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Physiological And Biochemical Characterization Of Indigenous Rhizobacterial Isolate As Plant Growth-Promoting Agent And Biocontrol Agent Towards White Root Rot (*Microporus Rigidospor)* Of Nutmeg Plants (*Myristica fragrans* Houtt)

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Abstract :
This study aims to determine the effect of the biological seed treatment using indigenous rhizobacteria on nutmeg plants to control white root fungal disease. This research was conducted at the Laboratory of Seed Science and Technology, Department of Agrotechnology, Faculty of Agriculture and at the Laboratory Education Department of Biology, Syiah Kuala Darussalam University, Banda Aceh. The study was conducted from January 2020 to September 2020. This study used a non-factorial completely randomized design (CRD). Rhizobacterial treatment consisted of 18 isolates and one control. The variables observed included Rhizobacterial Antagonist Test against white rot fungal disease Pathogens (Multiple Culture Methods), Ability of Agents to Produce Hydrogen Cyanides (HCN), Peroxidase Activity Test, Analysis of Siderophore Compounds, Production of Indole Acetic Acid (IAA) by Rhizobacteria, and Ability of Rhizobacteria to Dissolve Phosphates. The results showed that the highest percentage of inhibitory power of rhizobacterial isolates against Rigidoporus microporus was obtained in isolates Alue Selaseh 4/5 (65.54%) and Alue Selaseh 7/5 (61.19%). Only Alue Selaseh 3/1, Alue Selaseh 4/5, Alue Selaseh 5/5 and Alue Selaseh 7/5 isolates were able to produce HCN compounds, while Alue Selaseh 3/1 isolates were also able to increase higher peroxidase activity. The best IAA content was found in isolates Alue Selaseh 3/11, Alue Selaseh 4/5, Alue Selaseh 5/5, Alue Selaseh 5/7 and Alue Selaseh 7/6. There are 14 isolates capable of dissolving phosphates, namely Alue Selaseh 3/1, Alue Selaseh 3/11, Alue Selaseh 5/3, Alue Selaseh 5/5, Alue Selaseh 5/6, Alue Selaseh 5/8, Alue Selaseh 6/1, Alue Selaseh 7/1, Alue Selaseh 7/2, Alue Selaseh 7/3, Alue Selaseh 7/4, Alue Selaseh 7/5, Alue Selaseh 7/6 and Alue Selaseh 8/2. All isolates produced siderophore compounds with higher activity found in Alue Selaseh 5/6 rhizobacterial isolates.

Key Word : *Indole Acetic Acid, Phosphate, Hydrogen Cyanide, Pathogens, Siderophore*

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I. Introduction

Nutmeg is a plantation commodity that has high economic value and plays a very important role for people in various regions, especially those in eastern Indonesia. Indonesia is the largest nutmeg exporter in the world with a market share of 60-75%. Furthermore, nutmeg production in Indonesia in 2019 amounted to 37,490 tonnes Ha-1. As much as 99% of the nutmeg in Indonesia is produced by smallholder plantations from North Maluku, North Sulawesi, West Papua, and Aceh province, where these areas are the centers of land for nutmeg production1. Aceh is one of the nutmeg central production provinces in Indonesia. Especially Aceh Barat Daya (Southwest of Aceh) District (Abdya) is the second nutmeg production area in Aceh Province, while South Aceh District is one of the centers for growing nutmeg that can be relied on as a source of livelihood for the local community. The nutmeg commodity is very promising for farmers because of its high economic value2.

The area of the nutmeg plant in Abdya district is around 2,697 ha spread over 8 sub-districts of 9 sub-districts in Abdya district.3 The average nutmeg production in Abdya District reaches 500-600 kg ha-1, compared to the standard nutmeg production, which is still relatively low because the potential for good nutmeg production is capable of producing up to 1.2 tons ha-1. The low production of nutmeg in Abdya Regency is due to disease infection. One of the pathogens is white root rot fungal disease which is caused by the pathogen Rigidoporus microporus, which has destroyed the nutmeg plant in the South West of Aceh. It is estimated that 50% of the nutmeg plants that have died as a result of the fungi are around 1,348 ha of the total area of nutmeg plantations in South West of Aceh.

Many farmers still use synthetic fungicides with chemical active ingredients to control pathogens in nutmeg plants. Therefore, the use of synthetic chemicals is not all effective in use, it can even lead to the emergence of new pathogen resistance, and is less selective4. In other words, control methods that are able to control pathogens and are environmentally friendly are increasingly urgent to study. One of the efforts that can be developed is to utilize the natural resources (biological control) of indigenous rhizobacteria in the plant rhizosphere.

Utilization of specific location (indigenous) rhizobacteria as candidates for biocontrol agents has been shown to effectively control disease-causing pathogens in plants. Rhizobacteria Pseudomonas fluorescens and Bacillus subtilis effectively inhibit the growth of Phytophthora palmivora fungi that cause pod rot in vitro in vitro in the laboratory5. The tested Bacillus spp and P. fluorescens strains were able to reduce the growth of pathogenic fungi R. microporus with peat power of 72.69-90.40% 6. The use of P. fluorescens and Bacillus spp rhizobacteria was effective in controlling white root fungal disease in rubber plants in disease endemic areas[7].

II. Material And Methods

The research was carried out at the Laboratory of Seed Science and Technology, Department of Agrotechnology, Faculty of Agriculture and at the Laboratory Education Department of Biology, Syiah Kuala Darussalam University, Banda Aceh. The study was conducted from January 2020 to September 2020. This study used a non-factorial completely randomized design (CRD). Rhizobacterial treatment consisted of 18 isolates and one control. The research data were calculated and analyzed using analysis of variance (ANOVA) and continued with further tests using the Duncan New Multiple Range Test (DNMRT) at the 5% significant level if the F test showed a significant effect.

Rhizobacterial Antagonist Test against White Root Rot Pathogens (Multiple Culture Method)

The rhizobacteria of the selected isolated biocontrol agent candidates were tested for their antagonistic ability to fight pathogens that cause white root fungal disease. Tests were carried out on PDA media in a 9 cm diameter petri dish. The distance between the inoculation point between the pathogen and the antagonistic rhizobacteria was 3 cm. The test was incubated at room temperature (28-29 0C), then observed every day for up to 7 days.

Ability of the Agent to Produce Hydrogen Cyanide (HCN)

Determination of the presence of hydrogen cyanide formation by bacterial biocontrol agents is carried out according to the procedure described by Bakker and Schippers 8. The materials used as the medium were 4.4 g of glycine, 2 g of picric acid, 8 g of sodium carbonate, 15 g of agar, 30 g of Trythip Soy Broth 1000 mL of sterile water and pieces of sterile filter paper (1x1 cm), then put Glycine, Trythip Soy Broth and agar into in 1000 mL of sterile water then autoclaved and poured into petridis. Furthermore, a solution for detecting HCN. CDS is made consisting of 2 g of picric acid and 8 g of sodium carbonate dissolved in 200 mL of sterile water. The sterile filter paper pieces were put into the CDS solution. The bacteria were scratched on the glycine medium, then a piece of filter paper was placed in the center of the petridis lid. Furthermore, the incubation was carried out at 24 ° C for 4 days. During incubation, the bacteria that produce HCN causes the filter paper to change color from yellow to light brown (+ little HCN), brown (++ medium HCN) and brick red (+++ high HCN) and blackish red (++++ HCN is very abundance). Observation of the agent's ability to produce hydrogen cyanide (HCN) was executed qualitatively.

Peroxidase Activity Test

Peroxidase activity was measured using nutmeg leaves aged 174 days after planting. The test was carried out by crushing 1 gram of nutmeg seedling leaves using a mortar in 0.01 M phosphate buffer pH 6 with a ratio of 1: 4 (g/ml). Nutmeg leaves are crushed in cold conditions (approximately 100C). Then, the crushed seed leaves were filtered using gauze bandage and put the filter results into a test tube, then centrifuged for 30 minutes. The result of centrifugation is used as an enzyme preparation and diluted in a ratio of 1: 3 (1 ml of enzyme preparation + 3 ml of 0.01 M phosphate buffer pH 6). Enzyme preparations that have been diluted, homogeneous using vortex for 5-10 seconds. Prior to the measurement of the peroxidase enzyme activity, a pyrogallol solution was made first by mixing 10 ml of 0.5 M pyrogallol with 12.5 ml of 0.066 M pH 6 phosphate buffer and then diluting it with distilled water until the volume became 100 ml. For the measurement of peroxidase enzyme activity, 0.2 ml of diluted enzyme preparations were added to the reagent consisting of 5 ml of 0.5 M pyrogallol solution and 0.5 ml of 1% H2O2 in a cuvette. Controls were prepared by inserting the above ingredients into the cuvette without enzyme preparation. The mixture is homogenized for 5 to 10 seconds and observed with a spectrophotometer at a wavelength of 420 nm. The absorbance value was observed every 30 seconds for 5 times. Calculation of the enzyme peroxidase activity unit, namely the absorbance value obtained is reduced by control.9.

Siderophore Compound Analysis

The production of siderophore from rhizobacterial isolates was tested by growing the bacteria in the test medium for 24 hours at room temperature. The composition per liter of media used was 20 g of sucrose, 2 g of L-Asparagine, 1 g of K2HPO4, and 0.5 g of MgSO4. The rhizobacteria suspension was centrifuged at a speed of 11,000 rpm for 30 minutes. The supernatant was filtered off with a 0.2 µm nitrocellulose membrane. The detection of siderophore production by rhizobacteria was carried out by adding 1 ml of 0.01 M FeCl to 3 ml of supernatant. In addition, as a comparison of the supernatant without the addition of FeCl. Siderophore detection was measured using a spectrophotometer (Novaspec II model) at a wavelength of 410 nm[10].

Production of Indole Acetic Acid (IAA) by Rhizobacteria Isolates

The ability of rhizobacterial isolates to produce IAA was analyzed using the method of Glickman and Dessaux 11. Rhizobacterial isolates were grown for 24 hours. To stimulate auxin synthesis, the amino acid of tryptophan 0.5 gL-1 was added to each media of rhizobacteria (Sucrose Peptone Agar or SPA). Bacterial culture was centrifuged at 10,000 rpm for 10 minutes, then the supernatant was separated from the bacterial sediment. Next the supernatant was filtered with a nitrocellulose membrane with a porosity of 0.2 µm, and analyzed for its LAA content. IAA content in the bacterial culture filtrate was detected using 12 gL-1 FeCl3 reagent in 7.9 M H2SO4. FeCl3 reagent (1 ml) and bacterial culture filtrate (1 ml) were added to the eppendorf tube (volume 2 ml), and the mixture was incubated in a dark room at 26 ° C for 30 minutes. After the incubation period, the ability of each rhizobacterial isolate to produce IAA was analyzed quantitatively and the absorbance value was read using a spectrophotometer at a wavelength of 550 nm.

Ability of Rhizobacteria in Dissolving Phosphate

Testing the ability of rhizosphere bacteria to dissolve phosphate using Pikovskaya's agar media with the addition of Tricalcium Phosphate (TCP) as a source of phosphate. The composition per liter of media used consisted of 5 g C3(PO4)2, 10 g glucose, 0.2 g NaCl, 0.2 g KCl, 0.1 g MgSO4, 2.5 mg MnSO4, 2.5 mg FeSO4, 0.5 g yeast extract, 0.5 g (NH4)2SO4, and 15 g agar. Then, the media is sterilized using an autoclave. The test medium was poured into a petridish (ϕ 9 cm), made a hole with a cork borer and filled with 0.2 mL of the tested rhizobacterial isolate suspension. The test media was incubated for 3-7 days in an incubation room at 28 ° C. The ability to dissolve phosphate was evaluated qualitatively based on the formation of a clear zone around the hole containing the bacterial suspension.

Observation

Measurement of the percentage of pathogen growth inhibition12 with the formula:

$$PP = (R1-R2)/R1 \ X\ 100\ \%$$

Note :

PP = Percentage of Pathogenic Colony Growth Inhibition (%)
R1 = Colony Radius of the Pathogen Growing Away from the Antagonist Agents (Cm)
R2 = Pathogenic Colony Radius Grows Toward the Antagonist Agent (Cm)

III. Result

Physiological and Biochemical Characterization of Rhizobacteria Isolates as Biocontrol Agents

The average results of the inhibition test results of various rhizobacterial isolates against the growth of the pathogenic Rigidosporus microporus colonies and the ability of rhizobacterial isolates to produce hydrogen cyanide (HCN) are presented in Table 1.

Table 1. The capability of inhibition of various rhizobacterial isolates against the growth of the pathogenic colony of Rigidosporus microporus and the capability of each rhizobacterial isolate to produce hyrogensianide (HCN).

Treatments	Capability of Various Rhizobacterial Isolates	
	Inhibition Percentage (%)	HCN** Production
Alue Selaseh 3/1	46.84 cde	+++
Alue Selaseh 3/10	34.37 ab	-
Alue Selaseh 3/11	35.58 abc	-
Alue Selaseh 4/1	41.69 bcd	-
Alue Selaseh 4/5	65.54 e	+++
Alue Selaseh 5/3	40.65 bcd	-

Alue Selaseh 5/5	54.17 de	++++
Alue Selaseh 5/6	40.12 bcd	-
Alue Selaseh 5/7	31. 27 a	-
Alue Selaseh 5/8	37.74 abc	-
Alue Selaseh 6/1	37.01 abc	-
Alue Selaseh 7/1	42.50 bcd	-
Alue Selaseh 7/2	38.89 abc	-
Alue Selaseh 7/3	39.37 abc	-
Alue Selaseh 7/4	36.52 abc	-
Alue Selaseh 7/5	61.19 e	++++
Alue Selaseh 7/6	34.66 abc	-
Alue Selaseh 8/2	33.15 ab	-

Note: ** For production: filter paper color : ++++ blackish red, +++ brick red, ++ dark brown, + light brown. The numbers followed by the same letter in the same HCN column are not significantly different based on the Duncan Multiple Range Test (DMRT) test at the test level: 0.05.

The characteristics of rhizobacteria that act as candidates for biocontrol agents are high to very high inhibiting ability of pathogens. 18 isolates tested based on Table 1, there were two isolates, namely Alue Selaseh 7/5 and Alue Selaseh 4/5 with high pathogen inhibiting activity (61.19 - 65.54% respectively). Then there was one isolate rhizobacteria that had moderate inhibition, namely Alue Selaseh 5/5 with an inhibition percentage of 54.17%. Meanwhile, the remaining 15 isolates had low inhibitory activity (<50% DH).

The results of the evaluation of rhizobacteria on their ability to produce Hydrogen Cyanide (HCN) compounds were different. The rhizobacterial isolates that have the ability to produce HCN are Alue Selaseh 3/1, Alue Selaseh 5/5, Alue Selaseh 4/5 and Alue Selaseh 7/5.

Seed treatment using indigenous rhizobacteria was able to increase peroxidase enzyme activity in nutmeg seeds. 18 rhizobacterial isolates were tested, it showed that the seed treatment using Alue Selaseh 3/1 rhizobacteria isolates obtained the highest peroxidase enzyme activity, namely 1.857 U mg protein-1. Although when compared with Alue Selaseh 5/8 rhizobacterial isolates, Alue Selaseh 7/2, and Alue Selaseh 7/5 were not statistically significant. However, it was significantly different from isolates of Alue Selaseh 3/10, Alue Selaseh 3/11, Alue Selaseh 4/1, Alue Selaseh 4/5, Alue Selaseh 5/3, Alue Selaseh 5/5, Alue Selaseh 5/6, Alue Selaseh 5 / 7, Alue Selaseh 6/1, Alue Selaseh 7/1, Alue Selaseh 7/3, Alue Selaseh 7/4, Alue Selaseh 7/6, Alue Selaseh 8/2 and control, but not significantly different from Alue Selaseh's rhizobacterial isolates 5/8, Alue Selaseh 7/2 and Alue Selaseh 7/5. The average results of the measurement of the peroxidase enzyme activity in nutmeg seeds are presented in Table 2.

Table 2. Effect of Seed Treatment with Various Rhizobacterial Isolates on Peroxidase Enzyme ActivityFollow up after 6 weeks.

Treatments	**Peroxidase Activities (U/mg protein)**
Control	0,641 a
Alue Selaseh 3/1	1,857 g
Alue Selaseh 3/10	1,177 bcd
Alue Selaseh 3/11	1,283 bcde
Alue Selaseh 4/1	1,247 bcde
Alue Selaseh 4/5	1,270 bcde
Alue Selaseh 5/3	1,307 bcdef
Alue Selaseh 5/5	1,220 bcde
Alue Selaseh 5/6	1,240 bcde
Alue Selaseh 5/7	1,197 bcde
Alue Selaseh 5/8	1,387 efg
Alue Selaseh 6/1	1,195 bcde
Alue Selaseh 7/1	1,190 bcde
Alue Selaseh 7/2	1,318 cdefg
Alue Selaseh 7/3	1,260 bcde
Alue Selaseh 7/4	1,173 bcd
Alue Selaseh 7/5	1,557 fg
Alue Selaseh 7/6	1,133 b

Alue Selaseh 8/2	1,193 bcde

Note: The numbers followed by the same letter in the same column are not significantly different based on the test (DNMRT) at the test level: 0.05.

Physiological and Biochemical Characterization of Rhizobacteria Isolates as Plant Growth Enhancers

The rhizobacterial isolates tested were able to produce IAA (Indole Acetic Acid), dissolve phosphate and produce siderophore compounds but with different content in each tested isolate is presented in Table 3.

Based on the evaluation results, all of the 18 isolates tested had the ability to produce IAA growth hormone in the range of 0.21 µg ml of filtrate-1 to 1.22 µg ml of filtrate-1. From 18 isolates that were tested, there were 4 isolates capable of producing high IAA (Indole Acetic Acid) growth hormone, namely Alue Selaseh 3/11, Alue Selaseh 4/5, Alue Selaseh 5/5, Alue Selaseh 5/7 and Alue Selaseh. 7/6 while other rhizobacterial isolates were able to produce IAA (Indole Acetic Acid) hormone less than 1.15 µg ml filtrate-1, but overall the Alue Selaseh 4/5 isolates were able to produce IAA with the highest concentration, namely 1.22 µg ml filtrate - 1. In Table (5), 18 isolates tested, 14 rhizobacterial isolates were able to produce phosphate which was provided in the form of TriCalcium Phosphate as a source of phosphate marked by the formation of halozone (clear zone) on the test media. Meanwhile, 4 rhizobacterial isolates (Alue Selaseh 3/10, Alue Selaseh 4/1, Alue Selaseh 4/5 and Alue Selaseh 5/7) were unable to dissolve phosphate.

Meanwhile, in the analysis of the ability of rhizobacteria in producing siderophore as a whole, all tested rhizobacterial isolates had the ability to produce siderophore, but each tested isolate contained different siderophore content. Overall, the bacteria that produce siderophores in large numbers tend to be found in Alue Selaseh 5/6 rhizobacterial isolates.

Table 3. Ability of Various Rhizobacterial Isolates to Produce IAA, Dissolve Phosphates and Produce Siderophores

Treatments	The ability of rhizobacteria as plant growth-promoting agents		
	IAA content (µg /ml filtrate)**	Dissolving Phosphate	Siderophore Production (Abs 550 nm)
Alue Selaseh 3/1	0,773 h	+	1,043
Alue Selaseh 3/10	0,630 fgh	-	0,928
Alue Selaseh 3/11	1,203 i	+	0,760
Alue Selaseh 4/1	0,477 cde	-	0,860
Alue Selaseh 4/5	1,220 i	-	0,618
Alue Selaseh 5/3	0,547 ef	+	0,142
Alue Selaseh 5/5	1,160 i	+	1,132
Alue Selaseh 5/6	0,215 a	+	1,335
Alue Selaseh 5/7	1,217 i	-	1,258
Alue Selaseh 5/8	0,414 bcd	+	1,215
Alue Selaseh 6/1	0,820 h	+	0,811
Alue Selaseh 7/1	0,340 b	+	1,091
Alue Selaseh 7/2	0,497 def	+	0,938
Alue Selaseh 7/3	0,775 h	+	1,242
Alue Selaseh 7/4	0,601 efg	+	0,972
Alue Selaseh 7/5	0,373 bc	+	0,962
Alue Selaseh 7/6	1,150 i	+	1,028
Alue Selaseh 8/2	0,733 gh	+	0,978

Note: The numbers followed by the same letter in the same column are not significantly different based on the test (DNMRT) at the test level: 0.05. For Phosphate: (+) positive reactions form a clear zone, (-) negative reactions do not form a clear zone.

IV. Discussion

Physiological and Biochemical Characterization of Rhizobacteria Isolates as Biocontrol Agents

Rhizobacteria which have high inhibiting ability to the growth of pathogenic colonies of *R. microporus* indicate that these rhizobacterial isolates are antagonistic. One of the abilities of rhizobacteria which are antagonistic to the pathogens of this test is thought to be related to the ability of each isolate to secrete secondary metabolites. The secondary metabolites that are anti microbial, such as hydrogen cyanides, chitinase, cellulase, lipase and protease enzymes. 15 isolates against the tested pathogens are still potential candidates for plant growth-promoting agents. The rhizobacteria group that has the ability to inhibit the growth

of pathogenic fungi colonies with moderate, low inhibitory activity and has no inhibitory power at all, so it has the potential to be developed as plant growth-promoting rhizobacteria [14]. This group of rhizobacteria probably has various other abilities such as producing IAA compounds, siderophore compounds, reducing manganese, and the ability to dissolve phosphates.

Apart from its ability to inhibit pathogen growth, HCN is also a secondary metabolite produced by antimicrobial properties of rhizobacteria. HCN is a secondary metabolite compound which is generally produced by *P. fluorescens* and is toxic to pathogenic fungi[15]. The test results of rhizobacterial isolates in producing HCN showed that there were three rhizobacterial isolates capable of producing HCN compounds, namely Alue Selaseh 5/5 and Alue Selaseh 7/5 isolates with the activity of producing very large amounts of HCN, while Alue Selaseh 3/1 and Alue Selaseh 4/ 5 isolate are with activity to produce high amounts of HCN. A total of four rhizobacterial isolates were able to produce HCN which could be identified by the occurrence of color changes on the test paper (filter paper). Rhizobacteria that were able to inhibit showed a positive reaction in producing varying amounts of HCN which could be detected based on the intensity of the color tested[16].

Physiological and Biochemical Characterization of Isola Rhizobacteria as Plant Growth Enhancers

Based on the results of the study, it was shown that some of the rhizobacterial isolates tested had the potential to promote plant growth. This can be seen from the results of the analysis of the rhizobacterial isolates such as the ability to dissolve phosphate, the ability to produce IAA and produce siderophore compounds. Candidate rhizobacteria as plant growth stimulating agents have the ability to produce IAA, siderophore and dissolve phosphate, while this is beneficial for the physical and chemical properties of soil in plant rhizosphere [17,18], thus rhizobacteria also can play a very important role in increasing plant growth[19].

Based on the analysis results showed that all rhizobacterial isolates were able to produce IAA in media with the amino acid tryptophan added with different content in each isolate. This is consistent with the results of studies [20] and [21], the ability to produce IAA is determined by the type of rhizobacteria tested and the ability to colonize plant roots has implications for the amount of tryptophan amino acid obtained from plant root exudates. The production of IAA by rhizobacteria will only occur if the concentration of the amino acid tryptophan in the plant root area is high enough. Based on [22] and [23], it can be seen that IAA is able to stimulate various processes of plant growth and development, however its effect is also determined by its concentration. On the other hand, the effect of low IAA concentration makes lateral root formation, reduces primary root length, and increases root hair formation.

In addition, the results of rhizobacterial testing as plant growth promoters showed that more than 50% of the tested isolates had the ability to dissolve phosphate. The ability to dissolve phosphate is indicated by the formation of a clear zone (halo) around the hole containing a suspension of rhizobacteria on media containing Tricalcium Phosphate [24]. The ability to dissolve phosphate by bacteria varies depending on each isolate tested[25]. Phosphate compounds present in the plant growing environment are not always available to plants, so the presence of phosphate solubilizing bacteria in the plant rhizosphere helps provide phosphate compounds for plants, so that the ability of rhizobacterial isolates to dissolve phosphate is one of the physiological characters of rhizobacteria related to its role as a plant growth promoter[26].

Based on the test results of rhizobacterial isolates in producing siderophore, it showed that all rhizobacterial isolates tested were able to produce siderophore but in different numbers for each rhizobacterial isolate. Of the 18 rhizobacterial isolates tested, Alue Selaseh 5/6 rhizobacteria isolates had the ability to produce high amounts of siderophore compared to other rhizobacterial isolates. The production of siderophore by rhizobacteria is one of the characters and mechanisms in suppressing pathogen growth. The mechanism of rhizobacteria as pathogenic antagonists is carried out through competition against iron which is also used for the growth of other microbes[27]. Rhizobacteria that produce siderophores and antibiotics are more effective at suppressing disease than rhizobacteria that produce siderophores or antibiotics alone[28].

V. Conclusion

From the results of this study it can be concluded that the rhizobacterial isolates that have high inhibitory power are Alue Selaseh 4/5 and Alue Selaseh 7/5 with inhibitory activity (61.19-65.54%). The ability of rhizobacterial isolates to produce HCN compounds was obtained by 3 isolates with high HCN production, namely Alue Selaseh 5/5 isolates, Alue Selaseh 7/5 isolates, Alue Selaseh 4/5 isolates and Alue Selaseh 3/1 isolates. The peroxidase enzyme activity of 18 rhizobacterial isolates tested contained one AS 3/1 rhizobacterial isolate with peroxidase enzyme activity of 1.857 U mg protein -1. The role of rhizobacteria as Plant Growth-Promoting Agen was observed based on IAA production.The ability to dissolve phosphate and siderophore production was obtained in isolates of Alue Selaseh 3/11, Alue Selaseh 4/5, Alue Selaseh 5/5, Alue Selaseh 5/7, and Alue Selaseh 7/6 with IAA production was higher than that of other isolates. The ability to dissolve phosphate was obtained by almost all test isolates capable of producing phosphate except Alue Selaseh 3/10,

Alue Selaseh 4/1, Alue Selaseh 4/5 and Alue Selaseh 5/7. As for the production of siderophore, all tested rhizobacterial isolates were able to produce siderophore compounds but in different amounts.

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