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Biological activity of rhizobacteria isolated from rhizosphere *Acacia crassicarpa* A. Cunn ex Benth. in timber plantations

Zul, D.[1*], Mustava, M.[1], Diva, A. R.[1], Siregar, B. A.[2], Gafur, A.[2] and Tjahjono, B.[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau, Pekanbaru 28293, Indonesia; [2]SMF Corporate R&D, PT. Arara Abadi, Perawang, Riau 28772, Indonesia.

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Abstract *Acacia crassicarpa* is widely planted in timber plantations for pulp and paper industry. However, *A. crassicarpa* is relatively susceptible to diseases. To control diseases and promote the growth of *A. crassicarpa*, one of the efforts is utilized rhizobacteria or is known as plant growth-promoting rhizobacteria (PGPR). The biological activity of 27 isolates from the rhizosphere of *A. crassicarpa* was explored the ability of isolates to produce siderophores, hydrogen cyanide (HCN), indole acetic acid (IAA), and antagonism assays. The ability of isolates to produce siderophores and HCN was determined qualitatively by inoculation of the isolates on iron-free chrome azurol S agar and King's B medium, respectively. IAA production was estimated colorimetrically by culturing the isolate on nutrient broth containing L-tryptophan as a precursor. The characteristic of the isolates capable of producing IAA is the color change of the culture after the addition of Salkowski's reagent. Antagonisms assay on pathogens that cause leaf blight and wilt were performed by using a cross-streaking method and dual culture method, respectively. It was found that 23 isolates showed the ability to produce siderophores as indicated by the formation of orange halo around colonies. According to the color change of the filter paper to light brown and dark brown, five isolates were found to produce weak and moderate HCN. Seven isolates produced IAA with a concentration in the range of 108.07-73.78 ppm. Isolate 010B performed the best ability to inhibit the growth of *Fusarium* sp. (61.10%) and *Xanthomonas* sp. (28.14%). The study results it can be concluded that there were two isolates with potential as biocontrol and growth promoter agents.

Keywords: Acacia, HCN, IAA, Rhizobacteria, Siderophores

Introduction

Rhizobacteria are soil bacteria that live in the rhizosphere and are known to stimulate plant growth. Several rhizobacteria have been isolated such as *Achromobacter xylosoxidans* isolated from corn rhizosphere (Danish *et al*., 2020), *Bacillus stratosphericus* and *Bacillus subtilis* isolated from *Vallisneria*

[*] **Corresponding Author:** Zul, D.; **Email:** delita.zul@lecturer.unri.ac.id

natans rhizosphere (Wang *et al*., 2021), and *Klebsiella grimontii* and *Enterobacter tabaci* from the potato rhizosphere (Aloo *et al*., 2020). Rhizobacteria that stimulate plant growth are called plant growth-promoting rhizobacteria (PGPR). The ability of rhizobacteria to encourage the growth of the plant is indicated by the production of the hormone Indole-3-acetic acid (IAA), gibberellin acid, ethylene, and cytokinins. A previous study described that DCY^{11T} isolates isolated from the rhizosphere of *Panax ginseng* produced 17.6 g/mL IAA in an L-tryptophan medium (Huo *et al*., 2020). In addition, rhizobacteria can also act as biocontrol agents.

The role of rhizobacteria as biocontrol agents is supported by their ability to produce siderophores. Siderophore is a compound capable of chelating Fe^{3+} (Ahmed and Holmström, 2014). Fe is an essential element for microorganisms, as a catalyst for enzymatic processes, oxygen metabolism, electron transfer, DNA and RNA synthesis (Aguado-Santacruz *et al*., 2012). The siderophore-producing rhizobacteria compete with pathogens for a limited amount of Fe. The lack of Fe in the environment causes disrupted growth. It makes the population does not reach a certain threshold. This causes quorum sensing, which plays a role in stimulating the pathogenicity of the pathogen, does not work. Quorum sensing is related to the expression of virulence factors in plant pathogens such as *Xanthomonas* sp. (Li *et al*., 2019). Five isolates of *Bacillus* isolated from potato rhizosphere produced catechol-type siderophores (2.26-4.21 mgl^{-1}) and salicylate-type siderophores (1.71-4.21 mgl^{-1}). The isolates were able to control banana pathogens (Kesaulya *et al*., 2018).

Rhizobacteria can act as bioherbicides because of their ability to produce hydrogen cyanide (HCN). HCN is a toxic compound, volatile, and can prevent weed growth. HCN will interfere with weed photosynthesis by reducing oxygen in the cytochrome respiratory electron transport chain. Lawrance *et al*. (2019) suggested that H6 rhizobacteria isolates were able to produce HCN. The isolate is able to cause discoloration and depletion of root hair development as well as necrotic lesions in prickly amaranth.

A. crassicarpa is a forestry plant that is widely planted by timber plantation companies. The log production in Indonesia in 2016–2019 increased by an average of 16.12% compared to the previous year. The increase in log production continued until 2019 which reached 41,457.58 m^3. The most widely planted plants were *Acacia* sp. (77.36%), followed by *Eucalyptus* sp. (21.35%), and *Shorea* spp. (0.64%) (BPS, 2019). The increased log production is aimed at meeting the needs for pulp and paper raw materials. The main obstacle to meeting the demand for logs is the limited stock of wood produced in the forest. Therefore, *A. crassicarpa* is intensively planted by plantation companies.

Other problems faced by timber plantation companies in pulpwood plantations are pests and diseases that inhibit plant growth. In order to promote plant growth, timber plantation companies apply fertilizer in the first year after planting. Plant disease control is generally carried out through chemical methods, which can cause new problems such as pests or secondary diseases. Hence, it is necessary to develop integrated disease control by using PGPR (Saharan and Nehra, 2020). There were 27 rhizobacteria isolates that have been isolated from the rhizosphere of *A. crassicarpa* in various timber plantation areas. Yet, the biological activity of these isolates as PGPR is unknown. For this reason, further exploration was carried out to determine their potential as a PGPR.

Materials and methods

Subculture of isolates collection

As many as 27 isolated isolates from the rhizosphere of *A. crassicarpa* were analyzed for their biological activity. The isolates are a collection of the Laboratory Plant Protection Departement (PPD) of the Research and Development (R&D) Division of PT. Arara Abadi Perawang, Riau, Indonesia. Subculture of isolates was carried out by inoculating the isolates from vials on sterile nutrient agar using the streak plate method. The culture was incubated for 48 hours at 28^{o}C.

Analysis of isolate ability to produce siderophores

Rhizobacterial isolates aged 24 hours were inoculated on Chrome Azurol Sulphonate (CAS) agar and incubated for 24 to 72 hours. Siderophore-producing isolates were characterized by the formation of an orange halo around the isolate colonies. CAS agar was prepared by mixing 100 mL of MM9 solution, 750 mL of ddH_2O, 32.24 g of piperazine-N,N'-bis [2-ethanesulfonic acid] and slowly stirring and maintaining the pH of the solution between 6-6.8. The color of the solution will change if the pH of the solution increases above 6.8. Then, 15 g of agar was added. The medium solution was sterilized at 121 ^{o}C for 15 minutes. The sterilized medium was cooled to 50 ^{o}C. 30 mL sterile cassamino acid solution, 10 mL 20% glucose solution, and 100 mL blue dye solution was added slowly on the glass wall while stirring. The medium was poured aseptically into a petri dish (Louden *et al*., 2011). Instruments and materials for making CAS agar must be free of Fe.

Analysis of isolate ability to produce HCN

The activity of the isolates in producing HCN was analyzed using King's B liquid medium made by dissolving 20 g of peptone in 1000 mL of distilled water, 1.5 g of K_2HPO_4, 1.5 g of $MgSO_4.7H_2O$, and 10 mL of glycerol (Rane *et al*., 2007). Two mL of rhizobacterial isolates were inoculated into a test tube containing 8 mL of King's B medium. At the edge of the test tube, filter paper that has been cut into rectangles with a size of 3x1cm was placed. It was saturated with 1% picric acid and 10% Na_2CO_3. The filter paper was saturated by placing the filter paper on a glass vessel and adding 1% picric acid. Subsequently, the filter paper was turned over and placed in a new vessel and dripped with 10% Na_2CO_3 (Lorck, 1948). The test tube was tightly sealed using cotton and plastic wrap to prevent the gas produced by the isolate did not come out. Cultures were incubated for 48 hours at room temperature. During incubation, the color changes of the filter paper were observed with the observation periods of 4, 8, and 48 hours. Qualitatively, the ability of isolates to produce HCN was indicated by a change in the color of filter paper from yellow to light brown or yellow-brown, dark brown, and reddish-brown (Kremer and Souissi, 2001).

Analysis of isolate ability to produce IAA

Rhizobacteria isolates were inoculated into a test tube containing 5 mL of nutrient broth enriched with L-tryptophan 100 g/mL. The cultures were incubated at a stirring speed of 150 rpm at room temperature for three days in the dark. The rhizobacterial cell cultures were centrifuged at 6000 rpm for 10 minutes and one mL supernatant was transferred into a sterile test tube. Then, 4 mL of Salkowski's reagent was added and afterward the mixture was incubated for 60 minutes in the dark at room temperature. The solution turning light red is an indication of IAA. The absorbance of the solution was measured using a spectrophotometer at a wavelength (λ) of 530 nm (Gordon and Weber, 1951). The concentration of IAA was determined by comparing it to the standard curve of IAA.

Isolate antagonism test against Fusarium sp.

The antagonism test of the isolates on *Fusarium* sp. was performed using the dual culture method. Pure culture isolates of rhizobacteria and *Fusarium* sp. were grown together on potato sucrose agar (PSA) with a distance of 3 cm between isolates. Isolates of rhizobacteria and *Fusarium* sp. were inoculated

into PSA with a diameter of 0.5 cm. The growth of *Fusarium* sp. was observed from day 3 to day 7 after inoculation. Observations were stopped when control colonies reached maximum growth. The percentage of inhibition was calculated using the formula from Fokkema and Skidmore (1976):

$$P = \frac{r1-r2}{r1} x\ 100\%$$

Inhibitory power of the isolate (P), colony radius of *Fusarium* sp. farthest from the rhizobacteria isolate (r1), and colony radius of *Fusarium* sp. closest to the rhizobacteria (r2). The antagonistic of the isolates was determined based on the clear zone formed between *Fusarium* sp. and rhizobacteria isolates.

Isolate antagonism test againts Xanthomonas sp.

The antagonism of *Xanthomonas* sp. isolate was analyzed using the cross streaking method. Rhizobacterial isolates of 48 hours were performed by horizontally streaking the middle of nutrient agar. Meanwhile, isolates of the pathogen *Xanthomonas* sp. which was also 48 hours was scratched vertically in the middle of nutrient agar in the same petri dish. As a control, *Xanthomonas* sp. inoculated in the middle of the nutrient agar horizontally. The growth of the two isolates was observed every day by measuring the radius of *Xanthomonas* sp. of the treatment and control sample. The percentage of inhibition was calculated using the formula (Bakker and Schippers, 1987):

$$I = \frac{D1-D2}{D1} x\ 100\%$$

Percentage of inhibition (I), the diameter of *Xanthomonas* sp. (control) (D1), and the diameter of *Xanthomonas* sp. towards the rhizobacteria (treatment) (D2).

Results

Siderophore production by rhizobacteria

As many as 23 isolates were able to produce siderophores from 27 isolates tested (Table 1). The ability of rhizobacteria isolates to produce siderophores was indicated by the formation of an orange halo zone around the bacterial colonies on CAS agar (Figure 1).

Table 1. Isolate codes and their activity in producing siderophores

No	Isolate code	Siderophores production
1	003B	+
2	006B	+
3	008B	+
4	010B	+
5	042B	+
6	066B	+
7	068B	+
8	070B	+
9	072B	+
10	145B	+
11	146B	+
12	147B	+
13	149B	+
14	153B	+
15	156 B	+
16	221B	+
17	222B	+
18	224B	+
19	226B	+
20	AC1	+
21	CN3N1	+
22	CN3N2	+
23	RSKA4	+

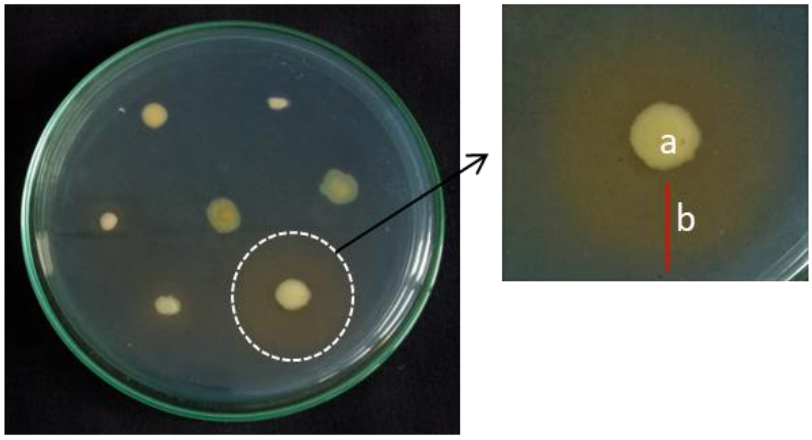

Figure 1. Orange halo zone formed around colonies grown on CAS agar, (a) rhizobacterial colony, (b) orange halo zone around colonies

HCN production by rhizobacteria

A total of five out 27 isolate collections were able to produce HCN (Table 2). HCN producing isolates were indicated by a color change on the filter paper. The filter paper before incubation was yellow (Figure 2a). It was found that 4 isolates had a low ability to produce HCN as indicated by a change in the color of the filter paper to light brown (Figure 2b). There was 1 isolate that produced moderate HCN as indicated by a change in the color of the filter paper to dark brown (Figure 2c).

Table 2. Isolation codes and their level of ability in producing HCN

No	Isolate code	Color changes	HCN production rate*
1	008B	Light brown	+
2	011B	Light brown	+
3	066B	Light brown	+
4	072B	Light brown	+
5	AC2	Dark brown	++

Description: *+ (low), ++ (moderate) in producing HCN

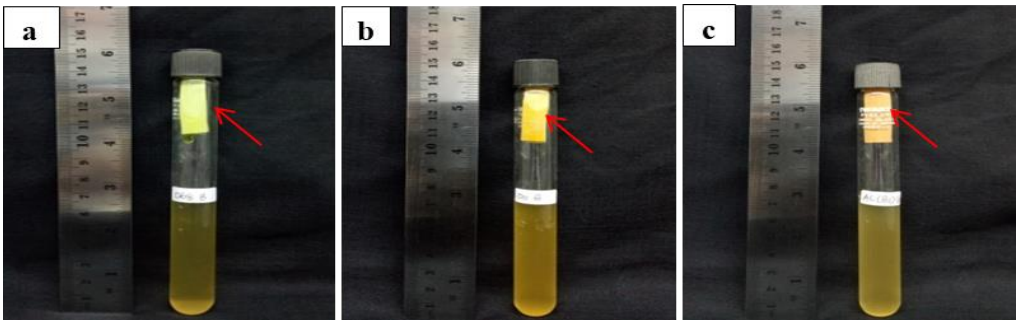

Figure 2. The HCN produced from rhizobacterial isolates is marked by the change in the color of the filter paper (a) the color of the filter paper before the culture was incubated, (b) the filter paper changed to light brown, (c) the filter paper became dark brown (indicated by arrows)

Production of IAA by rhizobacteria

Seven rhizobacteria isolates were able to produce IAA with varying concentrations. IAA production was indicated by the formation of pink and pink transparent colors after the addition of Salkowski's reagent (Table 3). There were 20 isolates without color change after the addition of Salkowaski's reagent. The compound resulted was tryptophan 2-oxindole.

Table 3. The concentration of IAA produced by rhizobacteria isolates and indicator color formed after the addition of Salkowski's reagent

No	Isolat code	IAA production (ppm)	Color indicator
1	145 B	108.07	Light red
2	RSKA 4	105.21	Transparent pink
3	153 B	80.92	Transparent pink
4	068 B	80.21	Transparent pink
5	CN3N (B2)	75.92	Transparent pink
6	146 B	75.21	Transparent pink
7	AC (A1) S3	73.78	Transparent pink

***Rhizobacteria resistance against Fusarium* sp.**

Antagonism test on *Fusarium* sp. employed dual culture method. A test resulted positive as indicated by the formation of a clear zone around the rhizobacterial colonies (Figure 3a). Eight rhizobacteria isolates had inhibitory power of *Fusarium* sp. with various antagonism abilities (Table 4). Isolates 008B, 010B, 224B, 226B, and CN3N1 showed a consistent increase in inhibition from 3-7 DAI. Meanwhile isolates 004B, 072B, and 156B showed a decrease in inhibition at 7 DAI.

Table 4. Inhibition of mycelium diameter of *Fusarium* sp. by rhizobacteria isolates

No	Isolate code	Inhibition diameter (%)		
		3 DAI*	5 DAI*	7 DAI*
1	004B	27.46 ±3.40	12.76 ±3.38	9.06 ±3.05
2	008B	35.08 ±3.04	52.38 ±2.06	59.99 ±3.33
3	010B	37.68 ±2.07	58.54 ±1.21	61.10 ±3.85
4	072B	29.81 ±3.03	57.14 ±3.57	56.66 ±3.33
5	156 B	10.52 ±0	19.04 ±2.06	5.55 ±1.92
6	224B	0 ±0	17.27 ±2.13	24.44 ±1.92
7	226B	24.55 ±3.03	43.20 ±2.13	45.55 ±1.92
8	CN3N1	29.62 ±3.21	49.95 ±3.13	53.33 ±3.33
9	CONTROL	0 ±0	0 ±0	0 ±0

*Description: DAI: Days after inoculation

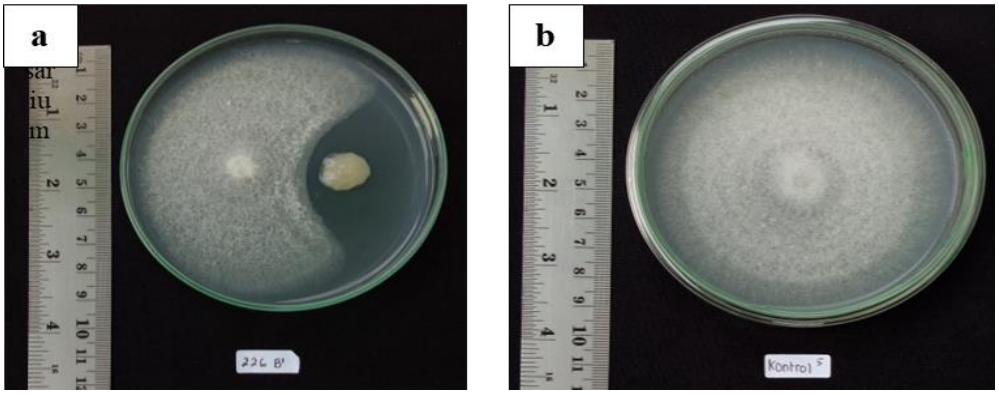

Figure 3. Antagonism activity of rhizobacteria isolates againts *Fusarium* sp. (a) *Fusarium* sp. isolate 226B, (b) Control

***Rhizobacteria resistance against Xanthomonas* sp.**

The ability of 27 rhizobacteria isolates to inhibit the growth of *Xanthomonas* sp. was analyzed. The antagonism test used the cross streaking method. An antagonism test on *Xanthomonas* sp. showed that all rhizobacteria isolates had inhibitory power. The inhibitory power of isolates was various. Isolate 010B showed the best inhibitory activity (Table 5 and Figure 4).

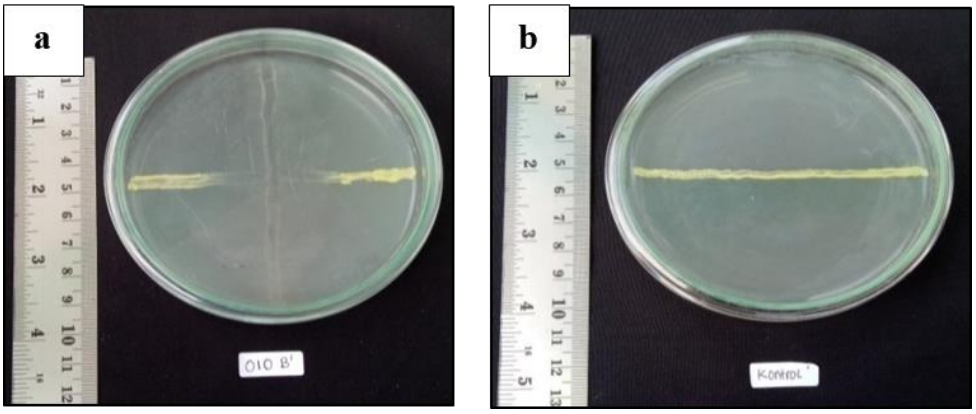

Figure 4. Cross streaking method of rhizobacteria isolates and *Xanthomonas* sp. a) *Xanthomonas* sp. isolate 010B, b) Control

Table 5. Percentage of inhibition of rhizobacteria againts *Xanthomonas* sp.

No	Isolate code	Inhibition (%)	
		1 DAI*	2 DAI*
1	003B	4.81 ±1.28	7.77 ±1.11
2	004B	6.66 ±1.11	7.4 ±0.64
3	006B	6.66 ±1.11	7.03 ±0.64
4	008B	14.44 ±0	25.92 ±0.64
5	010B	11.48 ±0.64	28.14 ±0.64
6	011B	9.25 ±0.64	9.62 ±1.28
7	042B	7.03 ±0.64	7.4 ±1.28
8	066B	6.66 ±1.11	7.4 ±0.64
9	068B	6.29 ±0.64	6.66 ±1.11
10	070B	8.88 ±1.11	8.14 ±0.64
11	072B	7.77 ±1.11	17.77 ±1.11
12	145B	5.18 ±0.64	5.92 ±1.28
13	146B	5.92 ±0.64	6.66 ±1.11
14	147B	7.4 ±0.64	7.4 ±0.64
15	149B	9.62 ±0.64	8.88 ±1.11
16	153B	7.4 ±0.64	8.51 ±0.64
17	156 B	7.77 ±1.11	7.77 ±1.11
18	220B	6.29 ±0.64	7.03 ±1.28
19	221B	6.66 ±0	9.62 ±1.28
20	222B	9.99 ±1.11	9.99 ±1.11
21	224B	8.14 ±0.64	7.77 ±1.11
22	226B	8.51 ±0.64	8.14 ±1.28
23	AC1	9.25 ±1.28	10.37 ±0.64
24	AC2	2.59 ±0.64	4.81 ±0.64
25	CN3N1	7.4 ±0.64	6.29 ±0.64
26	CN3N2	9.62 ±0.64	9.62 ±0.64
27	RSKA4	7.4 ±0.64	7.4 ±0.64
28	CONTROL	0 ±0	0 ±0

*Description: DAI: Days after inoculation

Discussion

In this study, 27 isolates of rhizobacteria were tested for their potency as PGPR. The parameters were the production of siderophores, HCN, IAA, and antagonism test on *Fusarium* sp. and *Xanthomonas* sp. The assays began with an analysis of the isolates' ability to produce siderophores. Siderophores are iron-chelating agents that are secreted by microbes due to the lack of Fe. In general, siderophores produced by microorganisms are beneficial for plants because they can inhibit pathogens' growth. The inhibition of pathogens is due to the ability of siderophores to bind Fe^{3+}. Pathogens become lack of Fe^{3+}. According to (Noumavo *et al*., 2016) pathogens and siderophore-producing PGPR compete to bind Fe. Generally, microorganisms produce hydroxamic, catecholate, and salicylate-type siderophores. The catecholate-type siderophores are characterized by the formation of a pink or purple zone, while the hydroxamate-type siderophores are characterized by the formation of an orange halo zone around bacterial colonies (Ahmed and Holmström, 2014). From 27 bacterial isolates tested, 23 isolates showed the ability to produce siderophores (Table 1). Siderophores production was indicated by the formation of an orange halo zone around the colonies grown on CAS agar (Figure 1). This showed hydroxamate-type of siderophores. The number of isolates capable of producing siderophores in this study was more than in previous studies. Dhull and Gera, (2018) succeeded in obtaining six siderophores producing isolates from 13 *Rhizobium* isolates isolated from root nodules of *Cyamopsis tetragonoloba* (L.) Taub. It is in line with Agustiyani *et al*. (2021) who succeeded in isolating 42 isolates of rhizobacteria from various types of rhizospheric soils with different vegetation. There were eight positive siderophores producing isolates as indicated by the formation of a yellow-orange halo zone around the colonies. Our study shows that Siderophores started to be produced after 12 hours of incubation and increased during 24-30 hours of incubation. In general, siderophores are produced when the pH of the environment is neutral. The same thing was found in this study, that a neutral pH of around 6.8 caused the decreasing solubility of Fe^{3+} and unavailability to microorganisms. This is a signal for bacteria to produce siderophores (Sayyed *et al*., 2005).

There were five rhizobacteria isolates that produced HCN (Table 2). HCN production was detected by changing the filter paper color to light brown or dark brown (Figure 2). Changes in the color of filter paper indicate the HCN production. Similar results were found in the study conducted by Agustiyani *et al*. (2021) who found that the color change of the filter paper from light brown to dark brown indicated the HCN. Meanwhile, a study carried out by Ezrari *et*

al. (2021) showed positive HCN production as indicated by a change in the color of the filter paper from yellow to orange or brown. In general, the darker the filter paper color, the higher the HCN concentration produced. The color change of the filter paper occurs due to the reaction between picric acid ($C_6H_3N_3O_7$) or sodium carbonate (Na_2CO_3) with cyanide produced by bacteria to form sodium cyanide (NaCN). NaCN is formed through the absorption of cyanide gas by NaOH or Na_2CO_3 solutions through a reaction between sodium and ammonia that initially form $NaNH_2$. It will react with carbon and produce sodium cyanamide (Na_2NCN) and eventually form NaCN, which is a type of cyanide (Knowles, 1976). Generally, HCN is used as a weed biocontrol agent. According to Riaz *et al*. (2021), several groups of rhizobacteria that colonize plant roots in the rhizosphere or known as deleterious rhizobacteria (DRB) can be used as weed biocontrol agents. The ability of rhizobacteria to control weeds is associated with their ability to produce HCN. HCN can inhibit root metabolism by entering the root system and limiting the cytochrome-C oxidase enzyme in the electron transport chain, which is required for the production of adenosine-tri-phosphate in root cells (Jain and Das, 2016).

Isolate 145B produced the highest IAA concentration (Table 3). Qualitatively, IAA-producing bacterial culture turns pink because of the interaction between Salkowski's reagent and IAA, $FeCl_3$, and H_2SO_4 to form a tris-(indole-3-aceto)-Fe (III) complex which forms a pink color. In this study, the medium used to induce IAA production was enriched with L-tryptophan. This indicated that the addition of L-tryptophan to the growth medium was an important factor in IAA biosynthesis. L-tryptophan is an amino acid as a monomer of protein. Thus, this amino acid can be easily used by microorganisms since the protein is easy to be broken down. This is supported by the fact that isolates of rhizobacteria associated with *Persea americana* Mill (Nadeem *et al*., 2012), isolates from the rhizosphere of *Triticum aestivum* L. (Sheirdil *et al*., 2019), and the rhizosphere of *Solanum lycoperium* L. Guerrieri *et al*. (2020) was found to produce IAA on L-tryptophan-enriched medium. The concentration of IAA produced by each isolate varied (Table 3). The difference in the IAA production was influenced by several factors including the isolate source, type of species, culture conditions, growth stage, substrate availability, genetic composition, and enzyme activity (Mirza *et al*., 2001; Khalid *et al*., 2001). In addition, the concentration of IAA produced by rhizobacteria is also influenced by incubation time. At the incubation time of 24 hours, the IAA produced was still in low concentration because the enzyme activity to convert tryptophan to IAA had not reached the optimal state. The highest concentration of IAA was found at an incubation time of 48 hours. This was because the isolates were in the logarithmic phase and the enzymes became more active in

line with the growth rate. Meanwhile, at 72 hours incubation, the isolates had entered the death phase and thus the production of IAA decreased sharply.

The antagonism test of rhizobacteria againts *Fusarium* sp. was performed by using the dual culture method (Figure 3). The results showed that from 3 DAI, *Fusarium* sp. was in the accelerated phase with maximum growth. In contrast, rhizobacteria isolates were in a stationary phase where the growth rate and cell death rate were balanced. In this phase, the surviving rhizobacteria isolates produced secondary metabolites that can inhibit the growth of *Fusarium* sp. The highest inhibition zone was found in isolates 008B and 010B with the zones of inhibition of 35.08% and 37.68%, respectively (Table 4). The inhibition of rhizobacteria isolates was indicated by the formation of a clear zone (Figure 3). The formation of a clear zone indicated an inhibitory mechanism by antifungal compounds produced by rhizobacteria that diffused into media. In general, antifungal compounds produced by bacteria cause abnormal hyphal growth which is characterized by swelling and shortening of hyphae. Swelling occurs because antifungal compounds penetrate into pathogenic cells. This causes the hyphae to not develop properly (Compant *et al*., 2005). Observations of five DAI showed *Fusarium* sp. is in the log phase. In this phase, the cell division of *Fusarium* sp. was very fast and constant. At the same time, the inhibitory ability of eight rhizosphere bacterial isolates increased, while the inhibitory ability of other isolates decreased (Table 4). The decrease in inhibition was indicated by the covering of rhizobacterial colonies by the mycelium of *Fusarium* sp. The observation of seven DAI showed that *Fusarium* sp. was in the deceleration phase. In this phase, its growth rate slowed down because the nutrients in the media are limited. In general, rhizobacteria isolates showed a decrease in inhibitory power but isolates 008B, 010B, 224B, 226B, and CN3N1 continued to show an increase in inhibitory power.

The antagonism test of rhizobacteria isolates against *Xanthomonas* sp. was done using the cross streaking method (Figure 4). In contrast to *Fusarium* sp., the incubation time for the inhibitory test on *Xanthomonas* was sp. shorter. It is because the growth of bacteria is faster than fungi. In general, from 1 DAI observation, rhizobacteria isolates showed varying inhibitory power (Table 5). The best inhibitory power was found in isolates 008B and 010B, with the zones of inhibition of 14.44% and 11.48%, respectively. Observations of 2 DAI showed a balance between growth rate and bacterial cell death. In this condition, nutrients availability will be very limited and the bacteria will produce secondary metabolites such as antibiotics. This can be observed in several bacterial isolates, which showed greater inhibition to suppress the

growth of *Xanthomonas* sp. The isolate that showed the highest inhibitory power was isolate 010B with a zone of inhibition of 28.14%.

In general, 23 isolates of rhizobacteria were able to produce siderophores as indicated by the formation of orange halo zone around colonies. Five rhizobacteria isolates produced HCN with varying levels. One isolate was able to produce moderate levels of HCN, and four other isolates had a low ability in producing HCN. There were seven isolates of rhizobacteria that produced IAA on growth media containing 100 g/mL L-tryptophan. Isolate 145B was able to produce the highest IAA concentration of 108.07 ppm. Meanwhile, the lowest IAA production was produced by isolate AC1 of 73.78 ppm. From five isolates that inhibited the growth of mycelium *Fusarium* sp., the highest percentage of inhibition was found in isolate 010B of 61.10%. Two isolates were able to inhibit the growth of *Xanthomonas* sp. with the highest percentage of inhibition found in isolate 010B with an inhibition zone of 28.14%. In conclusion, isolates 008B and 010B have the potential to be used as PGPR because of their ability to produce siderophores, HCN, as well as biocontrol agents in inhibiting the growth of *Fusarium* sp. and *Xanthomonas* sp.

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