Development of Biological Seed Treatment Using Indigenous Rhizobacteria to Control Fruit Rot (*Phytophthora palmivora* Butl.) In Cocoa (*Theobroma cacao* L.) Plants

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Abstract: *The long-term objective of this study is to obtain effective and efficient and environmentally friendly control methods that can be applied to cocoa farmers throughout Indonesia in general and especially cocoa farmers in Aceh Province. The research methods include exploration methods and descriptions and experimental methods in the laboratory and in the field. Detection, isolation, identification and molecular characterization of P. palmivora pathogens and bio-control agent rhizobacteria, study the mechanism of action of agents through physiological approaches and biochemical metabolic analysis of bio-control agents using exploratory methods and descriptions. While testing the effectiveness of rhizobacteria both acting as bio-control agents and as Plant Growth Promoting Rhizobacteria (PGPR) agents in laboratories, in greenhouses and in the field using experimental methods. Research Activities: include experiments on the detection and identification and isolation of P. palmivora fungal pathogens, isolation of rhizobacterial candidates for bio-control agents from the rhizosphere of cocoa plants, evaluation of the effectiveness of candidate bio-control rhizobacterial inhibitors on pathogen P. palmivora in vitro, and molecular characterization of pathogenic isolates and rhizobacterial isolates of bio-control agents. The results showed that rhizobacterial isolates from cocoa plantation farmers in Tripa Sub-district, Nagan Raya Regency had very high inhibitory activity (> 75%), namely RBT86, RBT88, and RBT89 rhizobacterial isolates, while rhizobacterial isolates had high inhibitory activity (61-75%) there are 7 isolates, namely RBT613, RBT614, RBT615, RBT71, RBT74, RBT84, and RBT810 rhizobacterial isolates. Isolation of rhizobacteria from farmers cocoa plantations in Glumpang Minyeuk Sub-district, Pidie Regency which showed very high inhibitory activity (61-75%) in the growth of test pathogenic colonies in vitro, namely RBGM82 and RBGMMS3 isolates. While rhizobacteria isolates RBGM36, RBGMS6, RBGM7IO, RBGM88, and RBGM36 isolates had high inhibitory activity (61-75%). Isolated rhizobacterial isolates from the cocoa farmers plantation areas in Tripa Sub-district, Nagan Raya Regency and Glumpang Minyeuk Sub-district, Pidie Regency did not show a significant effect on the inhibitory rate parameters on the growth of pathogenic causes of cocoa rot (P. palmivora).*
Keywords: *agent, bio-control, biological, cocoa, rhizobacteria*

I.   Introduction

Cocoa (*Theobroma cacao* L.) is one of the important plantation commodities, both as a source of foreign exchange, a source of income and as a driver of new economic growth in the area around cocoa plantations, Indonesia is the third largest country as a cocoa producer after Ivory Coast and Ghana. The area of Indonesian cocoa plantations reached 1,691,334 million hectares with a production of 777,500 tons (Ministry of Industry, 2007). The productivity of Indonesian cocoa is still relatively low at 0.78 tons Ha-1 while the potential of cocoa productivity per year reaches 11 tons Ha-1 (Corley, 1988).

Aceh Province is one of the centers of cocoa production among 32 other provinces in Indonesia which are listed as national cocoa production centers. The area of cocoa plantations reached 98,233 Ha with production of 32,403 tons, and productivity of 0.59 tons Ha^{-1} (Indonesian Plantation Statistics, 2017). Cacao plants are cultivated in almost all level II regions within the Aceh province, both in the form of government, private and community plantations.

One of the causes of low cocoa productivity in Indonesia as well as in Aceh Province due to pests and diseases, including fruit rot caused by *Phytophthora palmivora*. *Phytophthora* fruit rot (pod rot) is one of the main diseases that affect the cocoa production system in the world. This disease can cause loss of yield reaching 90%, especially in the rainy season or dry season (Rosmana *et al*., 2010). The fungus that causes rot of cocoa fruit consists of four species of *P. palmivora*, *P. megakarya*, *P. capsici* and *P. citrophthora* fungi (Bowers *et al*.,

2001; Drenth and Sendall, 2004). In Indonesia *Phytophthora palmivora* is the main species that attacks all phases of plant development so that besides causing fruit rot, it also causes cherelle wilt (Acebo-Guerrero *et al.*, 2012).

Symptoms of a disease that is typical of plants in the form of blackish fruit rot. At first, the affected fruit has small patches on the end of the fruit. Spots develop quickly covering the internal tissue and the entire surface of the fruit, including seeds (Guest, 2007). Infected fruit becomes totally rotten within 2 weeks (Jackson and Wright, 2001). Pathogens attack the internal tissues of the fruit and cause the cocoa beans to wrinkle and change color, eventually the fruit becomes black and mummy (Bowers *et al.*, 2001; Guest, 2007), and pathogens can enter the fruit, causing the seeds to rot and reduce quality (Sukamto and Punaguti, 2004).

Control of cocoa fruit rot is still not effective enough. Currently, cocoa farmers still use synthetic fungicides to control pathogenic fungi. But with increasing public awareness of environmental and health hazards, the use of synthetic chemicals has begun to be limited (Burges, 1998). Besides that synthetic pesticides are not all effective and can cause new resistance to pathogens, and are less selective. Non-adverse negative impacts on product and food safety, as well as phytotoxicity problems often occur in connection with the use of excessive fungicides (Charles 1997; Bruin and Edgington, 1980).

Control methods that are capable of eradicating pathogens and environmentally friendly are increasingly urgent to learn. One effort that can be developed is by utilizing natural resources (biological control) and indigenous rhizobacteria in plant rhizosphere. Microorganisms such as rhizobacteria symbiosis with plant root systems have been shown to be quite effective and efficient in reducing plant diseases. Thus it is expected to reduce farmers' dependence on synthetic chemical pesticides.

Biological control using rhizobacteria which acts as a bio-control agent (biological control) is an alternative as a substitute for synthetic chemical fungicides in disease control. Biological control is control by using one or more organisms other than humans to reduce the number of inoculums or activities to produce disease from a pathogen (Cook and Baker 1983; Baker and Scher, 1987). Biological control of pathogens in the form of a total or partial reduction of pathogenic populations by other organisms naturally occurs continuously in natural ecosystems (Agrios, 1997).

The use of indigenous location-specific rhizobacteria as candidates for bio-control agents as a result of the latest research has been shown to effectively control plant disease pathogens. Rhizobacteria of *P. fluorescence* and *Bacillus subtilis* effectively inhibited the growth of *P. palmivora* fungi causing cocoa fruit rot in vitro in the laboratory (Pratama *et al.*, 2013), Nasrun and Nurmansyah (2015) stated that the strains of *Bacillus* sp. and *P. fluorescence* that was tested was able to reduce the growth of pathogenic fungi *R. microporus* with peat power 72.69-90.40%. Effective use of rhizobacteria of *P. fluoresen* and *Bacillus* spp species controls white root fungal disease in rubber plants in disease endemic areas (Nasrun *et al.*, 2012).

In connection with some of the results of this study, this study was directed to study whether location-specific rhizobacteria (indigenous) in the cocoa root system can control the pathogens that cause fruit rot caused by pathogens *Phtophthora palmivora* still requires in-depth study. Therefore, the specific target to be achieved in this study is to get a method of detecting the attack of *phytophthora* fungal pathogens in plants and biological control techniques through the use of indigenous rhizobacteria, especially in cocoa plants, the habitat of the Aceh Province's agro-ecosystem. While the long-term goal is to get an effective and efficient and environmentally friendly control method that can be applied to cocoa farmers throughout Indonesia in general and especially cocoa farmers in Aceh Province. The targeted output from the results of this study is to obtain a method of early detection of pathogens that cause fruit rot in cocoa plants and their control techniques, so as to overcome the problem of fruit rot that has been faced by farmers, especially in cocoa plantation areas both private plantations and smallholder plantations in Aceh Province. Thus it is expected that cocoa productivity can increase and farmers' incomes will also increase which in turn will help improve the regional economy through the commodity trading activities.

The results of research that researchers have done so far using indigenous rhizobacteria, especially in the commodity of chili plants, have proven that some rhizobacterial isolates from both *Pseudomornas Bacillus* and *Sernatia* groups are quite effective in controlling pathogens that cause fruit rot. While in the cocoa commodity the results of a preliminary study of rhizobacteria from rhizosphere isolates from red chili from the *Pseudomonas* sp and *Bacillus* sp groups showed that in vitro effectively inhibited the growth of pathogenic colonies causing cocoa rotten disease (*P. palmivora*) isolated from people's cocoa plantations in Aceh Regency West. The results of this preliminary study indicate that if bio-control agent rhizobacterial isolates against *P. palmivora* fungal pathogens, direct isolation from the cocoa root system is expected to be the most effective and efficient bio-control agent isolate in the control of pathogens causing fruit rot. This is because the rhizobacteria are symbiotic and have adapted to the growth environment of the cocoa plant.

II. Research Methods

The research was conducted at the Seed Science and Technology Laboratory, Department of Agrotechnology, Laboratory of Plant Disease, Plant Protection Study Program, Faculty of Agriculture, Syiah Kuala University, Darussalam Banda Aceh. In addition, this research was also conducted at the Laboratory of Disease Research of the Spice and Medicinal Crops Research Institute Cimanggu Bogor and the Inter-University Central Laboratory of Bogor IPB, as well as other laboratories in accordance with the requirements in conducting research. Research is planned within 3 years. The first year will begin in May 2018 until November 2018. The research methods include exploration methods, namely isolation of fungal pathogens *Phytophthora palmivora* and rhizobacterial isolation of candidates for bio-control agents from several central cocoa plantation areas in the Aceh province and experimental methods to test and determine candidates for bio-control agents most effective and efficient in eradicating the pathogens that cause cocoa fruit rot. Tests carried out include tests in vitro and in vivo. The data obtained were analyzed based on standard statistical analysis procedures.

Research activities include: trials of detection and identification and isolation of *P. palmivora* pathogens, isolation of rhizobacterial candidates for bio-control agents from healthy cocoa rhizosphere areas, evaluation of the effectiveness of candidate rhizobacterial inhibitors of bio-control agents on *P. palmivora* fungal pathogens by in vitro, evaluation of possible role of rhizobacteria PGPR, and molecular characterization of pathogenic isolates and bio-control agent rhizobacterial isolates.

Detection and identification of pathogens *Phytophthora* fungi based on observations of disease progression in the field. Based on the survey results, it was found that the types of diseases and pathogens that developed in the cocoa plantation area of farmers were then determined and taken by pathogenic isolates by taking part of the cocoa plant infected with *Phytophthora* fungal disease. Isolation use Potato Dextrose Agar (PDA) medium. Pathogenic colonies that have been obtained are sub-cultured several times to obtain pure isolates. Then Koch's Postulate Test was carried out. Then it is stored and periodically rejuvenated. Pathogen isolation was carried out at the cocoa farms of Glumpang Minyeuk Sub-district, Pidie Regency and Kuala Tripa Sub-district, Nagan Raya Regency.

Bio-control agent candidates to be used as antagonistic agents and PGPR were isolated from the rhizosphere of the healthy cocoa plantations of Glumpang Minyeuk Sub-district, Pidie Regency and Kuala Tripa Sub-district, Nagan Raya Regency. From each sample 10 g of the roots were taken with the soil and put into an erlenmeyer flask with a volume of 250 cc containing 90 ml of 0.1 M buffer phosphate (pH 7.0) and 0.1% peptone. Dilutions of samples up to 103 and 104 were carried out, and grown on King's B (*Pseudomonas fluoresen*) and Tryptic Soy Agar (TSA) (*Bacillus* spp) medium plus 100 ppm cycloheximide, and incubated 48 hours at 30 °C. Single colonies that glowed on King's B medium under UV light were transferred to King's B medium and white was transferred to TSA media and then both bacteria were incubated for 24 hours at 30 °C.

The effectiveness test of the inhibition of candidate rhizobacterial bio-control agents on the growth of pathogenic colonies of *P. palmivora* fungi was carried out by a completely randomized design trial in a non-factorial pattern, rhizobacteria candidate bio-control agent (result of isolation). Each is a separate experiment and repeated 3 times. The data obtained were analyzed using SAS analysis program. To test the differences between treatment averages will be continued with the Duncan's Multiple Range Test (DMRT) distance test at a real level $\alpha = 0.05$. Rhizobacteria of isolated bio-control agent candidates, the ability of antagonism was tested against *P. palmivora* pathogens using dual culture techniques. Double cultures were prepared by placing small pieces (0.5 mm in size) of pathogens and antagonistic rhizobacteria (4 days old) on PDA media in petridish, the distance between pathogenic inoculation points and 3 cm antagonistic rhizobacteria. The test was incubated at a temperature of 25-27 °C for 7 days, then observed every day for up to 7 days. Three petri dish were prepared for each isolate. As a control, it is also prepared with only the pathogenic pathogen only. Observation of inhibition is done by measuring the distance between the edges of pathogenic colonies and antagonistic agents. Measurements are taken when the pathogenic colonies reach the edge of the petri dish. Determination of percentage of inhibition of pathogenic radius growth was carried out for each isolate. Measurements using formulas:

$$\text{PIRG}\ (\%) = [\ R_1 - R_2 / R_1\ ] \times 100\ \%,$$

where PIRG = Percent inhibition of radial growth (percentage of inhibition of colony growth), R_1 = radius of pathogenic colonies away from antagonists (cm), R_2 = radius of pathogenic colonies towards antagonists (cm). Assessment of rhizobacterial antagonism activity was determined based on percentage inhibition scale, namely: very high activity (++++ = > 75%), high activity (+++ = 61-75%), moderate activity (++ = 51-60%), low activity (+ = < 50%) and there is no activity (-). From the results of this experiment 10 of the most effective were chosen, for the next experiment. While the measurement of the rate of inhibition of pathogenic colonies growth is done by measuring the diameter length of pathogenic colonies every day due to inhibition by the rhizobacterial candidate bio-control agent. Measurements were carried out for 7x24 hours in mm day^{-1} units. The rate of inhibition of pathogenic colonies growth measurement uses a formula:

The rate of inhibition of pathogenic colonies growth (mm day^{-1}) = $\sum\limits_{0}^{7} [\frac{xi - xi - 1}{Ti}]$

where xi = the length of the diameter of the pathogenic colony of the 1st observation and Ti = observation time expressed in units of days.

III.   Result and Discussion

The results of rhizobacterial isolation from the root system of the farmers' plantations in Kuala Tripa Sub-district, Nagan Raya Regency, obtained 94 rhizobacterial isolates. All of the rhizobacterial isolates were then tested for their initial potential as bio-control agent candidates for cocoa rotten fruit pathogens (*Phytophthora palmivora* Butl.),using multiple test techniques (dual test) in Potato Dextrose Agar (PDA) media. From the results of the initial potential test 19 rhizobacterial isolates were obtained which showed the ability to inhibit the growth of *P. palmivora* pateogen colonies. While the remaining 75 rhizobacterial isolates did not show a significant percentage of inhibition of the growth of pathogenic *P. palmivora* colonies. The pathogen results of the tests in vitro are presented in Table 1. The results of pathogenic isolation of *Phytophthora palmivora* from farmers' cocoa plants that were found were rhizobacteria which showed a high inhibitory power to the growth of pathogenic isolates from the cocoa farms of Glumpang Minyeuk sub-district (Figure 1).
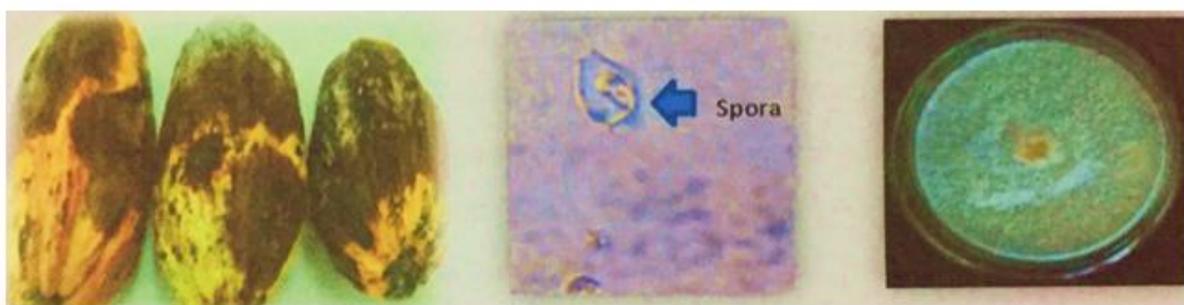

Figure 1. Pathogen Isolates Causes of Cocoa Fruits Rot (*P. palmivora*) Isolation Results from Cocoa Farmers in Glumpang Minyeuk District, Pidie Regency.

While the results of rhizobacterial isolation from the rooting system of farmers cocoa plantations in Glumpang Minyeuk Subdistrict, Pidie Regency were obtained by 84 rhizobacterial isolates. All of the rhizobacterial isolates were then tested for their initial potential as bio-control agent candidates for cocoa rotten fruit pathogens (*Phytophthora palmivora*), using a double test technique in the Potato Dextrose Agar (PDA) media. From the results of the initial potential test obtained 15 rhizobacterial isolates which showed the ability to inhibit the growth of pathogenic *P. capsici* colonies. While the remaining 79 rhizobacterial isolates did not show a significant percentage of inhibition of the growth of pathogenic *P. palmivora* colonies. Rhizobacteria which show high inhibitory power to the growth of pathogenic colonies test results in vitro are presented in Table 2.

All rhizobacterial isolates which have a high inhibitory power to the growth of test pathogen colonies from the two regions are then used in the study to be studied further as candidates for bio-control agents. While rhizobacterial isolates that do not show significant inhibitory power to the growth of the test pathogen colonies will be studied further for rhizobacterial candidates that promote plant growth.

The results of the analysis of variance (Test F) inhibition of isolate rhizobacterial isolates from the root system of cocoa plants in Kuala Tripa Sub-district, Nagan Raya Regency and Glumpang Minyeuk Sub-district, Pidie Regency showed that various rhizobacterial isolates tested had a significant in vitro inhibitory growth effect. The average percentage of inhibition of rhizobacterial bio-control agent candidates resulting isolation from cocoa plantations of Glumpang Minyeuk farmers, Pidie Regency and Kuala Tripa, Nagan Raya Regency to test pathogens is presented in Table 1 and Table 2.

Table 1 shows that the inhibitory power of each rhizobacterial isolate varies with the growth of test pathogenic colonies in vitro. In general there are several rhizobacterial isolates which have very high inhibitory power and some isolates have a high inhibitory power to the test pathogens. Rhizobacterial isolates from the cocoa plantation area of Kuala Tripa Sub-district, Nagan Raya Regency which have very high inhibitory power (>75%), namely RBT86, RBT88, and RBT89 rhizobacterial isolates. While rhizobacterial isolates that have high inhibitory activity (61-75%) there are 7 isolates, namely RBT613, RBT614, RBT615, RBT71, RBT74, RBT84, RBT810 isolates. While the remaining isolates only have a low category of inhibitory power.

Table 1. The Inhibition of Various Rhizobacterial Isolates to the Growth of Pathogenic Colonies Cause of Cocoa Fruit Rot (*P. palmivora*) by In Vitro in Kuala Tripa Sub-district

No	Rhizobacterial Isolate	The inhibitory power of Rhizobacteria (%)	Inhibition Rate Growth of Colony (mm/day)	Rizobacterial Inhibition Activity	The ability to dissolve phosphate
1	Isolate RBT33	25.56 a	1.10	+	-
2	Isolate RBT34	12.22 a	1.15	+	-
3	Isolate RBT311	10.00 a	1.15	+	+
4	Isolate RBT46	15.56 a	1.12	+	+
5	Isolate RBT47	10.00 a	1.19	+	-
6	Isolate RBT410	22.22 a	1.14	+	-
7	Isolate RBT66	25.56 a	1.11	+	-
8	Isolate RBT613	71.11 b	1.09	+++	-
9	Isolate RBT614	70.20 b	1.10	+++	+
10	Isolate RBT615	72.22 b	1.05	+++	-
11	Isolate RBT71	73.33 b	1.07	+++	-
12	Isolate RBT72	17.78 b	1.16	+	-
13	Isolate RBT74	71.11 b	1.08	+++	+
14	Isolate RBT82	26.67 b	1.16	+	+
15	Isolate RBT84	74.44 b	1.09	+++	+
16	Isolate RBT86	77.78 b	0.90	++++	-
17	Isolate RBT88	77.78 b	0.99	++++	+
18	Isolate RBT89	87.22 b	0.82	++++	+
19	Isolate RBT810	66.11 b	0.90	+++	-
	DMRT 0.05	17.30	F test		

Description: inhibitory activity is very high (> 75%), high inhibitory activity (61-75%), moderate inhibitory activity (51-60%), low inhibitory activity (<50%) and no inhibitory activity (-); the numbers in the column with the same letters are not significantly different based on the DMRT test level of 5%.

The results of isolation of rhizobacterial isolates from the cocoa farms of Glumpang Minyeuk Sub-district, Pidie Regency from the results of antagonistic tests with pathogen test showed that the results varied with the inhibitory power (Table 1). Several rhizobacterial isolates showed very high inhibitory activity (> 75%) on the growth of test pathogenic colonies by in vitro, namely RBGM82 and RBGM83 isolates. While rhizobacteria isolates RBGM36, RBGMS6, RBGM710, RBGM88, and RBGM36 isolates had high inhibitory activity (61-75%). While the remaining rhizobacterial isolates only had inhibitory power with moderate and low activity (51-60%) and (<50%).

Table 2. The Inhibition of Various Rhizobacterial Isolates to the Growth of Pathogenic Colonies Cause of Cocoa Fruit Rot (*P. palmivora*) by In Vitro in Glumpang Minyeuk Sub-district

No	Rhizobacterial Isolate	The inhibitory power of Rhizobacteria (%)	Inhibition Rate Growth of Colony (mm/day)	Rizobacterial Inhibition Activity	The ability to dissolve phosphate
1	Isolate RBGM36	69,44ab	0.88	+++	-
2	Isolate RBGM37	43,89ab	1.03	+	-
3	Isolate RBGM56	61,67ab	0.93	+++	+
4	Isolate RBGM61	35,00 a	0.96	+	+
5	Isolate RBGM65	28,33 a	0.97	+	+
6	Isolate RBGM66	51,76ab	0.95	++	+
7	Isolate RBGM79	59,44ab	0.94	++	+
8	Isolate RBGM710	61,67ab	0.94	+++	-
9	Isolate RBGM81	36,11 a	0.92	+	+
10	Isolate RBGM82	87,22 b	0.90	++++	-
11	Isolate RBGM83	75,10ab	0.88	++++	-
12	Isolate RBGM88	62,78ab	0.93	+++	-
13	Isolate RBGM89	39,44ab	1.01	+	+
14	Isolate RBGM810	53,89ab	0.96	++	+
15	Isolate RBGM81	69,44ab	0.87	+++	+
	DMRT 0.05	0,37	F test		

Description: inhibitory activity is very high (> 75%), high inhibitory activity (61-75%), moderate inhibitory activity (51-60%), low inhibitory activity (<50%) and no inhibitory activity (-); the numbers in the column with the same letters are not significantly different based on the DMRT test level of 5%.

The results of analysis of variance (Test F) showed that isolated rhizobacteria from the cocoa farmer plantations in Kuala Tripa Sub-district, Nagan Raya Regency did not show a significant effect on the inhibitory rate parameters on the growth of pathogens causing cocoa rot (*P. palmivora*). The difference in rhizobacterial isolates tested by in vitro was not followed by differences in the rate of inhibition of growth of test pathogenic

colonies. The results turned out to be the same as the results of analysis of variance (F Test) the rate of growth inhibition of the test pathogenic colonies in the rhizobacterial isolates from Glumpang Minyeuk Sub-district, Pidie Regency.

| ISOLATE RBT84 | ISOLATE RBT86 | ISOLATE RBT88 | ISOLATE RBT89 |

Figure 2. Performance of inhibition of Rhizobacterial Isolates from Cocoa Plantation in Kuala Tripa Sub-district, Nagan Raya Regency against Pathogens Causing Cocoa Fruit Rot (*P. palmivora*)

| ISOLATE RBGM82 | ISOLATE RBGM83 | ISOLATE RBGM36 | ISOLATE RBGM811 |

Figure 3. Performance of inhibition of Rhizobacterial Isolates from Cocoa Plantation in Glumpang Minyeuk Sub-district, Pidie Regency against Pathogens Causing Cocoa Fruit Rot (*P. palmivora*)

Antagonistic rhizobacterial isolates against test pathogens from in vitro test results showed that rhizobacterial isolates were potential candidates for biocontrol agents. The ability of rhizobacteria to act as a candidate for bio-control agents is related to its ability to compete with various pathogens and the synthesis of secondary metabolites such as antibiotics, siderofor, hydrogen cyanide (HCN) and the synthesis of various enzymes. The results of the study showed that the rhizobacterial group acting as a bio-control agent had the ability to produce enzymes such as chitinase, 1,3-glucanase, 1,4 glucanase, cellulase, lipase, protease, and inducyl-aminocyclopropane-carbocylate (ACC) deaminase (Baharum *et al*., 2003 , Huang & Chen, 2004, Gohel *et al*., 2004; Diby, 2004; Sutariati 2006; Kumar *et al*., 2007). The results of the analysis of the ability to produce cyanide acid (HCN) as one of the toxic compounds for pathogens were actually produced by all rhizobacterial isolates which have a high inhibitory power against the test pathogens. The antagonism of rhizobacteria both in vitro and in vivo against test pathogens indicates that the rhizobacterial group has the potential to be a candidate for bio-control agents. The results of previous studies have also been reported that some rhizobacteria from both the *Pseudomonas* group, *Bacillus Serratia* and other isolates have been shown to be very effective in controlling plant-causing pathogens. Rhizobacteria *Pseudomonas* spp. is proven to be effective in controlling various pathogens such as *R. solani* (Kumar *et al*., 2007), *C.capsici* (Sutariati, 2006), *P. infestan*, *F. oxyporum, F. speiceris* (Siddqui *et al*., 2005), dan *Phythitum apphanidermatum* (Ramamoorthy *et al*., 2002). The bio-control agent of the *Bacillus* spp. group is effective against the control of fungi *C. capsici* (Sutariati, 2006), *R. solani* (Szczech & Shoda, 2004), dan *F. udum* (Siddiqui *et al*., 2005). The bio-control agent of the *Serratia* spp. group is reported to be effective in controlling several pathogenic fungi such as *C. orbiculare*, *C. capsici* (Sutariati, 2006), *Phythium ultimunm* (Benhamou *et al*., 2000), dan *P.capsici* (Shen *et al*., 2002). The results of the study by Shen *et al*., (2002) showed that *S. plymuthica* A21-4 rhizobacteria were very potential as bio-control agents for the control of pathogenic fungi *P.capsici*.

Have long reported that *P. putida* isolates were able to synthesize indole acetic acid (IAA). Indole acetic acid is also produced by *P. aeruginosa* (Kumar *et al*., 2007). In addition to synthesizing indole acetic acid (Diby, 2004; Sutariati, 2006) it is known that *P. fluorescens* isolates also produce gibberellin (Diby 2004; Egamberdieva, 2005) and cytokinin. Likewise the strains of *Bacillus* spp, are able to synthesize indole acetic acid (IAA) (Sutariati, 2006, Egamberdieva, 2005), gibberellin and cytokinin. The ability to synthesize IAA was also found in rhizobacterial isolates from the *Serratia* spp. group.

IV.   Conclusion

Rhizobacterial isolates from the cocoa plantation area of the farmers in Kuala Tripa Sub-district, Nagan Raya Regency, which have very high inhibitory power (> 75%), are RB86, RBT88, and RBT89 rhizobacterial isolates. While rhizobacterial isolates with high inhibitory activity (61-75%) there were 7 isolates, namely RBT613, RBT614, RBT615, RBT71, RBT74, RBT84, and RBT810 rhizobacterial isolates.

Rhizobacterial isolates from the cocoa plantations area of the farmers in Glumpang Minyeuk Sub-district, Pidie Regency showed very high inhibitory activity (> 75%) on the growth of test pathogenic colonies by in vitro, namely isolates RBGM82 and RBGM83. While rhizobacteria isolates RBGM36, RBGM56, RBGM710, RBGM88, and RBGM36 isolates had high inhibitory activity (61-75%).

Rhizobacterial isolates from the cocoa plantation area in Kuala Tripa Sub-district, Nagan Raya Regency and Glumpang Minyeuk Sub-district, Pidie Regency did not show a significant effect on the inhibitory rate parameters on the growth of pathogenic causes of cocoa rot (*P. palmivora*).

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