

Indigenous Rhizobacteria treatment in controlling diseases *Phytophthora palmivora* and increasing the viability and growth of cocoa seedling

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Abstract. Rhizobacteria play a positive role as biocontrol agents as well as Plant Growth Promoting Rhizobacteria (PGPR) agents. The research objective was to obtain indigenous rhizobacteria isolates on cocoa plants that have the potential to inhibit the attack of *P. palmivora* fungal pathogens, and act as PGPR in vitro and in vivo. The results of the study concluded that isolates TRI 7/1, TRI 8/8, GM 7/9 and GM 7/10 had the highest ability to inhibit the growth of pathogen. The lowest disease severity (20%) was obtained in the seedlings treated using isolates TRI 7/1 and TRI 8/8. Rhizobacterial isolates GM 3/6, GM 5/6, GM 7/9 and GM 8/8 produce high amounts of IAA. Rhizobacteria isolates GM 5/6, GM 7/9 and GM 8/8 has very high peroxidase enzyme activity. High production of HCN compounds was obtained in rhizobacteria isolates TRI 3/3, TRI 4/10 and TRI GM 8/11. All rhizobacterial isolates gave an increase in the value of maximum growth potential, germination and vigor values for growth strength compared with the control. The rhizobacteria treatments using isolates TRI 7/1, TRI 8/8, GM 7/9 and GM 7/10 were able to increase plant height, stem diameter and number of leaves at 30, 40, 50, 60, and 70 DAP compared to control treatment.

Keywords: agent, biocontrol, pgpr, cocoa, rhizobacteria

INTRODUCTION

Cocoa is an important plantation commodity, both as a source of foreign exchange, a source of income and as a driver for new economic growth in areas around cocoa plantations. Indonesia is the third largest country as a cocoa producer after Ivory Coast and Ghana. Aceh Province is one of the centers for cocoa production among 32 other provinces in Indonesia which are listed as national cocoa production centers. Cocoa plantation area reaches 98.233 hectares with a production of 32.403 tons, and a productivity of 0.59 tons Ha⁻¹ [1]. Cocoa plants are cultivated in almost all level II regions in Aceh Province, both in the form of government, private and community plantations.

One of the causes of low cocoa productivity in Indonesia and in Aceh Province is due to pests and diseases, including pod rot caused by the pathogen *Phytophthora palmivora* Bult. *Phytophthora* pod rot disease is one of the main diseases affecting the world cocoa production system. This disease can cause yield losses of up to 90%, especially in the rainy season or dry season [2,3,4]. To date, control of pod rot disease is not effective enough. Currently, cocoa farmers generally still use synthetic chemical fungicides to control pathogenic fungi. However, with increasing public awareness of environmental and health hazards, the use of synthetic chemicals is starting to be limited [5,6].

One of the efforts that can be developed is to utilize the natural resources (biological control) of indigenous rhizobacteria in the plant rhizosphere. Microorganisms such as rhizobacteria that are in symbiosis with plant root systems have been shown to be quite effective and efficient in reducing plant diseases. Biological control using rhizobacteria

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as biocontrol agents (biological control) is an alternative as a substitute for synthetic chemical fungicides in disease control [7,8]. Biological control is a control using one or more organisms other than humans to reduce the amount of inoculum or disease-producing activity of a pathogen [9,10].

Utilization of specific location (indigenous) rhizobacteria as candidates for biocontrol agents. The results of the latest research have been proven to effectively control disease-causing pathogens in plants. Rhizobacteria *P. fluorescence* and *Bacillus subtilis* were effective in inhibiting the growth of *P. palmivora* fungi that cause pod rot in vitro and in vivo [6,11]. *Bacillus* sp. and *P. fluorescence* which were tested to reduce the growth of the pathogenic fungus *R. microsporus* with peat power of 72.69-90.40% [12]. The use of rhizobacteria of *P. fluorescent* and *Bacillus* spp species was effective in controlling white root fungal disease in rubber plants in disease endemic areas [13].

In connection with some of these research results, this study aims to obtain indigenous rhizobacterial isolates on cocoa plants that have the potential to inhibit the attack of *P. palmivora* and rhizobacteria which act as PGPR in vitro and in vivo. Thus, it is hoped that cocoa productivity and farmers' income can increase, so that it can improve the regional economy through the commodity trading activities.

METHODOLOGY

Rhizobacteria mechanism as biocontrol and PGPR agents.

The mechanism of inhibition of biocontrol agents against pathogens was observed based on the ability of these agents to produce extracellular enzymes (chitinase, cellulases and proteases), production of siderophore compounds, production of hydrogen cyanides (HCN) and the ability of the agents to produce manganese (Mn). The mechanism of action of rhizobacteria as PGPR was observed through the production of IAA, peroxidase activity and the ability to dissolve phosphate. All analyzes were performed using standard analytical procedures.

Experiments to determine the effect of seed treatment using biocontrol agent candidate rhizobacteria on viability and vigor of seed growth strength was carried out using a non-factorial completely randomized design. The experiment was carried out by planting 50 cocoa seeds in soil mixed with compost (1: 1 / v / v) in a 25 x 20 x 5 cm plastic box. Germination

observations were carried out until the 21st day after seeding. Eighteen rhizobacterial isolates were used (potential isolates from previous experiments). The observed variables included maximum growth potential (MGP), germination capacity (GC), vigor index (VI), growth simultaneously (GS), time required to reach 50% relative total germination (T_{50}) and relative growth speed (RGS). The procedure for testing the viability and vigor of seed growth strength refers to the provisions of the International Seed Testing Association [14]. The data from the observations were then analyzed using ANOVA, using the SPSS 25.0 analysis program. If the results of the analysis of variance (Test F) show that the treatment has a significant effect, the data analysis is continued with the DMRT further test procedure to compare the difference in the average treatment.

Seed treatment with biocontrol agent candidate rhizobacteria.

Rhizobacteria isolates were grown in solid potato dextrose agar (PDA) medium and incubated for 48 hours. The growing bacterial colonies were suspended in sterile distilled water until they reached density population 10^9 cfu mL⁻¹ [15] or equivalent to the reading of the absorbance value $OD_{600} = 0.164$ using a spectrophotometer.

Cocoa seeds were disinfected with 96% alcohol for 3 minutes, then washed 3 times with sterile distilled water, and dried in a laminar airflow cabinet for one hour. A total of 10 seeds were immersed for 24 hours in a suspension of each rhizobacterial isolate (250 mL) at 26 ° C. After treatment, the seeds are again dried in a laminar airflow cabinet and ready for use. The seeds that have been treated with rhizobacteria are then germinated in a plastic bowl measuring 27 x 56 x 5 cm (length x width x height) filled with soil and sterile compost (1: 1, v / v) as germination media. The soil and compost media were previously sieved with a 5 mesh sieve. Each treatment was planted 10 seeds and repeated three times. Observations were made on the viability and vigor parameters of the seed growth strength.

Experiments to determine the ability of biocontrol agent candidate rhizobacteria to control disease, the experiment used cocoa seeds that had previously received seed treatment with biocontrol agent candidate rhizobacteria. Seedlings observed for disease severity were one month old. Each experimental unit used 5 plant seeds. Furthermore, the seeds that were 2 months old were inoculated with *P. palmivora*. The *P. palmivora* inoculum was prepared by growing it on PDA media. After 7 days of age, the mycelium

and the media were cut using a 10 mm diameter Corkbord. Inoculation is carried out on the roots of the seedlings by first being covered with a pinprick 5 times, the inoculum is attached to the place where it was injured then covered with soil. Observation of disease severity (DS) was carried out by counting the number of infected seeds *P. palmivora* of the total number of seeds observed (infected and healthy seeds). Furthermore, it is calculated using an analog formula [16]: $KP = (a / a + b) \times 100\%$, DS (disease severity), a (number of infected seeds), and b (number of healthy seeds). The severity of disease is expressed in percent. Furthermore, it is observed plant height, stem diameter and number of leaves at 30, 40, 50, 60, and 70 days after planting (DAP).

RESULTS AND DISCUSSION

Inhibition of rhizobacteria isolates against of *P. palmivora*. in vitro and ability to produce growth regulatory substances

Based on Table 1, it can be seen that the rhizobacterial isolate from Kualat Tripa Nagan Raya has a very high inhibiting ability compared to other isolates, namely (DH > 75%),

namely isolate TRI 8/8 and TRI 7/1 were followed by GM 7/9 and GM 7/10 isolates and GM 3/6 isolates with high inhibitory power (DH 61-75%). While the results of the analysis of IAA production, isolates GM 3/6, GM 5/6, GM 7/9 and GM 8/8 produced higher amounts of IAA (1.578-1.672 μ / ml filtrate) while other isolates only produced in relatively lower amounts (Table 1). The results of the analysis of the ability of rhizobacteria to dissolve phosphate, from 15 isolates of rhizobacteria, there were 12 isolates showing the ability to dissolve phosphate. Rhizobacteria GM 7/9 has very high peroxidase activity compared to other isolates 3.491 (U / mg / min), followed by GM 8/8 3.274 isolates (U / mg / min). Meanwhile, 13 other isolates had lower peroxidase activity than GM isolates 7/9 and isolates 8/8. The results of the analysis of the ability to produce HCN compounds from 15 isolates of rhizobacterial candidates for biocontrol agents showed that only 3 isolates had the ability to produce HCN compounds, namely isolates TRI 3/3, TRI 4/10 and isolate GM 8/11. While the rest, 12 isolates did not have the ability to produce HCN compounds.

Table 1. Inhibition of various Rhizobacterial isolates on the growth of pathogenic colonies *P. palmivora*. in vitro and ability to produce IAA growth regulators in media containing triptophan amino acids, dissolving phosphates, and production of HCN compounds

Rhizobacteria Treatment	Ability parameters of various Rhizobacterial isolates				
	Inhibition (%)	Content of IAA (μ / ml filtrate) **	Phosphate Solvent *	Peroxidase (U / mg / min)	HCN Production **
Control	0.00 f	0.000	-	0.859 ab	-
Isolate TRI 3/3	2.87 d	0.666	-	0.805 a	+++
Isolate TRI 3/4	2.87 d	1.191	+	2.095 de	-
Isolate TRI 4/10	2.87 d	0.558	+	0.824 a	++++
Isolate TRI 6/6	60.16 c	1.258	+	2.035 de	-
Isolate TRI 7/1	75.50 ab	0.843	+	2.225 de	-
Isolate TRI 8/4	2.87 d	0.777	+	2.103 de	-
Isolate TRI 8/8	76.17 a	0.737	-	2.119 de	-
Isolate GM 3/6	64.06 bc	1.578	+	2.588 ef	-
Isolate GM 5/6	11.87 d	1.634	+	1.374 bc	-
Isolate GM 7/9	74.72 ab	1.672	-	3.491 h	-
Isolate GM 7/10	74.61 ab	1.015	+	2.604 ef	-
Isolate GM 8/1	2.87 d	0.557	+	2.875 fg	-
Isolate GM 8/3	2.77 d	0.215	+	2.836 fg	-
Isolate GM 8/8	2.87 e	1.648	+	3.274 gh	-
Isolate GM 8/11	2.87 e	0.311	+	1.725 cd	++

Note: very high inhibitory activity (++++ => 75% DH), high inhibitory activity (+++ = 61-75% DH), moderate inhibitory activity (++ = 51-60% DH), low inhibitory activity (+ = <50% DH) and no inhibitory activity (-). * for phosphate solvent activity: + positive reaction, halo form, - negative reaction, not halo form. ** The numbers in the column with the same letter are not significantly different based on the DMRT test at α = 0.05. ** for HCN production: filter paper color, +++ brick red, ++ dark brown, + light brown, and -yellow.

The ability of rhizobacterial isolates to act as candidates for biocontrol agents is closely related to their ability to compete with various pathogens and the synthesis of secondary metabolites such as antibiotics, siderophores, hydrogen cyanide (HCN), manganese (Mn) production and synthesis of various enzymes such as extracellular enzymes (cellulases, proteases, and chitinases). In addition, the ability of rhizobacterial isolates to induce systemic resistance to pathogens is also one of the characteristics of rhizobacteria that can act as candidates for biocontrol agents. The results showed that rhizobacteria, which act as biocontrol agents, have the ability to produce enzymes such as chitinase, 1,3-glucanase, 1,4 glucanase, cellulases, lipases, proteases, and inducyl-aminocyclo-propane-carbocylate (ACC) deaminase [18, 19, 20, 21].

The results of the analysis of the ability to produce cyanide acid (HCN) compounds as one of the compounds that are toxic to pathogens in this study were not in line with the ability of rhizobacteria which have high inhibitory power against the tested pathogens. This is thought to be the weapon of the rhizobacterial isolate not by producing HCN compounds, but by producing various other compounds. The antagonism properties of good rhizobacteria in vitro against the test pathogens indicate that this group of rhizobacteria is a potential candidate

for biocontrol agents. The results of previous studies have also reported that some good rhizobacteria from the *Pseudomonas*, *Bacillus* *Serratia* groups and other isolates have been shown to be very effective in controlling disease-causing pathogens in plants. Rhizobacteria *Pseudomonas* spp. proven to be effective in controlling various pathogens such as *R. solani* [22] *P. infestans* [23] and *F. oxyporum* f. *speiceris* [24]. Biocontrol agent from the *Serratia* spp. group. reported to be effective in controlling several pathogenic fungi such as *C. orbiculare* [25], *C. capsici* [26], and *Phythium ultimum* [27]. Rhizobacteria *S. plymuthica* A21-4 is very potential as a biocontrol agent to control the pathogenic fungi *P.capsici* [28].

Rhizobacterial treatment against viability and vigor of cocoa seeds

Apart from its role as a biocontrol agent, indigenous rhizobacteria also play a role as PGPR. The results of the analysis of the ability of indigenous rhizobacterial isolates isolated from the Kuala Tripa area, Nagan Raya and Glumpang Minyeuk districts, Pidie Jaya Regency to increase the viability and vigor of the growth strength of cocoa seeds showed that differences in rhizobacterial isolates used were followed by differences in viability and vigor values of the resulting growth of kako seeds.

Table 2. Average maximum growth potential, germination capacity, simultaneity of growth, vigor index, relative growth speed, time required to reach 50% relative total germination of cocoa seeds from pre-planted seed treatment using indigenous rhizobacteria

Rhizobacterial Treatment	Total Viability	Potential Viability	Vigor Strength Growth			
	MGP (%)	GC (%)	VI (%)	GS (%)	RGS (%)	T50 (day)
Control	83.33ab	63.33a	26.67	46.67a	53.26	8.75
Isolate TRI 3/3	96.67bc	90.00cde	43.33	70.00cde	59.24	7.58
Isolate TRI 3/4	100.00c	96.67de	26.67	83.33de	84.64	8.33
Isolate TRI 4/10	96.67bc	90.00cde	46.67	80.00cde	83.25	8.33
Isolate TRI 6/6	100.00c	96.67de	33.33	76.67de	85.81	7.33
Isolate TRI 7/1	96.67bc	96.67de	36.67	83.33de	89.16	7.92
Isolate TRI 8/4	100.00c	100.00d	33.33	83.33d	88.53	7.83
Isolate TRI 8/8	76.67a	76.67ab	30.00	63.33ab	69.36	8.50
Isolate GM 3/6	96.67bc	80.00bcd	26.67	70.00bcd	71.53	7.33
Isolate GM 5/6	90.00abc	76.67abc	43.33	66.67abc	72.51	7.75
Isolate GM 7/9	93.33bc	80.00bcd	40.00	73.33bcd	75.07	7.50
Isolate GM 7/10	96.67bc	96.67de	43.33	86.67de	88.16	7.83
Isolate GM 8/1	93.33bc	90.00cde	33.33	76.67cde	79.27	8.08
Isolate GM 8/3	93.33bc	83.33b-e	36.67	66.67b-e	75.32	7.53
Isolate GM 8/8	83.33ab	70.00abc	33.33	53.33abc	66.11	8.42
Isolate GM 8/11	96.67bc	83.33b-e	43.33	66.67b-e	77.74	7.83

Note: The numbers followed by the same letter in the same column are not significantly different at the 0.05 level (DMRT). PTM (maximum growth potential), DB (germination capacity), IV (vigor index), KST (simultaneous growth), KCT-R (relative growth rate), and T50 (time needed to reach 50% relative total germination).

Table 2 shows that isolates TRI 4/10 and TRI 8/4 produced total viability values of cocoa seeds which were observed based on the highest maximum growth potential compared to untreated seeds and seeds treated with rhizobacterial isolates GM 8/8 and TRI 8/8. Meanwhile, compared to other prayers, the difference in viability was insignificant.

The same thing was also found in the germination benchmarks, where all rhizobacterial isolates used in the pre-planting cocoa seed treatment could increase the germination value higher than untreated seeds and seeds treated with rhizobacterial isolates TRI 8/8, GM 8/8, and GM 5/6 (Table 2). The results of observations on the vigor measure of the growth strength of cocoa seeds which were observed based on the synchronous growth showed that all rhizobacterial isolates used in the pre-planting kako seed treatment provided added value to the synchronization of seed growth, except for the seeds treated with GM 5/6 rhizobacteria. While the vigor index variable,

The ability of rhizobacterial isolates which gave an increasing effect on the value of viability variables and vigor in growth strength of cacao seeds was closely related to the ability of these isolates to act as PGPR rhizobacteria. This is as shown in the results of the analysis of the ability of rhizobacteria to the production of IAA growth regulators in this study. All rhizobacterial isolates demonstrated their ability to produce IAA. The treatment of seeds with

plant growth-promoting rhizobacteria isolates plays a very important role, especially beneficial in the process of seed germination under stressful environmental conditions [29]. Root colonization by rhizobacteria increases the growth and development of the root system, is resistant to abiotic stress, and the absorption and utilization of nutrients is more efficient [29]. *P. putida* isolates were able to synthesize indole acetic acid (IAA) [30].

Apart from synthesizing indole acetic acid [21,26,32], it is known that *P. fluorescens* isolates also produce gibberellins [21,33,34] and cytokinins [35,36]. Likewise, the *Bacillus* spp. strain was able to synthesize indolasetic acid (IAA) [21,33,34,36], gibberellin [37] and cytokinins [38]. The ability to synthesize IAA was also found in rhizobacterial isolates from the *Serratia* spp. group. [39].

Treatment of Rhizobacteria on growth of cocoa seeds

Tables 3, 4 and 5 show that Rhizobacterial isolates have the ability to increase growth such as plant height, stem diameter and number of leaves compared to control treatments. Table 3 shows pthe treatment of isolates TRI 3/4, TRI 4/10, TRI 7/1, TRI 8/8, GM 7/9, and GM 7/10 were able to increase plant height compared to the control treatment on observations 30, 40, 50, 60 and 70 DAP. to increase stem diameter compared to control treatments on observations of 30, 40, 50, 60 and 70 DAP.

Table 3. Average height of cacao plants treated by pre-planted seeds using indigenous Rhizobacteria

Rhizobacterial Treatment	Plant Height (cm)				
	30 DAP	40 DAP	50 DAP	60 DAP	70 DAP
Control	17.68 a	18.58 a	18.88 a	19.07 a	19.30 a
Isolate TRI 3/3	18.65 ab	19.23 abc	19.59 ab	19.73 ab	20.08 ab
Isolate TRI 3/4	20.96 bcd	21.51 de	21.67 bc	21.23 bc	22.78 c
Isolate TRI 4/10	20.58 bcd	21.15 de	21.46 bc	21.84 bc	22.09 c
Isolate TRI 6/6	19.80 abc	20.51 bc	20.87 bc	21.45 bc	21.73 bc
Isolate TRI 7/1	22.04 d	22.32 e	22.74 c	23.05 c	23.48 c
Isolate TRI 8/4	19.75 abc	20.58 bc	20.84 bc	21.23 bc	21.70 bc
Isolate TRI 8/8	22.08 d	22.61 e	22.94 c	23.11 c	23.39 c
Isolate GM 3/6	18.78 ab	19.85 abc	20.31 bc	20.87 bc	21.29 bc
Isolate GM 5/6	18.28 ab	18.98 a	19.49 ab	19.77 ab	20.39 bc
Isolate GM 7/9	22.04 d	22.13 e	22.34 c	22.86 c	22.91 c
Isolate GM 7/10	21.08 cd	22.23 e	22.67 c	22.79 c	23.05 c
Isolate GM 8/1	20.18 abc	21.36 de	21.45 bc	21.68 bc	22.87 bc
Isolate GM 8/3	20.54 abc	21.46 de	21.89 bc	22.36 bc	22.90 bc
Isolate GM 8/8	20.27 abc	21.40 de	21.68 bc	22.22 bc	22.75 bc
Isolate GM 8/11	19.24 abc	20.53 bc	20.82 bc	21.19 bc	21.55 bc

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

Table 4. Average diameter of cocoa stems result of pre-planted seed treatment using indigenous rhizobacteria

Rhizobacterial Treatment	Stem Diameter (cm)				
	30 DAP	40 DAP	50 DAP	60 DAP	70 DAP
Control	3.75 a	4.27 a	4.36 a	4.49 a	4.55 a
Isolate TRI 3/3	4.32 bc	4.34 a	4.44 a	4.57 a	4.76 ab
Isolate TRI 3/4	4.15 bc	4.31 a	4.42 a	4.52 a	4.76 ab
Isolate TRI 4/10	3.98 a	4.31 a	4.49 a	4.58 a	4.62 ab
Isolate TRI 6/6	3.69 a	4.21 a	4.30 a	4.32 a	4.74 ab
Isolate TRI 7/1	4.65 c	4.81 bc	4.92 ab	5.01 b	5.03 b
Isolate TRI 8/4	4.09 bc	4.30 a	4.54 ab	4.61 a	4.79 ab
Isolate TRI 8/8	4.69 c	4.90 c	5.02 c	5.15 b	5.21 b
Isolate GM 3/6	4.05 bc	4.47 ab	4.49 ab	4.59 a	4.79 ab
Isolate GM 5/6	3.89 a	4.33 a	4.40 ab	4.41 a	4.69 ab
Isolate GM 7/9	4.60 c	4.81 bc	4.98 bc	5.03 b	5.05 b
Isolate GM 7/10	4.58 c	4.80 bc	4.94 bc	5.03 b	5.09 b
Isolate GM 8/1	3.97 ab	4.26 a	4.46 ab	4.74 a	4.89 ab
Isolate GM 8/3	3.81 ab	4.27 a	4.45 ab	4.48 a	4.82 ab
Isolate GM 8/8	3.81 ab	4.38 a	4.47 ab	4.67 a	4.75 ab
Isolate GM 8/11	3.98 ab	4.47 ab	4.65 ab	4.68 a	4.82 ab

Note: The numbers followed by the same letter in the same column are not significantly different at the 0.05 level (DMRT)

Table 5. The average number of cocoa leaves from pre-planted seed treatment using indigenous Rhizobacteria

Rhizobacterial Treatment	Number of Leaves (Unit)				
	30 DAP	40 DAP	50 DAP	60 DAP	70 DAP
Control	4.00 a	5.13 a	5.60 a	6.13 a	6.73 a
Isolate TRI 3/3	4.33 ab	5.33 a	6.13 ab	6.20 a	7.00 ab
Isolate TRI 3/4	4.60 bc	5.80 bc	6.00 ab	6.80 ab	7.33 ab
Isolate TRI 4/10	4.60 bc	5.60 bc	6.33 bc	6.86 ab	7.33 ab
Isolate TRI 6/6	4.26 a	5.53 bc	5.40 a	6.26 a	7.00 ab
Isolate TRI 7/1	5.00 c	6.33 c	6.73 c	7.66 b	8.26 b
Isolate TRI 8/4	4.13 a	5.40 bc	5.80 a	6.53 a	7.20 ab
Isolate TRI 8/8	4.73 bc	6.40 c	7.06 c	7.60 b	8.20 b
Isolate GM 3/6	4.33 ab	5.13 bc	6.20 bc	7.20 b	7.66 ab
Isolate GM 5/6	4.13 a	5.60 bc	6.20 bc	6.60 ab	7.13 ab
Isolate GM 7/9	5.00 c	6.20 c	6.80 c	7.26 b	8.20 b
Isolate GM 7/10	4.86 bc	6.20 c	6.86 c	7.53 b	8.06 b
Isolate GM 8/1	4.20 ab	5.60 bc	5.66 a	6.53 ab	7.13 ab
Isolate GM 8/3	4.53 ab	5.60 bc	6.13 ab	6.66 ab	7.26 ab
Isolate GM 8/8	4.20 ab	5.53 ab	6.40 bc	6.86 ab	7.60 ab
Isolate GM 8/11	4.33 ab	5.60 bc	6.26 ab	6.80 ab	7.46 ab

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

Table 4 shows that the treatment Rhizobacteria have the ability to increase stem diameter compared to control treatment. treatment rhizobacterial isolates TRI 7/1, TRI 8/4, TRI 8/8, GM 7/9, and GM 7/10 were able to increase stem diameter compared to control treatments on observations of 30, 40, 50, 60 and 70 DAP.

In Table 5 shows that the treatment Rhizobacteria have the ability to increase the number of leaves compared to the control treatment. treatment rhizobacterial isolates TRI 7/1, TRI 8/4, TRI 8/8, GM 7/9, and GM 7/10 were able to increase the number of leaves compared to the control treatment on

observations 30, 40, 50, 60 and 70 DAP. The results of the evaluation of the role of rhizobacteria as growth promoters in the growth phase of seedlings at 30, 40, 50, 60 and 70 DAP had a very significant effect compared to control treatment, this was evident both in the observation of seed height, stem diameter and number of leaves. Rhizobacteria not only act as biocontrol agents but also act as pgpr, pgpr produced by rhizobacteria can affect root colonization capacity and increase plant growth [40]. PGPR can directly promote plant growth by increasing the uptake of certain nutrients from the environment (such as nitrogen fixation and phosphate mobilization) or the production

of phytohormones, such as indole-3-acetic acid (IAA), gibberellic acid, or phytohormone regulators such as cytokinins. and 1 – amino – cyclopropane – 1 - carboxylate (ACC) deaminase [41,42].

Treatment of rhizobacteria on the percentage of disease severity in cocoa seeds

The results of evaluating the ability of rhizobacterial isolates to control pod rot disease showed that the differences in rhizobacterial isolates given to the seed treatment before planting were followed by the severity of the disease caused in the inoculated seedlings. Seeds derived from seeds treated with rhizobacteria isolates TRI 7/1 and TRI 8/8 resulted in the lowest disease severity compared to seed treatments using other isolates, which was around 20%. Meanwhile, the seeds treated using isolates TRI 4/10, GM 8/1, TRI 3/4, and isolates TRI 6/6 showed a severity level of 33.33% -46.67%. Meanwhile, other indigenous rhizobacteria isolates showed disease severity above 66.66% (Figure 1).

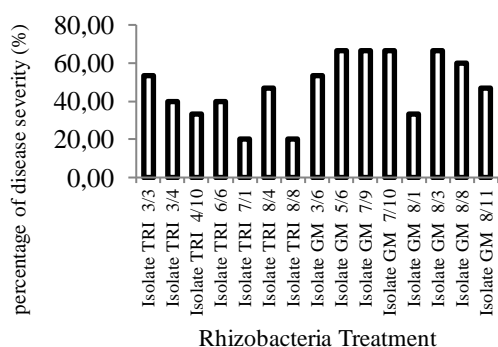


Figure 1. Percentage of disease severity for various rhizobacterial treatments on cocoa seedlings

The low severity of disease in cocoa seedlings was due to the fact that pre-planting seed treatment using rhizobacterial isolates TRI 7/1 and TRI 8/1 had been able to induce systemic resistance. One of the abilities of rhizobacteria is to induce plant systemic resistance. Biocontrol agent rhizobacteria have been reported to induce plant systemic resistance to disease-causing pathogens, which in turn increases the yield resulting from long-term disease control [17,44].

CONCLUSION

In summary, there were rhizobacteria isolates TRI 7/1, TRI 8/8, GM 7/9 and GM 7/10 has the highest inhibition of *P. palmivora*. isolates TRI 7/1 and TRI 8/8 has the lower disease severity rate (20%), isolates GM 3/6, GM 5/6, GM 7/9 and GM 8/8 produced high amounts of IAA

(1,578-1,672 μ / ml filtrate), isolates GM 5/6, GM 7/9 and GM 8/8 has very high peroxidase enzyme activity. Isolates TRI 3/3, TRI 4/10 and TRI GM 8/11 had the highest production of HCN activity. All rhizobacterial isolates increased viability and vigor of cacao germination compared with the control. The rhizobacteria treatments using isolates TRI 7/1, TRI 8/8, GM 7/9 and GM 7/10 were able to increase plant height, stem diameter and number of leaves at 30, 40, 50, 60, and 70 DAP compared to control treatment.

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