderma*	6.57	1.34	4.50
MB 6.2/*Trichoderma*	6.80	1.32	4.40
MT 6.1/*Penicillium*	6.19	1.26	4.08
MT 9.2/*Gliocladium*	6.11	1.24	3.98
MT 5.3/*Penicillium*	6.15	1.20	3.77
MT 1.1/*Aspergillus*	6.88	1.19	3.72
MT 10.4/*Gliocladium*	6.58	1.18	3.66
MT 6.3/*Aspergillus*	5.94	1.18	3.66
MT 3.2/*Penicillium*	6.55	1.15	3.51
MB 6.1/*Rhizopus*	6.19	1.10	3.25
MB 2.1/*Rhizopus*	6.22	1.09	3.19
MT 2.1/*Fusarium*	6.36	1.09	3.19
MT 3.1/*Penicillium*	5.63	0.92	2.30
MT 8.5/*Aspergillus*	6.68	0.88	2.09
MT 2.3/*Aspergillus*	4.97	0.88	2.09
MT 7.2/*Penicillium*	5.37	0.72	1.26
MT 6.2/*Aspergillus*	6.61	0.63	0.79
MT 5.4/*Aspergillus*	6.25	0.57	0.47
MT 4.2/*Aspergillus*	5.17	0.22	-1.36
MB 3/*Gliocladium*	6.05	0.20	-1.47

Based on the table of phosphate ability test results, the concentration value produced by each isolate is different - even with the same genus. This is because each fungus isolates have the ability to produce organic acids. Phosphate production is also influenced through the incubation period. The longer incubation period can cause a reduction in the number of nutrients present in the growing

media. Phosphate concentration is also influenced by environmental factors or conditions of each sampling location [8].

Table 2 shows that the highest phosphate concentration was from the genus Glioclodium (MB 9.2) with a 6.65 ppm concentration value, while the lowest phosphate concentration was the genus Glioclodium (MB 3) with -1.47 ppm concentration value. However, the results of this study are lower compared to research by Lailasari, which was conducted in 2019, stated that the phosphate solubility test of Rhizozfer fungus isolates on candlenut stands highest phosphate concentration was 9.79 ppm from genus Rhizopus, and the lowest phosphate concentration was 4.32 ppm from genus Penicillium. While the study by Rahim, Iradhatullah, [9] showed that the highest phosphate concentration of cacao plant fungus isolates was 3.46 ppm.

Fungus isolates with genus Aspergillus had phosphate concentrations from -1.36 ppm to 5.34 ppm. The results obtained in this study are higher when compared with the study by Elfiati [10], which obtained and isolated in five ecosystems with the value from 2.88 ppm to 3.81 ppm. These isolates produce phosphorus that can be used by plants.

Fig. 2 explains that the higher the absorbance (ppm), the higher the concentration, indicating the amount of phosphate dissolved in the soil that can be utilized by plants. Based on [11], the mixed microbial culture of phosphate solvents can increase the effectiveness of inorganic phosphate solvents in the soil; thus, it can be utilized by plants. The beneficial association occurring between plant and microbes in rhizosphere determines the health of plant and fertility of soil [12,13].

There is a difference between the pH of the fungus supernatant isolates and control with pH from-4.97 to 6.88. Generally, phosphate dissolution occurs at acidic pH or below 6. However, the results obtained are not much different if compared with the study by Lailasari, [8], where pH obtained ranged from 4.32 to 6.93. There is a tendency that at acidic pH, the level of phosphate dissolution varies in value when compared with isolates tested [14]. Organic acids produced by fungi can cause a decrease in pH in liquid pikovskaya media. Organic acids are beneficial for increasing phosphate absorption. The higher the production of organic acids, the higher the rate of phosphate absorption.

In neutral or alkaline soils that have high phosphate content, microorganisms are able to dissolve phosphate and make it available to plants. In contrast, acid soils are usually poor in calcium ions. Thus phosphates are deposited in the form of iron compounds. One way to improve phosphorus amount is to inoculate seeds or soil with solvent microorganisms. Research by Sethi and Rao (1968) showed that fungus is a better agent in dissolving phosphate than bacteria [2].

CONCLUSION

Based on the results of research, it can be concluded that the fungi isolate with the highest phosphate dissolving ability was 6.65 ppm at pH 6.74 from isolate MB 9.2 and genus Glioclodium. The relationship of absorbance with the concentration of phosphate solubility was directly proportional to the increase in the blue intensity of the low concentration phosphate solution to a high concentration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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