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Potential of PGPR isolated from rhizosphere of pulpwood trees in stimulating the growth of *Eucalyptus pellita* F. Muell

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Abstract *Eucalyptus pellita* is an alternative plant for *Acacia crassicarpa* and *Acacia mangium* as raw materials for pulp and paper. *E. pellita* is a plant that easily adapts to various environmental conditions, but its growth rate is lower than that of *A. crassicarpa* and *A. mangium*. The growth rate of *E. pellita* can be stimulated by applying plant growth-promoting rhizobacteria (PGPR). A study was conducted to characterize and analyze the ability of 25 PGPR isolates that isolated from rhizosphere of *E. pellita* seedlings in stimulating the growth of *E. pellita*. Characteristics of PGPR were analyzed consisted of the isolates' ability to produce IAA, siderophores, phosphate solubilization, ability as a biocontrol, hypersensitivity test using tobacco plants, and compatibility test between isolates. Stimulating the growth of *E. pellita* seedlings was carried out with the following treatments: inoculation of a consortium of five selected isolates with or without the addition of fungicides. As a comparison, *E. pellita* seedlings were also treated without inoculation of a PGPR consortium, and some seedlings were added with fungicides, as well as fungicides and fertilizer. Five isolates were selected which were applied for stimulating the growth of *E. pellita*. The selected isolates were known to produce IAA, siderophores, phosphate solubilization, and be able to inhibit the growth of *Ralstonia solanacearum* or *Cylindroclaium* sp.. In addition, the isolates were not pathogenic, because they did not cause necrotic on tobacco plants. The five isolates were employed as a consortium because the isolates were mutually compatible. The most effective treatment in stimulating the growth of *E. pellita* was the inoculation of PGPR consortium with the addition of fungicide which caused an increase in plant height of 46.92% and stem diameter of 22.88% compared to control. In contrast, treatment with the addition of fungicides actually inhibits the growth of *E. pellita*.

Keywords: *Eucalyptus pellita*, IAA, PGPR, Phosphate Solubilization, Siderophores

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Introduction

Global demand for pulp and paper raw materials continues to increase every year. As a result, timber plantations, which in 1990 covered an area of around 167.5 million ha, increased to 277.9 million or an increase of about 6.95% of the total plantation forest area in 2015 (Payn *et al.*, 2015). The highest increase was found in East Asia followed by Europe, North Korea, South America, and Southeast Asia. Indonesia is one of the countries in Southeast Asia where the demand for pulp and paper raw materials continues to increase from year to year in line with the rapid growth of the pulp and paper industry. The demand for pulp and paper raw materials in Indonesia from 2013 to 2016 increase to 41.9 million m^3 (18.7%) with a production capacity of 10 million tons/year (Ministry of Industry 2016). The total area of timber plantations in Indonesia reaches 10.7 million hectares and about 2.59 million hectares of this area is peat soil (Ministry of Environment and Forestry, 2018).

Several types of pulpwood that are widely grown in Indonesia are *Acacia crassicarpa* and *Acacia mangium* (Inail *et al*., 2019). *A. crassicarpa* is the only acacia species that can grow well on peatlands that are known to be relatively wet. However, the survival rate of these species tends to decrease as a result of increased crop rotation. The survival rate of *A. crassicarpa* in the second rotation was lower (below 30%) than *A. crassicarpa* planted in the first rotation (Junaedi, 2018). In addition, on dry land, the growth rate of *A crassicarpa* is not optimal compared to *A. mangium* (Harwood and Nambiar, 2014). *A. mangium* is a fast-growing plant. This plant reaches 10 to 15 m in height in the first two to three years and grows faster to 25 m at the age of five years. The average increase in plant height ranged from 1.8 to 5.8 m/year. The average plant diameter increased rapidly to 15 cm for stands less than three years old and the plant diameter reached about 25 cm at the age of eight years (Krisnawati *et al*., 2011). Currently, *A. mangium* is facing a very serious threat due to root rot disease caused by *Ganoderma* sp. and *Ceratocystis* sp. wilt disease. resulting in significant crop damage and high plant mortality (Brawner *et al.*, 2015; (Harwood and Nambiar, 2014; Harwood *et al*., 2015). To maintain the productivity of the pulp and paper industry, *E. pellita* emerged as a substitute species for *A. crassicarpa* and *A. mangium*. *E. pellita* can adapt well to various environmental conditions. However, *E. pellita* has a relatively slow growth compared to *A. mangium* (B áez-Aparicio *et al*., 2021; Nambiar *et al*., 2018) and *E. pellita* is also susceptible to bacterial wilt disease caused by *Ralstonia solanacearum*. The wilt disease caused losses in nurseries in the timber plantation area of Riau during the 2010-2011 period which reached Rp. 16 billion (Siregar *et al*., 2020). Bacterial wilt disease control is carried out in

various ways, such as eradicating disease using synthetic chemicals, selecting Eucalyptus clones that are resistant to pathogen attack, and early molecular detection of bacterial wilt disease in nurseries. However, these efforts have not shown effective results.

One of the efforts to stimulate the growth and protection of *E. pellita* from disease attack is by applying microorganisms called Plant Growth Promoting Rhizobacteria (PGPR). PGPR is known to stimulate plant growth because their ability to solubilize phosphate compounds (Kumari *et al*., 2018), auxin and IAA synthesis(Viscardi *et al*., 2016; Nadeem *et al*., 2016), ACC deaminase activity (Belimov *et al*., 2015), siderophore production *(*Kumar *et al*., 2019), as N-fixing bacteria (Kuan *et al*., 2016), and as a biocontrol agent (Habazar *et al*., 2018; Rajeela *et al*., 2018). PGPR has been widely applied to cultivated plants, such as tomatoes (Hernández-Montiel *et al.*, 2017), rice (Banik *et al*., 2019), soybeans (Omara *et al*., 2017), cucumber (Salim *et al*., 2021), and corn (Lin *et al*., 2019). PGPR application is able to stimulate maize growth in high salinity areas through the mechanism of phosphate solubilization and siderophore production (Ullah and Bano, 2015) and helps bean plants adapt to drought stress (Kumar *et al*., 2016). PGPR has also been widely applied to annual plants. A consortium of PGPR (*Azotobacter chroococcum*) with arbuscular mycorrhizae (*Ambispora leptoticha* and *Trichoderma harzianum*) was able to increase stem diameter, plant height, and dry weight (97%) of teak (*T. grandis*) in the nursery phase compared to control (Raghu *et al*., 2020). PGPR *Azotobacter* spp. and *Azospirillum* spp. are also able to increase the growth of *Eucalyptus grandis* (González-Díaz *et al*., 2019).

Isolates that have the potential as PGPR need to know their hypersensitivity to plants and their compatibility between isolates when the isolates are employed as a bacterial consortium. A hypersensitivity test is a test to determine the pathogenicity of isolates to plants (Mulaw *et al*., 2018). Compatibility test on microbial consortium is very important because a consortium of bacteria that interact synergistically and compatible can give better results than single bacteria. Nine isolates that were isolated from cassava showed negative hypersensitivity reactions to tobacco plants, indicating that these isolates were not pathogenic to plants (Safriani *et al*., 2020). The consortium between *Bacillus cereus*, *Bacillus subtilis,* and *Cyanobacteria* showed compatibility between isolates that could increase tomato growth and reduce disease (Yanti *et al*., 2021). The consortium between fungicides and PGPR showed good compatibility and was able to significantly increase plant growth and fiber production (Bhattacharyya *et al*., 2017). Because of the importance of maintaining the continuous productivity of pulp and paper raw materials, it is necessary to search for PGPR isolates that can stimulate plant

growth. This study aimed to analyze the potential of isolates isolated from rhizosphere of pulpwood and to determine their ability to stimulate the growth of *E. pellita*.

Materials and methods

Sampling

Rhizosphere was taken from 5 two-month-old *E. pellita* seedlings planted in pots. One fertile plant was selected from each tray, then the five soil samples along with the roots from the pot were put into a plastic bag and homogenized.

Isolation and morphological characterization

Twenty grams of rhizosphere mixture was dissolved in 180 mL of sterile water solution, shaken for 20 minutes at 200 rpm. A total of 100 µL cultures from the 10^{-4} dilution series were inoculated onto sterilized nutrient agar. The culture was then incubated at 37^{o}C for 3-4 days. A single bacterial colony was subcultured on fresh nutrient agar. Characteristics of colony morphology observed were its shape, surface, elevation, edge, and color.

IAA Production

The ability of isolates to produce IAA was measured colorimetrically using a spectrophotometer at a wavelength (λ) = 530 nm. One loop of the bacterial isolate was added to nutrient broth and incubated for 48 hours at 100 rpm at room temperature. After the incubation period, the cultures were centrifuged at 10,000 rpm for 20 min. Two mL of the supernatant was taken and 2 mL of Salkowski reagent (composition 150 mL H_2SO_4, 250 mL aquadest, 7.5 mL $FeCl_3.6H_2O$ 0.5 M) (Alotaibi *et al*., 2021) were added which was then left at room temperature for one hour in the dark. Isolates capable of producing IAA were characterized by a change in the culture color to pink. The concentration of IAA produced by the isolates was measured based on a standard curve using pure IAA.

Phosphate solubilization

Each isolate was grown by spotting on Pikovskaya agar containing tricalcium phosphate, with the following composition per liter: 10 g glucose; 5 g $Ca_3(PO_4)_2$; 0.5 g $(NH_4)_2SO_4$; 0.2 g KCl; 0.1 g $MgSO_4.7H_2O$; 0.002 g $MnSO_4$;

0.002 g $FeSO_4$; 0.5 g yeast extract, and 20 g bacto agar. The plates were incubated in an inverted position for 7 days and a halo zone around the colony was observed and the phosphate solubilization index was measured (Pikovskaya, 1948).

Siderophore production

The ability of bacteria to produce siderophores was carried out by the universal CAS test. Before starting the experiment, the apparatus was rinsed with 3 M HCl to remove iron and then rinsed with distilled water. CAS was prepared by dissolving 60.5 mg of CAS in 50 mL of distilled water, then mixed with 10 mL of an iron (III) solution containing (1 mM $FeCl_3.6H_2O$ in 10 mM HCl). The mixture was autoclaved for 20 minutes and cooled to 50 ^oC after adding 40 mL of hexadecyltrimethylammonium bromide (HDTMA, 72.9 mg) solution to the CAS indicator solution. At the same time, PIPES agar (pH 6.5–6.8) was containing 15.6 g of PIPES and 15 g of agar in 500 mL were also autoclaved. The two solutions were mixed with the addition of 30 mL filter-sterilized of 10% casamino acid and then the mixture was poured into a petri dish. Approximately 50 µL of culture was poured onto a 10 mm diameter paper disk placed in the center of a petri dish containing CAS Blue Agar. The cultures were incubated in the dark at room temperature for 7 days. Siderophore production by isolates was indicated by the formation of an orange halo zone around the colony *(Louden et al.,* 2011).

Antagonism test against R. solanacearum and Cylindrocladium sp.

Antagonism test against *R. solanacearum* was carried out by growing the isolate using pour plate method onto trypenyl tetrazolium cloride agar. Then, 200 µL of PGPR isolate that had previously been grown in nutrient broth was poured on paper discs at three different points on top of the *R. solanacearum* culture. The culture was incubated for 24 hours at room temperature. The inhibition of PGPR isolates against *R. solanacearum* was indicated by the formation of a halo zone around the isolates colony. Antagonism test on *Cylindrocladium* sp. was performed by dual culture method. Potato sucrose agar (PSA) pieces with a diameter of 0.5 cm covered with *Cylindrocladium* sp. hyphae was placed on the left side on top of fresh PSA at a distance of 3 cm from the edge of petri dish. Subsequently, the PGPR isolate was scratched lengthwise with a distance of 3 cm from the right side on the edge of petri dish. The cultures were then incubated at 26-28 ^oC for 48 hours. Inhibition of the isolates against *Cylindrocladium* sp. was determined by measuring the radius of

pathogen growth towards the PGPR isolate and the radius of pathogen growth towards the edge of its colony.

Hypersensitivity test

A hypersensitivity test was conducted to determine the pathogenicity of isolates against plants. Tobacco plants that were 2 months old were used as non-host plants. A total of 0.5 mL of PGPR liquid culture was injected on the lower surface of the leaves which were previously surface sterilized using alcohol. After an incubation period of 48 hours, the symptoms of tobacco attack were observed. The presence of necrosis on the leaves indicated that the inoculated isolates were pathogenic (Nawaningsih *et al*., 2011).

Compatibility test

The compatibility test aims to determine whether the isolates do not inhibit each other using the disc diffusion method. The isolates to be analyzed were first grown in 3 mL of nutrient broth and incubated for 24 hours with 100 rpm agitation. Hereinafter, 100 µL of isolate suspension (A) aged 24 hours was inoculated into nutrient agar by pour plate method. A total of 10 µL of the other isolate suspension (B) was poured onto sterile paper disc with a diameter of 5 mm placed on a nutrient agar that had been inoculated with isolate (A) by pour plate. Compatible isolates were characterized by the absence of a halo zone around the colony.

Ability test of PGPR in stimulating the growth of Eucalyptus pellita

E. pellita growth stimulation was carried out using a completely randomized design with 5 treatments, namely control (T1), PGPR application (T2), PGPR and fungicide application (T3), fungicide application (T4) and fertilizer and fungicide application (T5). Each treatment consisted of 6 replications. The PGPR application was carried out by inoculating a PGPR consortium of 2-day-old in each treatment onto a growing medium containing cocopeat, dolomite, Simplot, and Triple Super Phosphate (TSP) (Figure 1). The seeds of *E. pellita* used were 2 weeks old derived from plantlets of tissue culture propagation that had complete organs. The PGPR consortium consisted of five compatible isolates selected based on the results of the compatibility test. One ose of each isolate was inoculated into 50 mL of nutrient broth. The culture was incubated for 2 days at 28^{o}C and the population was counted to obtain 10^{8} cells/mL. A total of 1 mL of PGPR consortium suspension was

inoculated into the growing media. The addition of the PGPR consortium and observations were carried out every week for 75 days.

The growth of *E. pellita* seedlings was observed before and after inoculation of the PGPR consortium every week by observing plant height, the number of leaves, stem diameter, wet and dry weight of plants, and disease intensity. Plant height was measured from the base of the plant to the growing point. The diameter of the stem was measured using a caliper on the part marked with a height of 1 cm from the ground surface. The number of leaves was calculated by counting the fully formed leaves. Plant dry weight was measured by weighing the wet weight of the plants that had been separated from the soil when the plants were 75 days old. Then the plants were wrapped in newspapers and dried at 60℃ for 72 hours and their dry weight was measured.

Figure 1. *E. pellita* seed test plant

Data analysis

All data obtained are presented in the form of tables and graphs. The effectiveness of PGPR isolates in stimulating the growth of *E. pellita* was measured based on its growth parameters. Statistical data analysis used Analysis of Variance (ANOVA) and continued with LSD test with a 5% confidence level using SPSS version 22.

Results

Morphological colony of the isolates

This study obtained 25 isolates that have varied characteristics based on shape, edge, elevation, surface, and color colony observed on nutrient agar. The

colony characteristics of rhizobacteria isolates were dominated by round shape, smooth edges, convex elevation, shiny surface, and white color. Detailed morphological colonies of isolates are presented in Table 1.

Isolate ability to produce IAA, siderophores and phosphate solubilization

Twenty-four of the 25 isolates were able to produce IAA. Isolate EP6 produced the highest IAA and the lowest was shown by isolate EP29. Eighteen of the 25 isolates were able to solubilize phosphate with the highest phosphate solubilizing ability at isolate EP2. This is indicated by the presence of a halo zone around the colony (Figure 2A). There were 18 isolates capable of producing siderophores indicated by the presence of an orange halo zone around the colony (Figure 2B).

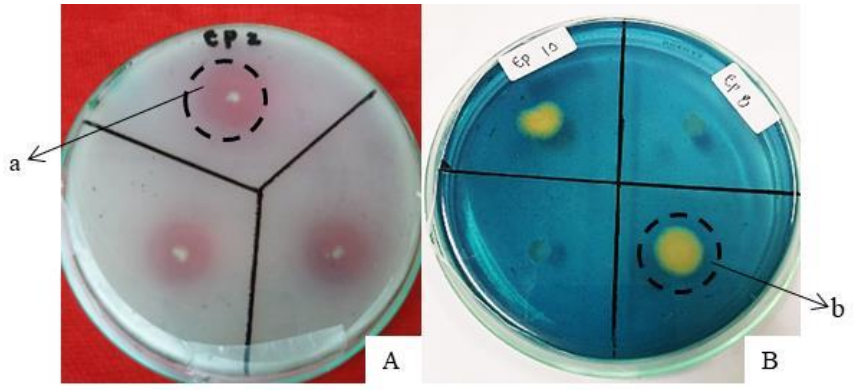

Figure 2. Phosphate solubilization (A) and siderophore production (B); (a) Halo zone, (b) Orange halo zone

***The ability of isolates to inhibit R. solanacearum and Cylindrocladium* sp.**

Nine out of 25 isolates were able to inhibit *R. solanacearum*. The highest inhibition was on isolate EP20. Of the 25 isolates, 16 isolates were able to inhibit *Cylindrocladium* sp. The highest inhibition against *Cylindrocladium* sp. was found in isolate EP11. Several isolates that did not show inhibition activity (Figure 3).

Table 1. Morphological and physiological characters of the isolates

Isolate code	Morphological colony					PGPR characters		
	Shape	Edge	Elevation	Surface	Colour	IAA (ppm)	Solubility index	Siderophore
EP1	Irregular	Undulate	Convex	not shiny	Yellowish white	1.25	16	+
EP2	Round	Undulate	Convex	shiny	Yellowish white	0.75	333	+
EP4	Concentric	Undulate	Convex	shiny	Yellowish white	0.42	24	+
EP5	Round	Entire	Drop-like	shiny	Orange	1.00	12	-
EP6	Irregular	Undulate	Convex	not shiny	A bit clear	4.33	24	+
EP7	Round	Entire	Umbonate	not shiny	White	0.25	18	-
EP8	Round	Entire	Raised	not shiny	Gray	1.58	0	-
EP10	Round	Entire	Raised	not shiny	Yellowish white	1.50	136	+
EP11	Irregular	Lobate	Hilly	not shiny	Yellowish white	0.58	58	+
EP12	Round	Entire	Convex	shiny	White	0.92	32	+
EP13	Round	Entire	Convex	shiny	Reddish orange	0.83	49	-
EP15	Round	Entire	Drop-like	shiny	Yellowish white	1.25	0	+
EP16	Filamentous	Undulate	Hilly	shiny	Yellowish white	3.25	8	+
EP17	Round	Undulate	Convex	shiny	Yellowish white	1.58	144	+
EP18	Round	Entire	Convex	shiny	Yellowish white	1.33	179	+
EP19	Concentric	Entire	Drop-like	shiny	White	0,42	34	+
EP20	Filiform	Ciliate	Raised	not shiny	White	1.00	0	+
EP21	Round	Entire	Raised	shiny	Orange	0.17	35	-
EP22	Round	Entire	Convex	not shiny	Yellow	2.25	0	+
EP24	Round	Entire	Convex	shiny	Yellowish orange	0.50	0	+
EP25	Filiform	Entire	Convex	shiny	White	0.00	70	+
EP26	Irregular	Undulate	Raised	shiny	A bit clear	2.25	0	+
EP27	Filiform	Entire	Convex	shiny	Yellowish white	0.75	30	-
EP28	Round	Entire	Drop-like	shiny	Orange	1.08	0	-
EP29	Concentric	Entire	Convex	shiny	Cream	0.08	41	+

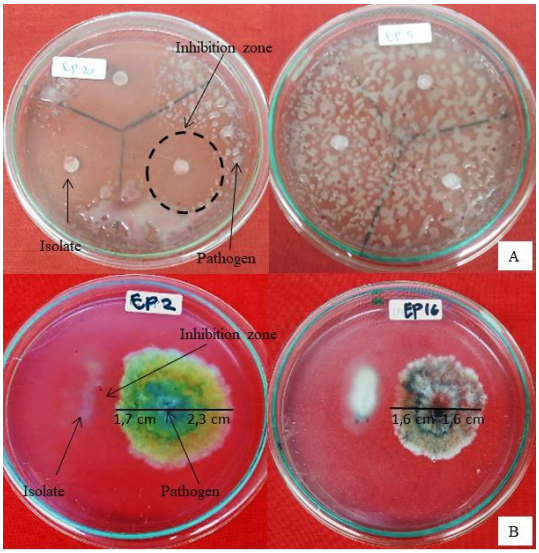

Figure 3. Antagonism test of isolates against *R. solanacearum* (A) and *Cylindroclaium* sp. (B)

Hypersensitivity test

PGPR isolates did not show any hypersensitivity reactions to tobacco plants. It is indicated by the absence of necrosis in tobacco leaves (Figure 4). Tobacco leaves that did not undergo necrosis indicated that the isolates inoculated on tobacco leaves were not pathogenic to non-host plants.

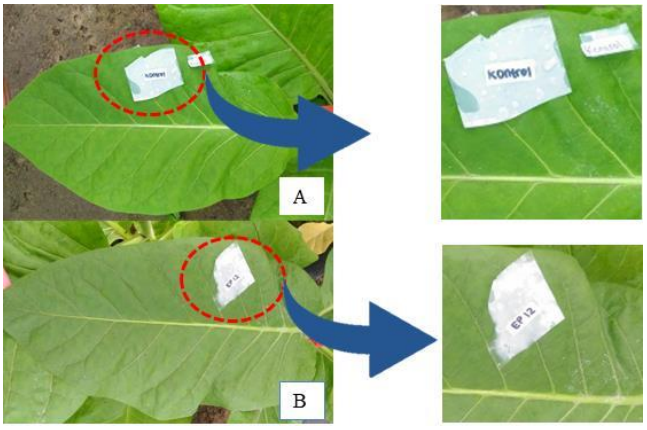

Figure 4. Hypersensitivity test of PGPR isolates. (A) control, (B) plants inoculated isolate

Compatibility between isolates

Compatibility test found that five isolates with codes EP1, EP12, EP18, EP19, and EP20 were compatible. This is indicated by the absence of a halo zone around the paper disc. Meanwhile, incompatible isolates formed a halo zone around the paper disc (Figure 5). This shows that these isolates can be used as a consortium of PGPR isolates.

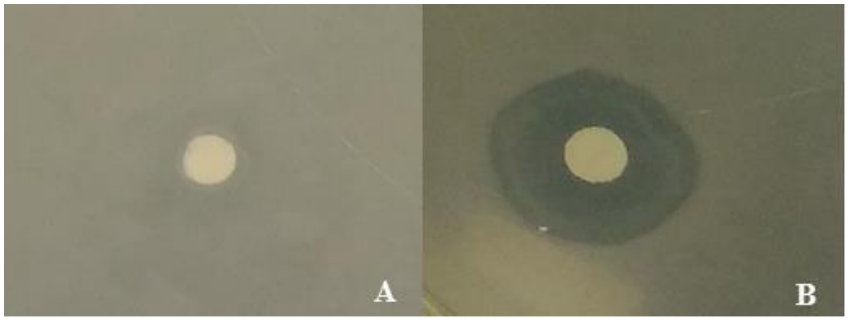

Figure 5. Compatibility test between PGPR bacterial isolates. (A) Compatible, (B) Incompatible

PGPR's ability to stimulate the growth of Eucalyptus pellita

The growth of *E. pellita* plants is presented in Figure 6. The plant height of each treatment showed varying results (Figure 6A). It is known that the inoculation of the PGPR consortium was able to increase the plant height of *E. pellita*. The order of plant height based on treatment was T3>T5>T2>T1>T4. Inoculation of the PGPR consortium with the addition of fungicides was able to increase the highest plant height. However, the addition of fungicides actually resulted in lower plant height than the control. Statistical analysis showed that the plant height of the five treatments had a significant effect with a significance of 0.000 at the 5% confidence level. The results of LSD test showed that inoculation of the PGPR consortium with the addition of fungicides and the treatment of fertilizers with the addition of fungicides were significantly different compared to the control. However, the addition of fungicide was not significantly different from the control.

Stem diameter of *E. pellita* also showed varying results in each treatment (Figure 6B). The highest stem diameter was obtained from inoculation of the PGPR consortium with the addition of fungicides and the lowest diameter was obtained from the application of fungicides. Application of fungicide resulted in lower diameter than control. Application of fungicides can inhibit the growth of *E. pellita* stem diameter. The stem diameter of the five

treatments had a significant effect with a significance of 0.016 at the 5% confidence level. The LSD test result showed that the stem diameter of the PGPR consortium inoculated with and without fungicide was significantly different from the control. However, the fungicide treatment with and without the addition of fertilizer was not significantly different from the control. The number of *E. pellita* leaves was different (Figure 6C). One-Way ANOVA statistical analysis showed that the number of leaves had no significant effect on the five treatments with a significance of 0.172 ($p<0.05$), causing further test did not proceed. That is, the five treatments had no significant effect on the number of leaves. It is known that inoculation of the PGPR consortium with the addition of fungicide resulted in the highest number of leaves and the fungicide treatment produced the lowest number of leaves. The highest dry weight of *E. pellita* plants was obtained by inoculation of the PGPR consortium with the addition of fungicides and the lowest dry weight was obtained in the control treatment (Figure 6D).

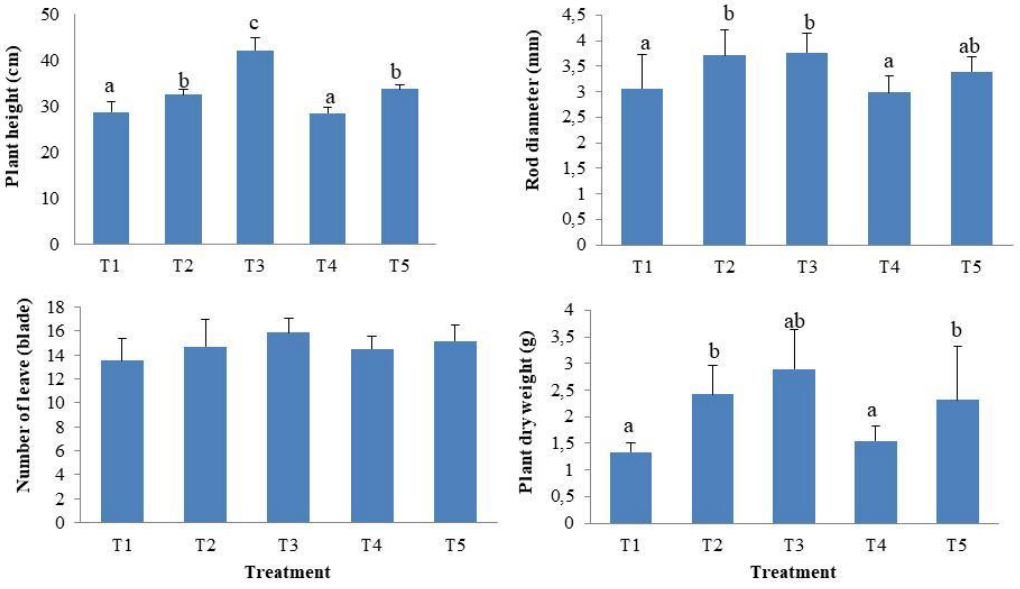

Figure 6. Growth of *E. pellita*. A. Plant height, B. Stem diameter, C. Number of leaves, D. Plant dry weight. T1= control, T2= PGPR, T3= PGPR+Fungicide, T4= Fungicide, T5= Fertilizer+Fungicide

The dry weight of plants from high to low was sequentially obtained from treatments T3, T2, T5, T4, and T1. Statistics showed that the dry weight of the plant was significantly different from the five treatments with a

significance of 0.001 ($p<0.05$). LSD further test showed that the dry weight of plants in inoculation of the PGPR consortium and inoculation of the PGPR consortium with the addition of fungicides and fertilizer treatment with the addition of fungicides were significantly different from the control. However, the fungicide treatment was not significantly different when compared to the control. Fertilizer treatment with the addition of fungicides was not significantly different from the PGPR consortium inoculation treatment. The effectiveness of PGPR application in stimulating growth is presented in Table 2.

Table 2. The effectiveness of PGPR application in stimulating the growth of *E. pellita*

Treatment	Accretion (%)			
	Plant height	**Stem diameter**	**Number of leaves**	**Plant dry weight**
T1 (Control)	0	0	0	0
T2 (PGPR)	13.14	20.98	8.64	81.19
T3 (PGPR+fungicide)	46.92	22.88	17.28	116.56
T4 (Fungicide)	-0.87	-2.50	7.41	15.19
T5 (Fertilizer +fungicide)	17.44	10.65	12.34	73.22

In general, PGPR inoculation was able to enhance the growth of *E. pellita*. Inoculation of the PGPR consortium with the addition of fungicide was the most effective treatment in stimulating the growth of *E. pellita* compared to other treatments, seen from the percentage accretion in the growth of *E. pellita*. This treatment was able to increase plant height by 46.92% and stem diameter about 22.88%. However, the fungicide treatment resulted in a lower percentage increase compared to the control on plant height and stem diameter parameters. This indicates that fungicide treatment can inhibit plant height and stem diameter of *E. pellita*. While the number of leaves increased in each treatment when compared to the control. The percentage increase in the number of leaves from the highest to the lowest was obtained by inoculation of the PGPR consortium with the addition of fungicides followed by fertilization treatment with additional functions, inoculation of the PGPR consortium, and fungicide treatment. Plant dry weight also showed an increase in each treatment. The highest increase in dry weight was obtained in the inoculation of the PGPR consortium with the addition of fungicides and the lowest was in the fungicide treatment (Figure 7).

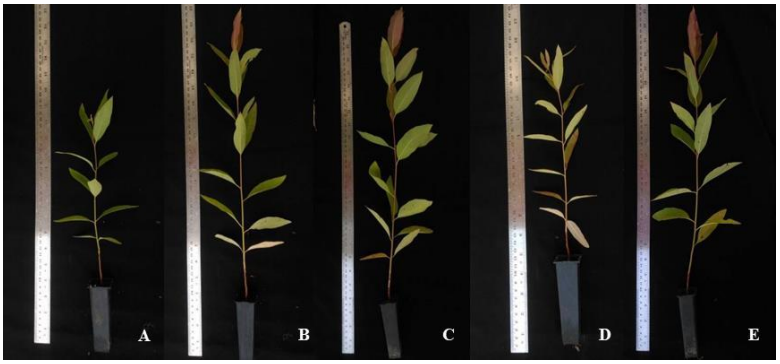

Figure 7. Growth of *E. pellita*. A. Control, B. PGPR application, C. PGPR+fungicide application, D. Fungicide application, E. Fertilizer+fungicide application

Discussion

PGPR is a group of microbes that have a mutualistic symbiosis with plant roots and can promote plant growth through phosphate solubilization, production of siderophores and phytohormones, ability to fix nitrogen, and act as biocontrol agents (Maougal *et al*., 2021). Of the 25 isolates isolated from a mixture of rhizospheres of *E. pellita* seedlings, some isolates were identified to have potential as PGPR. It is characterized by its ability to produce IAA, siderophores, phosphate solubilization, inhibit the growth of *R. solanacearum* and *Cylindroclaium* sp. (Table 1) and stimulating the growth of *E. pellita*. A hypersensitivity test of the isolated isolates against tobacco plants was carried out to determine the pathogenicity of these isolates to non-host plants. This study found that PGPR isolates showed a negative reaction to the hypersensitivity test, which means that they are not pathogenic to non-host plants. It is known from the absence of symptoms of necrosis in tobacco plants. Tobacco leaves that did not undergo necrosis indicated that the inoculated microbes were not pathogenic against non-host plants (Marwan *et al*., 2020).

Hypersensitivity reaction is a plant response to pathogen infection to inhibit the growth of pathogens (Mardhiana *et al.,* 2017). Warzatullisna *et al*. (2019) found that six out of seven endophytic isolates isolated from rice roots showed no symptoms of necrosis in tobacco leaves. Marwan *et al*. (2020) found that 12 test isolates (100%) showed negative hypersensitivity reactions with no symptoms of necrosis in tobacco leaves. Plant defense reactions against pathogenic infections can be through chemical mechanisms by producing phytoalexins. Phytoalexins will inhibit or stop the development of pathogens to fail plants from being infected with pathogens. Meanwhile, plants that are susceptible to pathogens can tolerate phytoalexins by detoxifying them into

non-toxic compounds to grow pathogens continuously. Another mechanism of plant defense against pathogens is the direct production of Pathogenesis Related-Protein (PR-protein). PR protein is a peroxidase enzyme that plays an important role in the lignification process to make plants being resistant to pathogen attack (Jain and Khurana, 2018).

Isolates employed in the form of a consortium need to be tested for compatibility. The PGPR compatibility assay aims to determine which isolates are compatible and do not inhibit growth between isolates when used in the form of a consortium. Compatible PGPR isolates were indicated by the absence of a halo zone formed around the colony, while incompatible isolates formed a halo zone around the colony. In a consortium, microorganisms can work together in a complex and synergistic manner. Thus, they can provide better results than single bacteria. Each species of bacteria has different physiological activities. The bacterial consortium can provide several physiological activities simultaneously which makes it much more effective in promoting plant growth and controlling pathogens (Ijaz *et al*., 2019; Syed *et al*., 2020).

This study obtained isolates capable of stimulating the growth of *E. pellita*. This can be seen from the increase in plant height, stem diameter, number of leaves, and dry weight of *E. pellita* plants with PGPR application treatment (T2) and PGPR mixed fungicide (T3) application. This indicated that the treatment was able to significantly increase plant height, stem diameter, and dry weight of *E. pellita* plants. This study is in line with the research of Moncada *et al*. (2021) who obtained the results that PGPR bacterial inoculants had no significant effect on increasing the number of plant leaves. The effectiveness of PGPR application in plants depends on the amount of inoculum and certain environmental conditions. Inoculation of the PGPR consortium with the addition of fungicides was counted to be the most effective treatment for enhancing *E. pellita* growth, since plant growth can be stimulated directly or indirectly by PGPR. Directly, PGPR contributes to providing nutrients through N fixation, producing phytohormones such as IAA, phosphate solubilization and siderophore production (Bhardwaj *et al*., 2014; Pahari and Mishra, 2017).

The PGPR consortium used in this study were EP1, EP12, EP18, EP19, and EP20 and were known to produce IAA 1.25; 0.92; 1.33; 0.42 and 1.00 ppm, respectively. A qualitative assay of IAA production detected by the addition of Salkowski reagent was indicated by the formation of pink color, due to the interaction between IAA and Fe to form a complex compound $[Fe_2(OH)_2(IA)_4]$. The intensity of the color indicates higher content of IAA produced by bacteria. IAA production by bacteria can occur due to the presence of tryptophan as a precursor. Tryptophan is converted to IAA using two pathways, namely the Indole-3-acetamide (IAM) and Indole-3-pyruvate (IpyA) pathways. The IAM

pathway can only be carried out by bacteria, while the IpyA pathway can be carried out by plants and bacteria (Jahn *et al*., 2021). *Pseudomonas putida* strain was known to produce IAA which significantly increased plant height, stem diameter, radical volume, dry biomass, and the number of the fruit of tomato (Hernández-Montiel *et al*., 2017).

Besides being able to produce IAA, the PGPR isolate used in this study was also able to solubilize phosphate with the highest phosphate solubility index found in the EP18 isolate. Santana *et al*. (2016) reported that phosphorus (P) is one of the macronutrients which is a growth-limiting factor needed by plants for its growth due to its relatively low availability in soil, especially in the tropics. Phosphate solubilizing bacteria in soil play a role in providing P by hydrolyzing insoluble organic and inorganic phosphorus compounds into soluble P forms that can be easily assimilated by plants (Kalayu, 2019). Phosphate solubilization by *Burkholderia ambifaria* and *B. tropica* was able to increase the growth and yield of chili plants (*Capsicum frutescents*). The high P-solulibizing microbial activity can increase the available P content in the soil, thereby increasing the absorption of plant nutrients (Worcapon *et al.*, 2013).

Most of the PGPR isolates used were able to produce siderophores. Siderophores produced by bacteria are beneficial for plants because they can increase plant growth by increasing the availability of iron in soil (Santana *et al*., 2016). Linu *et al*. (2019) found that siderophores producing *Pseudomonas aeruginosa* was able to stimulate the growth of chili plants. This was indicated by higher growth parameters in *P. aeruginosa* inoculated plants compared to controls. In addition, siderophores can also act as biocontrol agents. Siderophores can inhibit the growth of pathogens by binding to Fe^{3+} which causes the pathogen to lack Fe^{3+} (Niehus *et al*., 2017).The PGPR isolate used in this study was able to inhibit the growth of *R. solanacearum* and/or *Cylindroclaium* sp. The highest growth inhibition of *R. solanacearum* was found at EP20 and the highest growth inhibition of *Cylindroclaium* sp. was found in EP11 isolates.

In this study, it was found that the isolates isolated from a mixture of rhizospheres of *E. pellita* are potential as PGPR because it is able to produce IAA, solubilize phosphate, produce siderophores and inhibit the growth of *R. solanacearum* or *Cylindroclaium* sp. The PGPR consortium selected for application to *E. pellita* was EP1, EP12, EP18, EP19, and EP20 because these isolates were compatible. Combination treatment of PGPR and fungicide showed the most effective in stimulating the growth of *E. pellita*. The effectiveness of the combination was indicated by an increase in plant height and stem diameter compared to the control. On the other hand, the application of fungicides inhibits the growth of *E. pellita.*

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