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Mechanism of Indigenous Rhizobacteria Isolate Growth Inhibition of *Ganoderma boninense*

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Abstract— *Basal stem rot (Ganoderma boninense) is the main disease of oil palm. Previous research results obtained 7 isolates of rhizobacteria indigenous which have the best ability to control G. boninense in oil palm seedlings and increase growth. The purpose of this study was to characterize the ability of PGPR and biocontrol of indigenous rhizobacteria isolates and inhibitory testing of G. boninense in vitro. Characterization methods include (Phosphate solubility, siderophore production, chitinase activity, hemolytic activity and dual culture test). The results showed four isolates of RZ1A 2.1, RZ2E 2.1, RZ1E 2.1, and RZ2B 1.1 were able to dissolve phosphate. only isolate RZ2B 1.1 produced siderophore. Two isolates produced chitinase RZ1E 2.1 and RZ2E 2.1. All isolates did not produce hemolysis. The best three isolates were obtained from RZ1E 2.1, RZ2E 2.1 and RZ2E 1.2 which have inhibitory properties against G.boninense*.

Keywords— **characterization, indigenous rhizobacteria, *G. boninense.***

I. INTRODUCTION

Oil palm (Elaeis guineensis Jacq.) is a monocotyledon from the family Arecaceae (formerly Palmae) within the subfamily Cocosoideae It is a major crop that grows in the tropical areas mainly in Southeast Asia. Palm oil is used worldwide for processing food, cosmetics, phar- maceuticals, biodiesel and in oleo chemical industry The high economic value and its role in the plantation sector has caused the oil palm commodity to be widely cultivated in various regions in Indonesia. The national productivity of oil palm is 3.6 tons / ha / year (Direktorat Jendral Perkebunan, 2017). The productivity is still very low when compared to the optimal productivity of oil palm which should be able to reach 7 - 8 tons / ha / year (Fauzi *et al*., 2008).

One of the low productivity of oil palm plants is caused by plant pathogen attack. Basal Stem Rot (BSR) caused by *Ganoderma boninense* is a major disease in oil palm plantations in Southeast Asia including Indonesia (Chong *et al*., 2011). This disease is reported to cause losses of around 50-80% per ha Indonesia and Malaysia are the countries with the largest losses due to BSR estimated at 500 million USD / year (Rees, Flood, Hasan, Wills, & Cooper, 2012). BSR is difficult to control, because *G. boninense* is a soil-borne pathogen that has a wide range of hosts and has a special structure in the form of klamidospora and pseudosclerotia that have an impact on the ability to survive and infect target plant (Sanderson, 2005; Susanto *et al.*, 2013).

Control efforts that have been made to *G. boninense* namely physical control through sanitation techniques, and using synthetic functions have not shown maximum results because they can cause negative impacts on the environment such as the killing of non-pathogenic organisms, causing human health problems, animals, and the occurrence of resistance to pathogens (Puspita *et al*., 2013). Alternative control of *G. boninense* that is safe for the environment is to use biocontrol agents from groups of microorganisms (Bivi *et al.,* 2010). Microorganisms that have been widely reported as biocontrol agents are rizobacteria from the group Plant Growth Promothing Rhizobacteria (PGPR) (Beneduzi *et al*., 2012).

Rhizobacteria are a group of bacteria that can improve the quality of plant growth both directly and indirectly (Vishwakarma *et al.,* 2018). Rhizobacteria exert antagonistic effects on plant pathogens in several ways, namely siderophore production, chitinase enzymes, parasitism, competition for nutritional sources, inducing systemic plant resistance and being able to dissolve phosphates which can increase the availability of nutrients for plant growth (Verma *et al.*, 2010). Indigenous bacteria are better introduced to plants, because indigenous bacteria are more adaptable to the environment and more competitive than non-indigenous bacteria (Burelle *et al.*, 2006:Yanti *et al*., 2013).

Control of soil borne pathogens with rhizobacteria has been reported to be effective, some rhizobacteria that have been reported to have the ability as

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biocontrol agents against *G. boninense* include *Pseudomonas*, *Bacillus* spp, and *Burkholderia* (Suryanto *et al*., 2012; Buana *et al*., 2014). *Achromobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Ochrobactrum* and Providencia genera were able to be both non-pathogenic and possessed the ability to colonize the host plant (Gowtham *et al*., 2018).

Previous research (Rifai, 2018) obtained 7 indigenous rhizobacteria isolates isolated from the roots of PTPN IV oil palm plantations in Simalungun Regency as a result of in planta selection has the best ability to increase growth and resistance of oil palm seedlings to BSR disease. These isolates still need to be characterized for their ability to control BSR disease caused by *G. boninense*. This study aims to characterize the ability of PGPR and biocontrol of indigenous rhizobacteria isolates and inhibitory testing of *G. boninense* in vitro.

II. MATERIALS AND METHODS

This research has been done as an experiment at Laboratory of Microbiology, Department of Plant Protection, Faculty of Agriculture, and Biomedical Laboratory Faculty of Medicine University of Andalas, Padang, Indonesia from Januari to April 2019.

In Vitro Characterization of PGPR and Biocontrol of Rhizobacteria Isolates

a. Phosphate solubilization

The isolates' ability to solubilize tri-calcium phosphate was assayed using methods of Wahyudi *et al.*, (2011). The isolates was inoculated to Pikovskaya's Agar (Compositons per litre glucose 10g, Ca3 (PO4)2 5g, (NH4)2SO4 0.5g, KCl 0.2g,

b. Siderophore Production

Siderophore productions was determined using Chrome Azurol Sulphonate (CAS) agar medium described by (Husen, 2003). Each isolate was streaked on the surface of CAS agar medium and incubated at room temperature for 3 days. Siderophore production was indicated by orange halos around the colonies after the incubation.

c. Chitinase Test

Testing is done by dipping a 6 mm filter paper in indigenos rhizobacteria isolate suspension, then filter paper is placed on the surface of the media so that chitin is solid (15 g Bacto agar, 5 g glucose, 2 g peptone, 10 g colloidal chitin, 0.5 g K2HPO4, 0.5 g MgSO4, 0.5 g NaCl in 1 liter of distilled water (Cattelan *et al*., 1996).

d. Hemolytic assay

Hemolytic activity was determined using agar diffusion technique by Monteiro *et al.*, (2005) namely by using Blood Agar (TSA enriched with 5% of sheep blood pH of 7.3) where halo zone (hemolysis) around the colony observed as hemolytic activity.

e. Antagonist in vitro test

Rhizobacteria inhibition testing of *G.boninense* was carried out by the dual culture method by cutting *G.boninense* on a solid 5 mm diameter PDA using cork borer and placed on petridish containing mixed media (NA: PDA). Calculation of the radius of the colony was carried out at 7 days, until the Petri dish in the control treatment was fulfilled by *G. boninense*. The percentage of growth suppression of *G. boninense* is calculated by (Bivi *et al.*, 2010)**.**

$$\text{PIRG} = \frac{R1 - R2}{R1} \; x\,100\%$$

Where

R1: radius of the *G. boninense* colony in the direction towards the antagonist colony

R2: radius growth of *G. boninense* in the control plate

III. RESULT

Characteristics of PGPR and Biocontrol Indigenous Rhizobacteria Isolates

Rhizobacteria isolates of indigenous oil palm roots are character to determine their ability as Plant Growth Promothing Rhizobacteria (PGPR) and biocontrol in vitro. In this study, four rhizobacteria isolates of RZ1A 2.1, RZ2E 2.1, RZ1E 2.1, and RZ2B 1.1 were able to dissolve phosphate (table 1). This is indicated by the presence of clear zones produced by rhizobacteria isolates in *Pikovskaya agar* medium (Figure 1A). In this study, isolates that were able to dissolve adhesives were the *Bacillus* spp group. The *Bacillus* spp was group reported to have an advantage over other types of bacteria because it can excrete organic acids, such as formic acid, acetate and lactate which function to dissolve phosphate forms that are difficult to dissolve into forms available to plants so as to increase plant growth (Khan *et al.*, 2009; Mehrab *et al.*, 2010). *Bacillus* and *Arthrobacter* have ability solubilized phosphate (Vanissa *et al*., 2018).

Siderophore production showed that only isolate RZ2B 1.1 was able to produce siderophore with an orange zone around the disc paper on CAS media (Figure 1B). Siderophore production by rhizobacteria is one of the characters and a direct mechanism in suppressing the growth of pathogenic fungi. Siderophore directly stimulates the biosynthesis of antimicrobial compounds for the availability of minerals for bacteria that will suppress the growth of pathogens of *R. solani* and *F. oxysporum* which will induce the resistance of host plants (Wahyudi *et al.*, 2011). The ability of bacteria to produce siderophore also an important component in PGPR, because

siderophore are able to bind iron (Fe3 +) into siderophor-iron bonds that become available to plant (Prihatiningsih *et al*., 2017; Ferreira *et al*., 2019). Bacterial siderophores have a higher affinity for Fe than phytosiderophores and are able to remove Fe from Fe3+-phytosiderophore complexes (Aguado-Santacruz et al., 2012).

Testing the activity of indigenous rizhobacteria chitinase isolates obtained two isolates of RZ1E 2.1 and RZ2C 2.1 (Table 2). Isolates are able to produce chitinase enzymes with clear zones on chitin agar media (Figure 1C). Chitinase enzymes produced by indigenous rhizobacteria isolates play a role in the degradation or lysis of chitin which is the structure of *G. boninense* fungal cell wall. In line with the research of Wibowo *et al.*, (2017) which stated that 3 of 63 isolates of chitinase producing bacteria isolated from oil palm plantations showed three isolates TB04-05, SW0111, and SW02-08 were able to suppress the growth of *G. boninense* fungi. Azizah *et al*., (2015) also stated that *Serratia marcescens* KAHN 15.12 and *B. amyloliquefaciens* SAHA 12.07 are able to produce chitinase so that it can suppress the development of *G. boninense* fungal hyphae. Hemolysis activity showed that all isolates of negative activity did not form a hemilysis zone (table 1), meaning that it was not pathogenic in humans and animals, so it was safe to be used as a candidate for biologycal control agents (Figure 1D) Figueroa-Lopez *et al*., (2016) stated that pathogenic bacteria able to produce hemolysis so that it can cause disease for humans

Antagonist in vitro test

In vitro inhibition testing of rhizobacteria isolates against *G.boninense* in dual culture test showed six isolates were able to inhibit the growth of *G. boniense* with various inhibitory percentages at 7 days after inoculation. Three best rhizobacteria isolates were obtained, namely isolates RZ1E 2.1, RZ2E 2.1 and RZ2E 1.2.

Isolate RZ1E 2.1 has a high percentage of inhibition on the growth of *G. boninense* (Figure 2). Inhibition of growth of *G. boniense* with rhizobacteria in a dual culture test is thought to be influenced by the ability of rhizobacteria isolates to produce secondary metabolite compounds that can inhibit the growth of antifungal *G. boninense* fungi which is antifungal in accordance with the reported research (Bivi *et al.*, 2010; Suryanto *et al*., 2012; Buana *et al*., 2014).

The mechanism responsible for the biocontrol activity of plant pathogenic fungi is through the production of antifungal compounds (Lee *et al*., 2015). The results of research Bakhtiar *et al.*, (2012) reported that *B. subtilis* B10 isolated from oil palm roots was able to produce active compounds that are antifungal in order to suppress the growth of *G. boninense* in vitro. is antifungal so that it can suppress the growth of *G. boninense* in vitro. Other research results Parvin *et al.*, (2016) confirmed that *Pseudomonas aeruginosa* isolated from palm oil rhizosphere produces metabolite products such as phenazine so that it can inhibit the growth of *G. boninense. P. aeruginosa* were able to inhibit G. boninense growth with the percentage of inhibition radial growth (PIRG) values of 71.42% (Muniroh *et al*., 2019).

IV. FIGURES AND TABLES

Table 1. Phosphate solubility, siderophore production, chitinase activity, and hemolytic activity of indigenous rhizobacteria isolates

Isolates	Phosphate solubility	Siderophore Production	Chitinase activity	Hemolytic activity
RZ1A 2.1	+	-	-	-
RZ2E 2.1	+	-	+	-
RZ1E 2.1	+	-	+	-
RZ2E 1.2	-	-	-	-
RZ2C 2.1	-	-	-	-
RZ2B 1.1	+	+	-	-
RZIE 1.2	-	-	-	-

Table 2. Percentage of inhibition ratio from rhizobacteria isolates against G.boninense growth in vitro

Isolates	**PIRG (%)**
RZ1E 2.1	70.22
RZ2E 2.1	62.00
RZ2E 1.2	45.50
RZ2C 1.2	40.56
RZ2B 1.1	40.00
RZ1A 2.1	35.00
RZ1E 1.2	0
Control	0

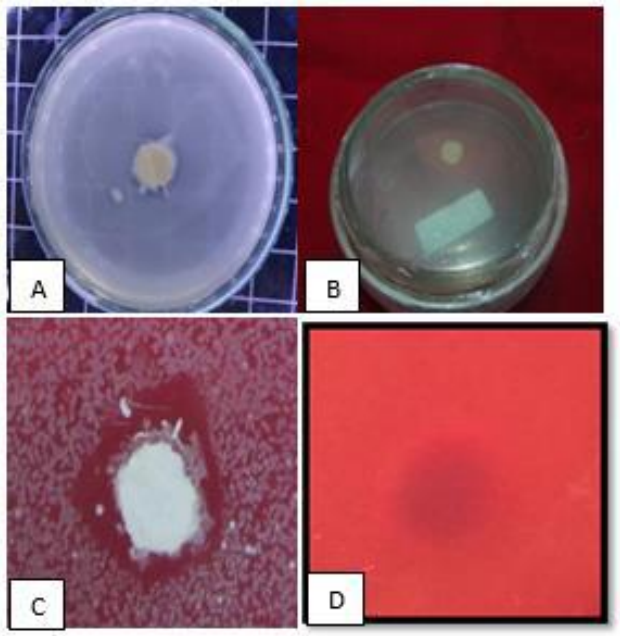

Fig.1: Character of indigenous rhizobacteria isolates (A) phosphate solubility (B) siderophore production (C) chitinase activity (D) hemolytic activity.

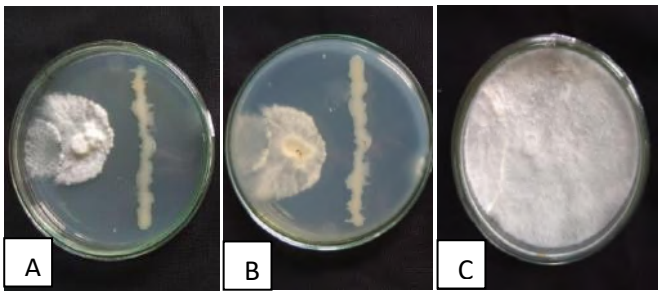

Fig.2: Effect of rhizobacteria isolates on the radial growth of G. boninense in the dual culture test (after 7 days of incubation) (A) Top, (B) bottom side of isolate RZ1E 2.1 and (C) G. boninense in control plate.

V. CONCLUSION

The results showed four isolates of RZ1A 2.1, RZ2E 2.1, RZ1E 2.1, and RZ2B 1.1 were able to dissolve phosphate. only isolate RZ2B 1.1 produced siderophore. Two isolates produced chitinase RZ1E 2.1 and RZ2E 2.1. All isolates did not produce hemolysis The best three isolates were obtained from RZ1E 2.1, RZ2E 2.1 and RZ2E 1.2 which have inhibitory properties against *G.boninense*

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