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Development of biological seed treatment utilization using indigenous rhizobacteria to control pod rot disease(*Phytophthora palmivora*) on cacao (*Theobroma cacao* L.)

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Abstract. *Cacao (Theobroma cacao L.) is one of the plantation commodities in Indonesia which has an important role in the national economy to increase the country's foreign exchanges. Aceh Province ranks first in Indonesia as a cacao production centre, however, pod rot disease caused by Phytotora palmivora is still a major problem in cacao productivity. The research was conductedstarted from April 2019 until February 2020 in Laboratory of Seed Science and Technology, Department of Agrotechnology and Laboratory of Plant Diseases, Department of Plant Protection, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh. The research was based on descriptive exploratory method and experimental method. The research was conducted as a non- factorial CompletelyRandomized Design (CRD) and a non- factorial Randomized Block Design (RBD) . The evaluated factors were 7 rhizobacterial isolates that had beeninitially tested and selected for their ability to inhibit pathogens. The results demonstrated that the biochemical physiological characterization under the isolates with negative results on the production of extracellulase enzymes, both protease and HCN, were able to produce the highest inhibitory rate, namely the treatment of TRI 3/4 isolates with very high activity (> 75%) with an inhibition rate of 0.70 mm/day , then followed by TRI 4/10 and TRI 3/3 isolates which had high inhibitory activity (61-75%), with inhibition rates of 0.66 mm/day and 0.57 mm/day, respectively. In observing the mechanism of action of rhizobacteria, physiologically all isolates can produce IAA, the highest concentration was obtained in the TRI 3/4. Whereas the ability to dissolve phosphate was only resultednegative in 2 isolates, namely TRI 3/3 and TRI 4/10, yet all isolates were able to produce siderophore. The highest result on the observation of peroxidase enzyme activity was found in TRI 7/, namely 2.23(U / mg protein). Isolate TRI 3/4 had the lowest percentage of symptomatic seedlings,namely 21.80%.*

Key words*: antagonism, pathogen, extracellular, physiological, enzyme*

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I. Introduction

Cacao(*Theobroma cacao* L) is one of the plantation commodities in Indonesia which has an important role in the national economy to increase the country's foreign exchange[1]. Currently, Indonesia's cocoa productivity remains below expectation, 0.80 tons per ha' while the potential productivity of cacaoreach 11 tons per ha[2]. Cacao has a real and reliable role in exteriorizingnational agricultural development programs.

Indonesia is the third largest cacao producer and exporter worldwide after Ghana and Ivory Coast. Aceh Province ranks first in Indonesia as a centreofcacao national production followed by North Sumatra and West Sumatra. Cocoa plantation area reaches 98,233 hectares with a production of 32,403 tons per annum, and productivity of 0.59 tons per ha [2]. Cacao plants are cultivated in almost all districts within the province of Aceh, the centre of cacao production in Aceh is in the districts of Pidie,Pidie Jaya, Bireuen, North Aceh, East Aceh, and Southeast Aceh.

Phytophthora palmivora is one of the causes of low cacao productivity both Indonesia and Aceh Province. Pod rot disease caused by*Phytophthora palmivora* is one of the primarydiseases affecting cacao production systems in the world. Thediseasecausingyield-lossup to 90%, particularly during rainy or dry season[3]. In Indonesia,*P. palmivora* is the main species infecting all phases of plant development, beside causing pod rot, it causes wilting as well[4].

To date, control of pod rot disease remains in ineffective way. Currently, cacao farmers in general still use synthetic fungicides to control pathogenic fungi.Synthetics fungicides managementwill eventually lead the pathogen resistance andless selective to non-targeted organism. Undesirable negative impacts on product and food safety, as well as phytotoxicity problems often occur in connection with the overutilization of fungicides[5,6].

Control methods for pathogens which are environmentally friendly are increasingly urgent to be developed. One possible mean is by utilizing natural resources (biological control) of indigenous rhizobacteria in plant rhizosphere. Microorganisms such as rhizobacteria have symbiosis with plant root systems that have

been prooven to be effective and efficient in reducing plant diseases. It is expected to reduce the dependence of farmers to thesynthetic chemical pesticides.

. The results of recent research have been indicated that the utilization of specific location (indigenous) rhizobacteria as candidates for biocontrol agentseffectively control disease-causing pathogens in plants. Rhizobacteria *Pseudomonas fluorescence* and *Bacillus subtilis* effectively inhibit the growth of *P. palmivora*, the fungi causing pod rot based on in vitro study in the laboratory[7]. The *Bacillus* sp. and *P. fluorescence* were tested and proven to able to reduce the growth of pathogenic fungi *Rigidoporus microporus* with inhibition rate of 72.69-90.49%[8]. The use of *P. fluorescent* and *Bacillus* spp. effective in controlling white root fungal disease in *Hevea brasiliensis* in an endemic disease areas[9].

Based on the description above, the specific aim in this study is to obtain early detection methods for phytophthora fungal pathogens on plants and biological control techniques through the use of indigenous rhizobacteria, especially in cacao plants in theagricultural ecosystem habitat of Aceh Province. Whereas, the long-term goal is to obtain an effective, efficient, and environmentally reliable disease management method to be applied by cacao farmers throughout Indonesia in general and particularly cacao farmers in Aceh Province.

II. Materials and Method

Location, duration and design of the research

The research was conducted at the Laboratory of Seed Science and Technology, Department of Agrotechnology, Faculty of Agriculture, Syiah Kuala University Darussalam, BandaAcehfrom April 2019 until February2020. It isconducted as a non-factorial completely randomized design (CRD) and a non-factorial randomized block design (CRD) studies. The evaluated factors in this study were 7 rhizobacterial isolates that had been iniatially tested and selected for their ability to inhibit pathogens. The research data were analysed using analysis of covariance, followed by F test if the results indicated significant effect, it would be further tested using the Duncan New Multiple Range Test at the 5% level.

Biochemical inhibiting mechanism physiological characterization of rhizobacteria as biocontrol agent

Due to the ability of rhizobacteria as biocontrol candicate, various types of extracellular enzymes such as proteases and the production of hydrogensianide compounds (HCN) will be produce forbiochemical inhibiting mechanism.

Physicological responses as physiological characterization of rhizobacteria as biocontrol agent

To determine the ability of rhizobacteria as a biocontrol candidate, each isolate was then evaluated for its ability to produce siderophore, production of *Indole Acetic Acid* (IAA), and its ability to dissolve phosphate.

Measurement of peroxidase activity

Measurement of peroxidase activity is calculated by destructing21 days cacao seedling (roots and stems included), then filtered using a filter cloth and centrifuged for 30 minutes at 5000 rpm at 4ºC. The supernatant is used as an enzyme stock.

For the enzyme observation, then pyrogallor solution was prepared and then diluted until the volume becomes 100 ml.For enzyme activity screening, 2 ml of diluted enzyme stock, a reagent consisting of 5 ml 0.5 M pyrogallor solution and 0.5 ml of H_2O_2 1% added into the cuvette. Blanks were prepared by inserting the above solutions into the cuvette without the enzyme stock. The mixture is homogenized for 5 to 10 seconds and screened using a spectrophotometer at a wavelength of 420 nm. The absorbance value was observed every 30 seconds during total time of 150 seconds. Calculation of the enzyme peroxidase activity unit (UEA), namely the absorbance value obtained is reduced by blanks[10].

Initial testing of rhizobacteria inhibition against growth of P. palmivora

The rhizobacteria that have been isolated are then selected. It was then tested for the potential of cacao pod rot pathogens growth inhibiting. The selection was conducted on PDA media by placing a 0.5 cm piece of pathogen in the middle of a petri dish and placing 4 rhizobacterial isolates 2.25 cm apart from the pathogenic isolate. The criteria for bacterial isolates to be selected as candidates for biocontrol agents are the earliest appearing rhizobacteria and the largest colony size, the clearestcolour and shape, and forming a zone between rhizobacteriainhibition and the surrounding pathogens.

R*hizobacterial antagonist test against cacao pod rot pathogen (multiple culture method)*

The biocontrol agent candidate rhizobacteria from isolation which have been selected, were tested for their antagonism ability against the pathogens causingpod rot using multiple culture techniques. Multiple cultures were prepared by placing small pieces of pathogens (size 0.5 cm), and antagonistic rhizobacteria that had grown well, the colonies had filled the petri for 4 days, the test was conducted on PDA media in 9 cm

diameter petri dishes. The inoculation point between the pathogen and the antagonistic rhizobacteria was 3 cm. The inoculated pertidishes were incubated at room temperature (28-29ºC), then observed every day for up to 7 days.

Antagonists testing on cacaoseedling

Testing is conducted by infecting pathogens at the base of the stem roots of cacao seedlings.

a. **The disease incidence** in seedlings was observed at 8 weeks after planting (WAP) by counting the number of infected seedlings from the total number of seedlings.

Antagonists testing on cacaopod

The prepared rhizobacterial isolate solution was sprayed to the entire surface of the cacao pods. Then the cacao pods were then perforated in the middle to be infected with pathogens and the ability of rhizobacterial isolates to suppress the development of pathogens was recorded.

a. **Spot area width** was observed and measured after the appearance of symptoms until day 7.

b. **The disease intensity** conducted by counting the number of infectedfruit based on theinfection damagecategories[10]presented in Table 1.

Table 1. Cacao disease intensity damage categories of pod rot disease.

No.	Category / score	Cacao pod damage level (%)
1	Healthy / 0	0%
2	Light / 1	(1-10%)
3	Medium / 2	(11-50%)
4	Heavy / 3	(51-75%)
5	Very heavy / 4	(<75%)

c. **Number of *Phytophthora palmivora* infected seeds** calculated by calculating the amount of infected cacao seeds one byone. The calculation is conducted after the incubation period was completed.

III. Result and Discussion

Biochemical inhibiting mechanism of rhizobacteria as biocontrol agent

The results of rhizobacteria isolates biochemical inhibiting mechanism analysisindicatedthat each isolate has differentcapabilities ininhibiting the growth of *P. palmivora*. The results of the rhizobacteria isolates biochemical inhibiting mechanism analysis are presented in Table 1.

Table 1.Biochemical inhibiting mechanism of rhizobacteria as biocontrol agentin exerting extracellular protease enzymes, producing hidrogen cyanide (HCN), inhibition rate (IR) and inhibition rapidity of each rhizobacterial isolate against thegrowth of *P.palmivora* fungal pathogens colonies.

Isolate	Extracellulase enzyme activity *)		IR (%)	Inhibitory activity against pathogens	Rhizobacteria inhibition rapidity (mm / day)
	Protease	HCN Production **			
TRI 3/3	+	+++	64.55 cd	+++	0.57abcd
TRI 3/4	-	-	76.99 d	++++	0.70d
TRI 4/10	-	++++	72.51 d	+++	0.66cd
TRI 6/6	-	-	37.41 ab	+	0.61bcd
TRI 7/1	-	-	19.58 a	+	0.50ab
TRI 8/4	+	-	58.86 bcd	++	0.46a
TRI 8/8	+	-	45.48 bc	+	0.51abc
HSD 0.05%			32.03		0.29

Note:
- *For extracellular protease enzymes:(+) positive reactions form a clear zone ,(-)negative reactions do not form a clear zone.
- ** For HCN production: filter paper colour, ++++ blackish red, +++ brick red, ++ dark brown, + light brown, and - yellow.
- Very high activity (++++ => 75% IR), high activity (+++ =61-75% IR), moderate activity (++ = 51- 60% IR), low activity (+ = <50 % IR) and no activity (-).
- Figures followed by the same letter in the same column are not significantly different in the Honest Significant Different (HSD) test α = 5%. The inhibition rate (IR) data were transformedwith arc sin.
- TRI = Tripa, 3/3 third dilution / third bacteria

Accordingto the results of the F test analysis indicated that the treatment of rhizobacterial isolates has a very significant effect on inhibiting the growth of the target pathogen, *P. palmivora*. The inhibition rateof each

rhizobacterial isolate on the growth of *P. palmivora* colonies indicates a different percentage, the average percentage of inhibition rateis presented in Table 1.Among the 7 rhizobacterial isolates tested, the isolate with the highest inhibition rateis found in TRI 3/4 followed by TRI 4/10 isolates and then TRI 3/3 with 76.99%, 72.51% and 64.55% inhibition rate, respectively.

The rhizobacteriainhibition capacity is also affected by their ability to secrete extracellular enzymes (protease). Extracellular enzymes are known to be compounds produced by rhizobacteria to suppress the growth of pathogens. This compound is a self-defense mechanism against thus the enzyme production is beneficial for plants. One of the mechanisms of rhizobacteria acting as biocontrol agents in inhibiting pathogen growth is the production of extracellular enzymes[11]. The mechanism in mean is the ability of rhizobacteria to degrade the cell walls of *Sclerotiumrolfsii* therefore pathogen development is disrupted[12], as strains of *Bacillus* spp. and *P. aeruginosa* producing extracellular enzymes of protease and cellulase which are antagonistic to pathogens[13].

In observing the ability of rhizobacteria to produce HCN compounds, there were 2 isolates that were positive for producing these compounds. HCN compounds are known to be toxic to pathogens that the results of secondary metabolites such as HCN compounds inhibit the growth of *Phytophthora capsici*[14]. HCN is a secondary metabolite compound which is generally produced by the bacterium *Pseudomonas flourescens* and is a toxin against pathogenic fungi[15].

Physiological inhibiting mechanism of rhizobacteria as biocontrol agent

The average resultsof physiologicalinhibiting mechanism of rhizobacteria as biocontrol agent indicates the various ability of physiological ability. The average result of physiological characterization to the IAA production, phosphate dissolving ability, and siderophore production are presented in Table 2.

According to the results of the F test analysis indicated that the ability of rhizobacteria to produce IAA has a very significant effect. Isolates that were able to produce the highest IAA content are found in isolates TRI 6/6 and TRI 3/4 with values of 1.26 and 1.19, respectively which are significantly different from TRI 3/3, TRI 4/10, TRI 7/1, TRI 8/4 and TRI 8/8 (Table 2). Indole acetic acid is known to be able to control various processes of plant growth and development, yet its effect also depends on the amount of concentration. The results demonstrated different abilities in producing IAA concentrations by rhizobacteria depending on the type of rhizobacteria. The ability to produce IAA is determined by the type of bacteria tested and its ability to colonize plant roots, which has implications for the amount of tryptophan amino acid from plant root exudates[16]. If the concentration of the amino acid tryptophan in the rhizosphere is high enough, the production of IAA by rhizobacteria will take place[17].

Table 2. Physiological characterization of rhizobacteria isolate ability to produce indole acetic acid (IAA), phosphate solvents and siderophore production

Isolate	IAA content (µg / ml filtrate) **	Phosphate Solvent *	Siderophore Production (Abs λ 550 nm)
TRI 3/3	0.67 ab	-	0.81
TRI 3/4	1.19 c	+	0.97
TRI 4/10	0.56 a	-	0.98
TRI 6/6	1.26 c	+	0.54
TRI 7/1	0.84 b	+	0.87
TRI 8/4	0.78 b	+	0.99
TRI 8/8	0.74 ab	+	0.86
HSD 0.05%	0.341		

Note: * for phosphate solvent activity: (+) positive reaction is forming halo, (-) negative reaction is not forming halo.

** Figures followed by the same letter in the same column are not significantly different at the HSD test $\alpha = 5\%$.

The results of the F test analysis indicated that the ability of rhizobacteria to dissolve phosphate from the 7 isolates, 5 isolates could dissolve phosphates, namely TRI 3/4, TRI 6/6, TRI 7/1, TRI 8/4 and TRI 8/8 while remaining 2 isolates, TRI 3/3 and TRI 4/10, were unable to dissolve phosphate. The ability to dissolve phosphate by rhizobacteria is very beneficial for plants growing on soils that are deficient in available phosphatethusthat it will be provided for the plants under the presence of rhizobacteria. The rhizobacteria characteristics to dissolve phosphateare related to its role as plant growth stimulator[18].

The ability of rhizobacteria to suppress pathogenic growth is also influenced by the ability of rhizobacteria to produce siderophores. The observations on the ability to produce siderophores indicated that all rhizobacterial isolates tested had the ability to produce siderophores. The ability to produce siderophores is a mechanism for microorganisms to survive under conditions of iron (Fe) deficiency. Rhizobacteria isolates with

the ability of inhibiting the pathogenic bacteria of *Pantoea stewartii* subsp. stewartii growth can be utilized as a candidate for antagonistic agents for controlling the disease[19].

Peroxidase activity measurement

The results of the F test analysis variety indicatedthat seed treatment using rhizobacterial isolates has a very significant effect on peroxidase activity. The average peroxidase activity is presented in Table 3.

Based on the results, it indicated that isolate treatment has a very significant effect on peroxidase activity. The highest value is obtained in the TRI 7/1 treatment, namely 2.23 which is not significantly different from TRI 3/4, TRI 6/6, TRI 8/4 and TRI 8/8, yet significantly different from the control, TRI 3/3 and TRI 4/10. Isolates that can produce high amounts of peroxidase enzymes can induce systemic resistance to plants by rhizobacteria, thus the plants will form a natural defence in case of penetration/infection by pathogens.

Peroxidase enzyme functioned in the process of plant defence. When plants were infected by diseases caused by pathogens, there would be physiological changes in the plants as the plant defence enzymes generally actively react. The formation of defence due to the activity of the peroxidase enzyme is determined by the sensitivity of the plant to a disease. Peroxidase is an enzyme that takes a role in plant resistance processes including hypersensitivity reactions, lignin formation, phenol synthesis, glycoproteins, binding and phytoalexin production[20]. Peroxidase enzyme activity is associated with the hardening process / lignin formation in plant cell walls thus the formation of plant cell walls will be stronger and capable of blocking the penetration of pathogens by the time of infection occurred.

Table 3. Peroxidase activity of the rhizobacteria isolates

Isolate	Peroxidase activity (U/ mg protein)
Control	0.86 a
TRI 3/3	0.81 a
TRI 3/4	2.10 b
TRI 4/10	0.82 a
TRI 6/6	2.04 b
TRI 7/1	2.23 b
TRI 8/4	2.10 b
TRI 8/8	2.12 b
HSD 0.05%	2.66

Note: The numbers followed by the same letter are not significantly different inHSDtest $\alpha = 5\%$.

Testing of the viability and vigour of the seeds

The results of the analysis of the F test variety indicated that the treatment of seeds using rhizobacterial isolates has a very significant effect on the parameters of the maximum potential growth and germination rate, while the parameters of simultaneous growth has a significant effect and on the vigour index meter, relative growth rapidity, and T_{50} have no significant effect. The maximum average growth potential, germinationcapacity, vigour index, growth uniformity, relative growth rapidity and T_{50}presented in Table 4.

Test results of seed viability and vigour using rhizobacteria indicates very good results to the MPG with a percentage of more than 75%, of all the tested isolates even 3 isolates reached a maximum MPG percentage of 100%, namely isolates TRI 3/4, TRI 6/6 and TRI 8/4. The 3 isolates with the highest MGP exhibited PTM results more than 90% in PM999chili plants variety on seed viability and vigour using rhizobacteria[21].Vigour indexresults are not significantly different, however the value that tended to be higher is obtained from the isolate treatment rather than the control, as well as the relative growth rapiditywhich statistically are not significantly different, yet in terms of value it could be seen that the isolate treatment had a different value which tended to be higher than controls. This indicates that pathogens are suppressed more by the treatment of rhizobacterial isolates.

Table 4. Average viability and vigour of seed due to treatment of rhizobacterial isolates

Isolate	Parameters of viability and vigour of seeds					
	MPG (%)	DR (%)	VI (%)	GU (%)	RGR (%)	T_{50} (day)
TRI 3/3	96.67b	90.00bc	43.33	70.00b	59.24	7.58
TRI 3/4	100.00b	96.67c	26.66	83.33b	84.63	8.33
TRI 4/10	96.67b	90.00bc	46.66	80.00b	83.24	8.33
TRI 6/6	100.00b	96.67c	33.33	76.67b	85.81	7.33
TRI 7/1	96.67b	96.67c	36.66	83.33b	89.15	7.91
TRI 8/4	100.00b	100.00c	33.33	83.33b	88.52	7.83
TRI 8/8	76.67a	76.67ab	30.00	63,33ab	69.36	8.50
Control	83.33a	63.33a	26.67	46.67a	53.26	8.75

Note: Maximum potential growth (MGP), germination rate (GR), vigour index (VI), growth uniformity (GU), relative growth rate (RGP) and time to reach 50% relative total germination (T_{50}).

Measurement of disease incidence in cacaa seedlings that have been treated with biocontrol agents

The results of the analysis of the F test variety indicated that rhizobacterial treatment has a very significant effect on the disease incidence in seedlings. The average percentage of disease incidence due to rhizobacterial treatment is presented in Table 8.

Based on Table 7, it can be stated that the percentage of disease incidence due to treatment of rhizobacterial isolates experienced a very significant decrease compared to controls who experienced disease incidence with the largest percentage (86.67%) which is significantly different from all other isolate treatments, the best emphasis is shown by the TRI treatment. TRI 7/1 with the lowest percentage (26.66%) which is significantly different from the TRI 3/3 treatment but not significantly different from the other 5 isolate treatments. The result demonstrated the use of rhizobacterial isolates have a decent effect on the resistance of seeds that have previously been infected and showing symptoms, thus it is expected that these rhizobacterial isolates can be utilized as biocontrol agents for suppressing the development of pathogens causing harm to the plant. One of the inhibition mechanisms carried out by rhizobacteria is the production of antibiosis, these compounds suppress the development of pathogens. Of all indigenous rhizobacteria isolates introduced to soybeans was able to slow down pathogen the incubation period[22].

Table 8. Average disease incidence due to rhizobacterial treatment

Isolate	Disease incidence in seedlings
TRI 3/3	53,33b
TRI 3/4	40.00ab
TRI 4/10	33,33ab
TRI 6/6	40.00ab
TRI 7/1	26.66a
TRI 8/4	46.66ab
TRI 8/8	26.66ab
Control	86.67c

Note: the numbers followed by the same letter in the same column are not significantly different at the α = %5 (DNMRT).

Measurement of disease incidence in cacao podthat have been treated with a biocontrol agent

The results of the F test analysisindicated that rhizobacterial treatment has a very significant effect on the disease incidence in fruit, whereas the observed parameters are the disease intensity, the spot areawidth,and the number of infected fruits. The mean percentage of disease intensity, spotarea width and number of fruits affected by rhizobacterial treatment are presented in Table 9.

From Table 9, it can be concluded that the highest percentage of disease incidence in cocoa pods occurred in controls, both the disease intensity, the spot area width and the number of infected seeds. The highest percentage of disease intensity of 91.67% occurred in the control treatment which is significantly different from the remaining seven treatments of rhizobacterial isolates, the lowest percentage value was obtained in the TRI 7/1 treatment, namely 40%yet all treatments indicates inhibition of pathogenic infection cacao pods. In the observation of thespot area width, it can be seen that the supression of the infection area development has the best results in the TRI 3/3 and TRI 6/6 with values of 7.18 and 7.17 which are significantly

different from TRI 3/4 and control treatments however not significantly different from TRI 4. / 10, TRI 7/1, TRI 8/4 and TRI 8/8. From the results obtained, the highest spot area width occurred in the control, namely 14.88, which is not significantly different from TRI 3/4 and TRI 8/8. In observing the percentage of the number of infected seeds, the highest value is shown by the control, which was 37.47% which was not different from the TRI 8/4, TRI 7/1 and TRI 4/10. From the data obtained, the TRI 3/4 and TRI 6/6 treatments have the lowest percentage of infected seeds, 21.80% and 21.93%, respectively which are not significantly different from TRI 3/3, TRI 4/10, TRI 8/4, TRI 8/8 and controls.

Table 9. Average disease intensity, spot area widthand number of fruits affected due to rhizobacterial treatment

Isolate	Disease intensity	Spot area width	Number of infected seeds
TRI 3/3	48,33ab	7,18a	24,40ab
TRI 3/4	50.00b	12.21bc	21,80a
TRI 4/10	45.00ab	8.94ab	31.47abc
TRI 6/6	48.33ab	7,17a	21.93a
TRI 7/1	40.00a	8,81ab	33.00bc
TRI 8/4	48.33ab	10,27ab	27.13abc
TRI 8/8	41.67ab	9,56abc	26.07ab
Control	91.67c	14.88c	37.47c

Note: the numbers followed by the same letter in the same column are not significantly different at the $\alpha = 5\%$ (DNMRT).

Based on this percentage data, it can be summarized that rhizobacterial treatment is effective in supressing the intensity of infection occuring on fruit in all observation parameters, namely disease intensity, spot area width and number of infected seeds, this proves that rhizobacteria can be utilized as a biocontrol agent that capable of inhibiting the intensity of the infection which in turn can reduce the losses experienced by farmers due to the pathogens infection causing disease, particularly those caused by *P. palmivora* . The competition between pathogens and antagonists caused a delay in the incubation period[23]. Presumably, it is associated to the environmental factors such as temperature, pH, water content, nutrients and enzymes[24].

IV. Conclusion

TRI 3/4 is a very potential isolate to be used for biocontrol agents due to its very high inhibition activity (> 75%) with an inhibition rate of 0.70 mm/day, followed by TRI 4/10 and TRI 3/3 which have high inhibitory activity (61-75%), with inhibition rates of 0.66 mm/day and 0.57 mm/day, respectively. Biochemical physiological characterization based on the action mechanism of rhizobacteria indicates that not all isolates are able to produce extracellulase enzymes, either protease or HCN. In observing the action mechanism of rhizobacteria, physiologically all isolates can produce IAA, the highest concentration is obtained in the TRI 3/4. Whereas the ability to dissolve phosphate is only negative in 2 isolates, TRI 3/3 and TRI 4/10, however all isolates can produce siderophore. The highest result in the observation of peroxidase enzyme activity is found in TRI 7/1 isolateswhich is significantly different from the treatment of TRI 3/3 and TRI 4/10 isolates. The observation of seed viability and vigourproved that the treatment of rhizobacterial isolates have a decent effect on all parameters, it demonstrates the higher value than the control. In measuring the disease incidence in seedlings and fruit, both the disease severity from the spot area width and the number of infected seeds, it is proven that isolate treatment can inhibit disease incidence (compared to controls), therefore rhizobacterial isolates can effectively be utilized to inhibit disease infection caused by *P. palmivora*.

References

[1]. Manalu, R. Processing of cocoa beans produced by smallholders to increase farmers' income. Journal of Economics & Public Policy, 2018; 9 (2): 99 - 111.
[2]. Central Bureau of Statistics (Badan Pusat Statistika). Indonesian Plantation Statistics. Jakarta: Central Bureau of Statistics; 2017
[3]. Rosmana, A,. Waniada, C. Junaid, M.Grassa, A. 2010. Role of Iridomyrmex cordatus Ant (Hyminoptera: Formicidae) in Spreading Cocoa Pod Rot Pathogen Phytophthora palmivora. Pelita Perkebunan. 2010; 26 (3): 169-176.
[4]. Acebo-Guerrero Y, Hernandez-Rodriguez A, Heydrich-Perez M, El-Jaziri M, Hernandez-Lauzardo AN. Management of black pod rotten in cacao (Theobroma cacao L.): a review. Podss. 2012; 67:41-48. doi: 10.1051/podss/2011065.
[5]. BilesC.L., B.D. Bruton,M.M. Wall, , and M. Rivas. *Phytophthora capsici* zoospore infection of pepper fruit in variuos physical environments. Pro. Okla. Acad. 1995; 75: 1-5.
[6]. Bruin, G.C.A., L.V. Edgington. Induction of fungal resistance to metalaxyl by ultraviolet irradiation. Phytopathology. 1982; 70: 459-560.
[7]. Pratama, S.W., S. Sukamto, I.N. Aisyah, and Y.V. Ervina. Growth inhibition of cocoa pod rot fungus *Phytophthora palmivora* by *Pseudomonas fluorescence* and *Bacillus subtilis* bacteria. Pelita Perkebunan. 2013; 29 (2): 120-127.
[8]. NasrunandNurmansyah. Potency of rhizobacteria and botanical fungicides to control white root fungus disease in rubber plant. J. TIDP2. 2015; 2(2), 61-68.

[9]. Nasrun, Nurmansyah, Burhanuddin, Zulkarnain. Utilization of vegetable pesticide formulations and biological agents to control white root fungal disease in rubber. Jakarta: Centre and Plantation Development Agency for Agricultural Research and Development, Ministry of Agriculture; 2012.
[10]. Baharudinand M. Asaad. Control effectiveness of *Phytophthora palmivora* with biological agents to increase cocoa productivity. Plantation Tower. 2017; 85 (1): 9-18
[11]. Chernin, L. and Chet L. Microbial enzyme in the biocontrol of plant pathogens and pests. In Burns RG, editor. Enzymes in the environment; activity, ecology, and application. Marcell Decker Inc. 2002; 171 - 178.
[12]. Zhang, Y.Q., M.L. Tao, W.D. Shen., Y.Z. Zhou., Y. Ding., Y. Ma. and W.L. Zhou. . Immobilization of L-asparaginase on the microparticles of the natural silk sericin protein and its characters. Biomaterials.2004; 25: 3751-3759.
[13]. Egamberdiyeva,D. Biological control of phytopathogenic fungi with antagonistic bacteria. Biocontrol of bacterial plant disease, 1st Symposium. 2005.
[14]. Mardiah. Seed treatment using plant growth-enhancing rhizobacteria on seed viability and vigour, seed growth and vegetative growth and red chili plant production (Masteral Thesis). Banda Aceh: Syiah Kuala University; 2015.
[15]. Ramamoorthy, V., T. Raguchander, and R. Samiyappan. Enhancing resistance of tomato and hot pepper to *Pythium* diseases by seed treatment with *Pseudomonads fluorescent*. European J. of Plant Pathol. 2002:108 (5): 429441.
[16]. Thakuria, D., N.C.Talukdar, C.Goswami, S.Hazarika, R.C.Boro, andM.R.Khan. Characterization and screening of bacteria from rhizosphere in rice grown in acidic soil from Assam. Curr. Sci. 2004; 86: 978-985.
[17]. Karnwal, A. Production of indole acetic acid by *Pseudomonas fluorescent* in the presence of Ltryptophan and rice root exudates. J. Plant Pathol. 2009; 91 (1): 61-63.
[18]. Faccini, G., S. Garzón, M. Martínes and A. Varela. 2004. Evaluation of the effect of a dual inoculum of phosphate-solubilizing bacteria and *Azotobacter chroococcum*, in crops of creole potato (papa "criolla"), "yema de huevo" variety (*Solanum phureja*). First International Meeting on Microbial Phosphate Solubilization. 2004 ; 301-308.
[19]. Rahma H., A. Zainal dan Suryati. Isolasi dan seleksi rizobakteri yang berpotensi sebagai agen pengendali *Pantoeastewartii* Subsp. Stewartii Penyebab Layu Stewart pada Tanaman Jagung. J. HPT Tropika. 2016; Vol. 16 (2): 124- 130.
[20]. Nicholson, R.L. and R. Hammerschmidt. Phenolic compounds and their role in disease resistance. Annual Review of Phytophatology.1992; 30: 369–389.
[21]. Taslim. The effect of seed treatment using plant growth-promoting rhizobacteria on the growth and production of PM999 chili (Undergraduate Thesis). Banda Aceh: Syiah Kuala University; 2016.
[22]. Yanti, Y, T. Habazar, Z. Resti, and D. Suhalita. 2013. Screening of Rhizobacteria Isolates from Roots of Healthy Soybean Plants for Control of Bacterial Pustules (X*anthomonas axonopodis* Pv. glycines). J. HPT Tropical . 2013;13 (1): 24 - 34.
[23]. Yudha, M.K., L. Soesanto and E. Mugiastuti.The utilization of four Trichoderma sp. isolates for controllingclubroot disease in chinese cabbage. Jurnal Kultivasi. 2016; 15 (3): 143-149.
[24]. Berlian, I., B. Setyawan., and H. Hadi.MekanismeAntagonisme Trichoderma spp. TerhadapBeberapaPatogenTular Tanah. Warta Perkaretan. 2013; 32 (2): 74-82.

Taslim, et. al. "Development of biological seed treatment utilization using indigenous rhizobacteria to control pod rot disease (Phytophthora palmivora) on cacao (Theobroma cacao L.)."*IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 13(9), 2020, pp. 01-08.