

The characterization of potential rhizobacteria isolates to inhibit *Phytophthora palmivora* and to increase the growth of cacao (*Theobroma cacao* L.)

Rahmat Barona¹, Syamsuddin², Syafruddin²

¹Department of Graduate Agroecotechnology, Syiah Kuala University, Banda Aceh, Indonesia

²Department, of Agrotechnology, Syiah Kuala University, Banda Aceh, Indonesia

Abstract: Cacao (*Theobroma cacao* L.) is one of the important industrial plantation commodities to increase the economical income of the community. Indonesia is included as the third largest producer of cacao after Ivory Coast and Ghana. This research is conducted in The Laboratory of Seed Science and Technology, Agroecotechnology Department, Faculty of Agriculture and Laboratory of Biology, Faculty of Teacher Training and Science, Syiah Kuala University, Darussalam, Banda Aceh. The study was conducted from April 2019 until February 2020 and used two research design. First, non-factorial complete random design and second non factorial group randomized design. The rhizobacteria treatments consisted of 8 isolates with one control. The result shows that the highest *Phytophthora palmivora* inhibition percentage of rhizobacteria isolates was found in GM 8/1 (71,18%) and GM 7/10 (68,89%). Only GM 8/1 and GM 8/8 which produced protease enzyme, GM 8/1 also produced HCN. The highest amount of IAA was found in GM 7/9, GM 8/8, GM 5/6, and GM 3/6. Only GM 7/10 and GM 8/8 which were able to dissolve the phosphate. All isolates produced siderophore content with the highest producing activity found in GM7/10 (0,972 Abs λ 550 nm). The rhizobacteria isolates treatment on cacao seeds before planting could increase the viability and vigor of the seeds. The lowest disease incidence caused by *P.palmivora* on cacao seeds was found in plant treated by GM 8/1(26,67%).

Key Word: Rhizobacteria isolates characterization, *Phytophthora palmivora*, Seeds treatment, Disease Incidence

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I. Introduction

Cacao (*Theobroma cacao* L.) is one of the important industrial plantation commodities that increase the economical income of the people. Indonesia is included as the third largest producer of cacao after Ivory Coast and Ghana. The cacao plantation in Indonesia reaches 1.600.648 hectares (ha) with the production of 783.978ton¹. Cacao productivity in Indonesia remains low namely 0,49 ton per hectare whereas the cacao potential productivity each year reaches around 2.5 tonperhectar². One of the cacao productions central in Indonesia is in Aceh Province. The cacao plantation in Aceh reaches 96.468 ha with the production of 44.181 ton, and the productivity of 0.46ton perhectare³.

The low productivity of the cacao in Indonesia caused by the rottenpods disease caused by certain species of fungi. There are species of fungi causing the rotten pods disease in cacao, including *P. palmivora*, *P. megakarya*, *P. capsici* and *P. citrophthora*^{4,5}. In Indonesia, *P.palmivora* is the main species that infect entire growing phase of the plants therefor besides causing the rotten pods, it cause the *Cherelle* withered⁶. Rotten pods disease caused by *P. palmivora* could cause the yield loss up to 90%, it become worse when it is raining or dry season⁷.

The disease management of cacao rotten pods is still not effective enough. Cacao farmers is currently using synthetic pesticides to control pathogenic fungi. However, the uncontrolled use of pesticidesleft residues on the plants and soil, causing various health problems and environmental pollution. In addition, synthetic pesticides are not all effective and can cause resistance, new pathogens, and less selective target⁸. Improving the disease management methods that can control pathogens and environmentally friendly is the challenge. One effortthat can be developed is byutilizing natural resources (biochemical control) of native rhizobacteria in the plant rhizosphere.

The utilization of location-specific rhizobacteria (indigenous) as a candidate for biocontrol agents has been proven to effectively control plant pathogens. Rhizobacteria *P. fluorescence* and *Bacillus subtilis* are effective in inhibiting the growth of *P. palmivor* pathogen colony causing cacao rotten pod in vitro⁹. The strain of *Bacillus* sp. and *P. fluorescence* has proven to be able to reduce the growth of R.

microporus pathogenic fungi with 72.69-90.40% inhibition rate¹⁰. Besides acting as a biocontrol agent, rhizobacteria also acts as biofertilizer and bio stimulant by producing plant growth hormones such as IAA, gibberellin, cytokinin, dissolving minerals, dissolving phosphates, and providing other nutrients^{11,12}.

In according with some of the results of this study, this research is aimed to observe the role of site-specific rhizobacteria (indigenous) in the cacao root system to control *P. palmivora* pathogens and to become the plant growth booster.

II. Material and Methods

Location, duration and design of the research

The research was conducted at The Laboratory of Seed Science and Technology, Agrotechnology Department, Faculty of Agriculture and Laboratory of Biology, Faculty of Teacher Training and Science, Syiah Kuala University, Darussalam, Banda Aceh. The study was conducted from April 2019 until February 2020. This study used 2 experimental designs, in which the first experimental design used was non-factorial Randomized Complete Design and the second was non factorial Randomized Group design. The rhizobacteria treatment consisted of 8 isolates and one control. The research data were analysed using variance (ANOVA) and continued with further test using the Duncan New Multiple Range Test (DNMRT) at 5% significance level if the F test showed a real effect.

Rhizobacterial antagonist test on cacao rotten pod pathogens

Rhizobacteria of the selected isolation biocontrol agents were tested for their antagonistic ability against pathogens in causing cacao pod rot. The test was conducted on PDA media in a 9 cm diameter petri dish. The point of inoculation between pathogens and antagonistic rhizobacteria is 3 cm. The inoculated petri dishes were incubated at room temperature (28-29°C), then observed every day until 7 days.

Proteolytic activity

To determine proteolytic activity, gelatine was used as a substrate. 5 g of agar powder was put into 400 ml of sterile distilled water and then be heated at a temperature of 50°C for 4 hours. Gelatine solution was prepared by adding 4 g of gelatine that has been dissolved in 50 ml of sterile aquades into the agar medium, to determine proteolytic activity, 5 g of saturated ammonium sulphate solution is put into the medium. The medium is autoclaved at 120°C for 30 minutes. After a bit cold, the medium is poured into a petri dish and four holes were made using cork borer. The hole was filled with 0.2 ml of bacterial suspension, then incubated at 28°C for 3 days. Proteolytic activity measurements were conducted qualitatively, proteolytic activity that can secrete protease enzyme is shown by the presence of a clear zone around the hole that contains a bacterial suspension.

The ability of agents to produce hydrogen cyanide (HCN)

Determination of the formation of hydrogen cyanide by bacterial biocontrol agents is conducted according to the procedure described by Bakker & Schippers (1987)¹³. The material used as a medium is 4.4 g glycine, 2 g picric acid, 8 g sodium carbonate, 15 g agar, 30 g King's Medium Base, 1000 ml sterile water and sterile filter paper pieces (1 cm x 1 cm). Glycine, King's Medium Base and agar were put into 1000 ml of sterile water and then autoclaved and poured into petri dish. Furthermore, a solution was made to detect HCN (CDS) consisted of 2 g of picric acid and 8 g of sodium carbonate dissolved in 200 ml of sterile water. Pieces of sterile filter paper were put into a CDS solution. Bacteria were scratched on the glycine medium, then filter paper pieces are placed in the middle of the lid of the petri dish. Then, incubation was conducted at 28°C for 4 days. The ability of agents to produce hydrogen cyanide (HCN) from the isolates tested was evaluated qualitatively, the ability of isolates to produce HCN can be observed during the incubation period, bacteria that produce HCN will cause filter paper discoloration from yellow to light brown (+ less HCN), brown (++ moderate HCN), brick red (+++ high HCN) and blackish red (++++ HCN very high).

The ability rhizobacteria to dissolve phosphate

Testing the ability of rhizosphere bacteria to dissolve phosphate using *Pikovskaya agar* testing media with the addition of tricalcium phosphate (TCP) as a source of phosphate. The composition of each litter of media consisted of 5 g $C_3(PO_4)_2$, 10 g glucose, 0.2 g NaCl, 0.2 g KCl, 0.1 g $MgSO_4$, 2.5 mg $MnSO_4$, 2.5 mg $FeSO_4$, 0.5 g yeast extract, 0.5 g $(NH_4)_2 SO_4$, and 15 g agar. Media were sterilized using autoclave. The test media was poured into a petri dish (ϕ 9 cm), a hole was made with cork borer and filled with 0.2 mL suspension of rhizobacterial isolates tested. The test media were incubated for 3-7 days in an incubation chamber at 28 ° C. The ability to dissolve phosphate is evaluated qualitatively based on the formation of a clear zone around the hole that contains a bacterial suspension.

Ability to produce indole acetic acid (IAA)

The ability of each rhizobacterial isolate to produce IAA was analyzed by the method of Glickman and Dessaux (1995)¹⁴. Rhizobacterial isolates were grown for 24 hours in a SPA (Sucrose Peptone Agar) medium. To stimulate auxin synthesis, 0.5 g L⁻¹ amino acid tryptophan is added to each medium. Bacterial culture was centrifuged at 10,000 rpm for 10 minutes, the supernatant was separated from bacterial deposits, filtered with millipore filter paper and analyzed for IAA. The content of IAA in bacterial culture filtrate was detected using *salkowskireagent* reagents. Reagent salkowski (1 ml) and bacterial culture filtrate (1 ml) were added to the test tube and the mixture was incubated in a dark room at 28°C for 30 minutes. The ability of each rhizobacterial isolate to produce IAA was analyzed quantitatively and read the absorbance value of the isolate suspension using a spectrophotometer at a wavelength of 550 nm.

Siderophore content analysis

Siderophore production from rhizobacteria isolates was tested by growing bacteria in a test medium for 24 hours at room temperature. The composition each litre of media was 20 g sucrose, Lasparagin 2 g, K₂HPO₄ 1 g, and MgSO₄ 0.5 g. Rhizobacterial suspension was centrifuged at 11,000 rpm for 30 minutes. The supernatant was filtered with a 0.2 µm nitrocellulose membrane. Detection of siderophore production by rhizobacteria is conducted by adding 1 ml of FeCl 0.01M to 3 ml of the supernatant and as a comparison of supernatant without adding FeCl. Siderophore detection was measured using a spectrophotometer (Novaspec II model) at a wavelength of 410 nm¹⁵.

Seed treatment with rhizobacteria

Cacao seeds were disinfected with 96% alcohol for three minutes, then washed three times with sterile distilled water, and dried in a laminar airflow cabinet for one hour. A total of 50 seeds were immersed for 24 hours in suspension of each rhizobacteria isolate (250 ml) at a temperature of 26°C. After treatment, the seeds were again air-dried in a laminar airflow cabinet and ready for use. Seeds that had been treated with rhizobacteria were germinated in pots that had contained 10 sterile soil mixtures per treatment with three replications.

Antagonistic testing of cacao seedlings

P. palmivora was inoculated into the cacao seedlings by scraping the soil around the base of the stem and injuring a small piece of cacao seedling stem using a needle, then the suspense spores placed around the base of the stem, then the base of the seedling stem was covered using soil.

Observation

- I. Measurement of pathogencolonies growth inhibition rate¹⁶, using this following equation:

$$IR = \frac{R1-R2}{R1} \times 100 \%$$

Note: IR = pathogencoloniesgrowthinhibition rate (%), R1 = radius of pathogen colonies grow away from antagonistic agents (cm), andR2 = radius of pathogen colonies grow into antagonistic agents (cm).

- II. Observation of seed viability and vigor, observed variables including:

- a. Maximum growth potential (MGP) was observed by counting all seeds that germinated on the last day of observation (21 DAP)¹⁷, using this following equation:

$$MGP (\%) = \frac{\sum \text{number of seeds showing growing symptoms}}{\sum \text{number of planted seeds}} \times 100\%$$

- b. The germination rate (GR) is calculated based on the percentage of Normal Sprout (NS) first count (11 DAP)and second count (21 DAP)¹⁷, using this following equation:

$$GR (\%) = \frac{\sum \text{NS count I} + \text{NS count II}}{\sum \text{number of planted seeds}} \times 100\%$$

- c. The vigour index (VI) is calculated based on the percentage of normal sprouts on the first count (11 DAP)¹⁸, using this following equation:

$$VI (\%) = \frac{\sum \text{NS count I}}{\sum \text{number of planted seeds}} \times 100\%$$

- d. Simultaneity growth (SG) is calculated based on the percentage of strong normal germination (SNG) on the 18th day after planting, i.e. the day between the first observation (11 DAP) and the second observation (21 DAP)¹⁷, using this following equation :

$$SG (\%) = \frac{\sum \text{strong normal germination (18 DAP)}}{\sum \text{planted seeds}} \times 100\%$$

- e. Relative growth rapidity (RGR) is calculated based on the total addition of normal germination every day during germination time (21 DAP)¹⁷, using this following equation:

$$RGR = \sum_0^{tn} \frac{N}{t}$$

Note: t= length of observation time, N = % of NS each observation day, tn= total of observation day the calculation of RGR for cacao is using this following equation :

$$\text{RGR Max} = \frac{100}{\sum \text{1st counting day}} = \frac{100}{11} = 9,09\%/\text{etmal}$$

$$\text{RGR Max} = \frac{\text{RGR}}{9,09} \times 100\%$$

- f. The period to reach 50% relative total germination (T_{50}) is calculated according to number of germinating seeds each day until it reaches 50% of relative total germination¹⁹, using this following equation:

$$T_{50}(\text{day}) = t_i + \left(\frac{(n_{50} - n_i)}{(n_j - n_i)} \right) (t_j - t_i)$$

Note : t_i = Time (days) of the lower limit before reaching 50% germination, t_j = The time between after the 50% seeds germinated, n_{50} = Number of seeds germinated (50% of total seeds germinated), n_j = The amount of germination of the upper limit after reaching 50% of total germination,

- III. Disease incidence is calculated based on symptoms²⁰, using this following equation:

$$I = \frac{n}{N} \times 100\%$$

Note : I =disease incidence, n = number of symptomatic plants, N = number of planted plants

III. Result

Characterization of rhizobacteria isolates as biocontrol agents

The average inhibition results of various rhizobacterial isolates to the growth of *P.palmivora* pathogen colonies and the ability of each rhizobacterial isolate to secrete protease enzymes and produce hydrogen cyanide (HCN) are presented in Table 1.

Table 1. The pathogen colonies growth inhibition rate (IR) of various rhizobacterial isolates against the growth of *P.palmivora* pathogen colonies, and the ability of each rhizobacterial isolate to secrete protease enzymes and produce hydrogen cyanide (HCN).

Treatment	The parameters of various rhizobacterial isolates ability		
	Inhibition Rate (%)	Protease	HCN Production**
GM 3/6	35,32 ab	-	-
GM 5/6	58,33 c	-	-
GM 7/9	52,38 bc	-	-
GM 7/10	68,89 c	-	-
GM 8/1	71,18 c	+	++
GM 8/3	24,08 a	-	-
GM 8/8	60,57 c	+	-
GM 8/11	52,81 bc	-	-

Note : *For extracellular protease: + positive reaction for clear zone, - negative reaction with nuclear zone.

**For HCN production: colour of filter: ++ dark brown, and – yellow. Number followed by the same letter on the same column is not significantly different according to 0,05 test level of Duncan Multiple Range Test (DMRT).

According to Table 1, the different activities of each type of rhizobacterial isolate in inhibiting the growth of *P.palmivora* pathogenic colonies. There are 2 rhizobacteria isolates which are categorized as high in inhibition rate (61-75%), they are GM 8/1 (71.18%) and GM 7/10 (68.89%). Whereas 4 rhizobacterial isolates were included in the moderate category (51-60%), namely GM 8/8 (60.57%), GM 5/6 (58.33%), GM 8/11 (52.81%), and GM 7 / 9 (52.38%) and 2 other isolates that are in the low category (<50%), namely GM 3/6 (35.32%) and GM 8/3 (24.08%).

As for the test results of the ability to secrete extracellular protease enzymes, there are 2 isolates that have the ability to secrete protease enzymes, they are isolate GM 8/1 and GM 8/8, the rest are not able to secrete extracellular protease enzyme. Whereas according to the results of the evaluation of the ability of rhizobacteria to produce hydrogen cyanide (HCN) compounds, GM 8/1 is the only isolate that can produce HCN compounds in moderate amounts.

Characterization of rhizobacteria isolate as plant growth booster

The results of rhizobacteria isolate test have a very significant effect on IAA content eventhough the effect is not significant on the siderophore production. The rhizobacteria ability to produce auxin Indole Acetic Acid (IAA) and siderophore is presented in Table 2.

According to various treatments, rhizobacterial isolates have the ability to produce different amount of IAA. There are 4 isolates that produce high IAA growth hormone, they are GM 7/9, GM 8/8, GM 5/6, and GM 3/6. Whereas other isolates produce IAA with the amounts less than 1.58 μ / ml filtrate.

Rhizobacterial isolates that can dissolve the phosphate provided in the form of TCP around the test media. There are 2 isolates that can dissolve phosphate, GM 7/10 and GM 8/8. Whereas, in the analysis of the ability of rhizobacteria to produce siderophore entirely, all isolates have the ability to produce siderophore yet in different amount, however the higher amount of siderophore production content is found in GM 7/10.

Table 2. The ability of various rhizobacterial isolates to produce auxin Indole Acetic Acid (IAA) in media containing the tryptophan amino acid, dissolving phosphate, and producing siderophore.

Treatment	The rhizobacteria as a plant growth booster ability		
	IAA content (μ /ml filtrate)**	Phosphate Solvents	Siderophore Production (Abs λ 550 nm)
GM 3/6	1,58 e	-	0,547
GM 5/6	1,63 f	-	0,762
GM 7/9	1,65 fg	-	0,836
GM 7/10	1,02 d	+	0,972
GM 8/1	0,56 c	-	0,922
GM 8/3	0,21 a	-	0,910
GM 8/8	1,67 g	+	0,893
GM 8/11	0,31 b	-	0,910

Note : * for phosphate solvent activity: + positive reaction, halo shaped, - negative reaction, no halo shaped. ** Number followed by the sama letter on the same column is not significantly different according to 0,05 test level of Duncan Multiple Range Test (DMRT).

Effect of seed treatment using rhizobacteria on viability and vigor of cacao seeds

The result of the rhizobacterial isolates treatment had a very significant effect on maximum growth potential, and germination rate. Whereas the growth rapidity is relatively significantly different, however it is not significantly different from the vigor index, simultaneity and time needed to reach 50% of the relative total germination. The average value of each viability and vigor observed at each level of rhizobacterial treatment presented in Table 3.

Table 3. Average viability and vigor value of cacao seeds in several treatments of rhizobacterial isolates

Treatment	Observed seed viability and vigor					
	MGP (%)	GR (%)	VI (%)	SG (%)	RGR (%)	T50 (day)
KONTROL	76,67 a	63,33 a	26,67	46,67 a	53,26	8,75
GM 3/6	96,67 c	80,00 bc	26,67	70,00 bc	71,53	7,33
GM 5/6	93,33 bc	76,67 abc	43,33	66,67 bc	72,51	7,75
GM 7/9	96,67 c	90,00 cd	40,00	73,33 bc	75,07	7,50
GM 7/10	83,33 ab	70,00 ab	33,33	53,33 ab	66,11	7,83
GM 8/1	93,33 bc	86,67 cd	33,33	76,67 c	79,27	8,08
GM 8/3	86,67 abc	83,33 bcd	36,67	66,67 bc	75,32	7,53
GM 8/8	96,67 c	96,67 d	43,33	86,67 c	88,16	8,42
GM 8/11	96,67 c	83,33 bcd	43,33	66,67 bc	77,74	7,83

Note : Number followed by the sama letter on the same column is not significantly different according to 0,05 test level of Duncan Multiple Range Test (DMRT).

According to the Table 3, the seed treatment using GM 3/6, GM 7/9, GM 8/8, and GM 8/11 resulted an increase in the maximum growth potential of 96.67% which are significantly different from GM 7/10 and control, but they are not significantly different from GM 5/6, GM 8/1, and GM 8/3.

While different things are shown in the germination rate, where the highest value is only found in the treatment of GM 8/8 with a value of 96.67%. Whereas the observations of relative simultaneity growth showed the similar thing with observations of germination rate, where the highest value of observations of relative simultaneity growth also found in the treatment of GM 8/8 with a value of 86.67%.

Effect of rhizobacteria treatment on the disease incidence percentage of *P. palmivora* on cacao seedlings

Table 4. Average disease incidence percentage of *P.palmivora*infection on cacao seedlings treated with various rhizobacterial isolates

Treatment	Percentage of <i>P.palmivora</i> disease incidence (%)
control	86,67 d
GM 3/6	60,00 bcd
GM 5/6	66,67 bcd
GM 7/9	73,33 cd
GM 7/10	53,33 bc
GM 8/1	26,67 a
GM 8/3	73,33 cd
GM 8/8	60,00 bcd
GM 8/11	46,67 ab

Note: Number followed by the sama letter on the same column is not significantly different according to 0,05 test level of Duncan Multiple Range Test (DMRT).

According to Table 4, the treatment of cacao seeds using rhizobacteria isolates can increase the resistance of cacao seedlings against *P.palmivora*infection. Of all the isolates tested, there is one of the best rhizobacterial isolates, GM 8/1 with only 26.67% disease incidence percentage which is significantly different from the GM 8/11, however it is not significantly different compared to other treatments.

IV. Discussion

Characterization of rhizobacteria isolates as biocontrol agents

According to the result presented in Table 1, rhizobacteria as candidates for biocontrol agents have various ability to inhibit the growth of pathogenic colonies. The difference of inhibition rate between the rhizobacterial isolates tested is due to differences in the rapidity of each isolate growth and its ability to compete in getting nutrients from growing media. The difference in ability is thought to be related to the ability of isolates to secrete secondary metabolites as antimicrobial, such as antibiotics, hydrogen cyanide (HCN) and synthesis of various cell wall degradation enzymes such as chitinase, cellulase, lipase, and protease²¹. While the rhizobacteria group that has moderate inhibition, or low and has no inhibition at all, still has the potential to be developed as a Plant Growth Promoting Rhizobacteria (PGPR). This rhizobacteria group might have various other abilities such as producing IAA content, siderophore content, reducing manganese, and the ability to dissolve phosphates²².

Rhizobacteria that can secrete the extracellular protease enzyme are also followed by the ability to inhibit the growth of *P. palmivora*. Extracellular enzymes have been known as one of the rhizobacterial mechanisms that act as biocontrol agents in inhibiting the growth of pathogens²³. Extracellular protease enzymes and cellulase produced by rhizobacteria strains of *Bacillus* spp. and *P. aeruginosa* are antagonistic to pathogens²⁴.

Volatile HCN compounds are secondary metabolites that are toxic to pathogens. From the evaluation results, rhizobacteria from isolate GM 8/1 turned out to produce HCN and inhibit the growth of pathogens *P.palmivora*. HCN produced by rhizobacteria can inhibit pathogens by breaking down the fungus cell wall thus pathogens will experience death²⁵. It is suggested that inhibiting pathogens rhizobacteria show positive reactions in producing varying amounts of HCN can be detected based on the tested color intensity²⁶.

Characterization of rhizobacterial isolate as plant growth promoter

Some of the rhizobacterial isolates tested were able to act as a plant growth promoter, according to the result of the rhizobacterial isolates physiological analysis such as the ability to produce IAA, dissolve phosphate and produce siderophore content.

According to Table 3, all of these rhizobacterial isolates have the ability to produce IAA even though in different amounts. The different ability to produce IAA from various rhizobacterial isolates depends on the tested isolate and the ability of each isolate to colonize plant roots^{27,28}, thus the ability to colonize plant roots has implications for the amount of tryptophan amino acids obtained from plant root exudates for IAA production by rhizobacteria²⁹.

Another characteristics of rhizobacteria as a plant growth booster besides being able to produce IAA is also to be able to dissolve phosphate. Phosphate is one of necessary contents for plants for growth and development, however phosphate are not always available to plants because they are bound to Al, Fe, and Ca in environment, thus presence of phosphate solvent bacteria in the plant's rhizosphere helps provide this content for plants. The *Pseudomonas* spp. and *Bacillus* spp. can produce organic acids such as acetic acid, and lactic acid which are soluble for phosphate and make it available for plant³⁰.

According to the test results GM 7/10 has the highest siderophore production capacity of compared to other isolates (Table 2). The ability of rhizobacteria to produce siderophore is an necessary to become plant growth booster, since siderophore is able to bind iron (Fe) to be iron-siderophore, which then available for the plants to absorb and at the end resulted in increased plant growth since Fe is an essential nutrient, necessary in photosynthesis, respiration and suppresses the pathogen infection. The inoculation of siderophore resulting *B.subtilis* rhizobacteria as biofertilizer subtilizes increases the number of leaves since it supports the nitrogen fixation and colonize the root widely³¹.

Effect of Seed Treatment Using Rhizobacteria on Viability and Vigor of Cacao Seeds

The treatment of cacao seeds using rhizobacteria isolates that were significantly different could increase the viability and vigor which tended to be different too. Increased viability and vigor of cacao seeds are caused by the rhizobacteria incorporation in the seed and the alleged ability of rhizobacteria to produce growth hormones such as Auxin Indole Acetic Acid (IAA) which in certain concentrations can be a trigger for plant growth³². One of the functions of IAA hormones for plants is to enhance the cell development needed in the process of germination of seeds. IAA-producing rhizobacteria such as *Neceria* sp., *P. fluorescens* and *Bacillus* sp., can colonize roots faster and metabolize organic compounds produced by plant root efficiently. Root colonization by rhizobacteria increases root growth and development, abiotic stress resistance, nutrients absorption and utilization efficiently³³.

The influence of rhizobacteria treatment on the disease incidence percentage in cacao seedlings

The results of this study indicate that cacao seedlings treated with rhizobacterial isolates experienced lower disease incidence compared to controls (Table 10). The low incidence of disease caused by *P.palmivora* on cacao seedlings is due to rhizobacteria that is capable of inducing resistance or increasing the resistance of cacao seedlings to *P. palmivora* pathogens. This indicates that the treatment of different isolates in seeds tends to increase the induction of systemic resistance of cacao plants to *P. palmivora*. There was an increase in the resistance status of C20 strain maize plants against downy mildew disease which was induced by soaking Abio2 rhizobacteria isolates in seeds before planting³⁴. The treatment of rhizobacterial isolates ST27d and ST17c inoculated on soybean plants was significantly capable of inhibiting the development of stem rot disease and increase plant height and number of soybean leaves³⁵.

V. Conclusion

In vitro test results show that the treatment of GM 7/10 and GM 8/1 isolates have a higher ability to inhibit the growth of pathogen *P. palmivora* than other treatments. Only GM 8/1 and GM 8/8 isolates produce the protease enzyme, whereas GM 8/1 isolates also produce HCN content. The highest IAA content is found in isolates GM 7/9 GM 8/8, GM 5/6, and GM 3/6. Only GM 7/10 and GM 8/8 isolates are capable of dissolving phosphate. All isolates produced siderophore content with the highest activity found in GM 7/10 (0.972 Abs λ 550 nm). Seeds treatment using rhizobacteria isolates is capable of increasing cacao seeds viability and vigor, the statistically highest viability and vigor increment is observed in GM 7/9 and GM 8/8 isolates. The statistically lowest *P. palmivora* disease incidence on cacao is observed in GM 8/1 (26,67%).

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