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Potential of salicylic acid rhizobacteria indigenous chili which is able to suppress Bacterial

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Abstract**—***Plant growth promoting rhizobacteria are a group of bacteria that actively colonize plant roots, increase plant growth and control plant pathogens. Some strains of rhizobacteria can produce salicylic acid and are responsible for the induction of ISR in plants. Salicylic acid is widely recognized as the key to plant resistance. This study aims to analyze the potential of salicylic acid from indigenous rhizobacteria isolates from the roots of chili plants. Rhizobacteria isolates were isolated from the roots of healthy chili plants and endemic bacterial wilt disease. The isolation results were tested in planta for the ability to control bacterial wilt disease (Ralstonia solanacearum). Salicylic acid levels were analyzed using the HPLC method. Results of the test twenty chili ingenus rizobakteria isolates were able to suppress the attack of bacterial wilt. The analysis showed that only nine of the twenty isolates contained salicylic RZ.1.1.AG4, RZ.1.1AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP3, RZ.2.1.AP4, dan RZ.2.2.AG2. Eleven other isolates namely namely RZ.1.3.AP1, RZ.1.4AG4, RZ1.4.AP4, RZ.2.1.AP2, RZ.1.5.AP4, RZ.2.3AG4, RZ.2.1.AG3, RZ.2.2.AG4, RZ.2.5.AP4, RZ.2.1.AG2 and RZ.2.3.AP1 do not produce salicylic acid. The highest concentration of salicylic acid from isolate RZ.1.1.AG4 at 20,95 ppm followed by RZ.1.1AP1 at 19.27 ppm, RZ.1.2.AP1 at 18.05 ppm, RZ.1.3.AG4 at 16.96 ppm, RZ.2.1.AG1 at 15.45, RZ.2.1.AP1 at 15.25, RZ.2.1.AP3 at 14.28, RZ.2.1.AP4 at 14.09, and RZ.2.2.AG2 at 13.45 ppm.*

Keywords**—** ***bacterial wilt disease, HPLC, rhizobacteria, salicylic acid.***

I. INTRODUCTION

Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most dangerous plant diseases that is widespread in the tropics and subtropics, and attacks many agricultural crops including tomatoes, peanuts, bananas, potatoes, tobacco and other *Solanaceae* tribes (Persley, 1985). Attack R. solanacearum on chilies can cause losses ranging from 0.8-10% (Simanjuntak *et al.*, 2014). Bacterial wilt disease is difficult to control because these bacteria are classified as soil-borne pathogens and have a wide host range, high genetic diversity (Suryadi and Machmud, 2002). Control *R. solanacearum* which have been recommended include the use of pathogen-free soil, crop rotation with resistant crops and non-host plants (Gnanamanickam, 2006), and the use of bactericides such as streptomycin (Rahaju and Sucahyono, 2000). However, these control techniques are still ineffective. As an alternative to control Xag is with microorganisms as biocontrol agents (Manuela, *et al.*, 1997), like a group Plant growth promoting rhizobacteria (PGPR). PGPR is a heterogeneous group of bacteria in the rhizosphere complex, on the root surface (rizoplan) and associated in the root (endophyte) (Soesanto, 2008)

The plant rhizosphere is home to various species of bacteria which are generally known as rhizobacteria. Microorganisms that have been widely reported to be capable of being biocontrol agents are the group plant growth promoting rhizobacteria (plant growth promoting rhizobacteria) known as PGPR. PGPR has the ability to antagonize plant pathogens in several ways, namely the production of antibiotics, siderophores, chitinase enzymes, β-1,3-glucanase, cyanide, parasitism, competition for nutritional sources and ecological niches and induces systemic plant resistance (Fernando *et al*., 2005). Besides

being able to control plant pathogens, PGPR is also a group of bacteria that can improve the quality of plant growth either directly or indirect (Joseph *et al*., 2007). PGPR is capable of producing or changing the concentration of plant hormones such as indolasetic acid (indoleasetic acid = IAA), gibberellic acid, cytokinins, and ethylene or their precursors (1-aminocyclopropene 1 carboxylate deaminase (ACC deaminase)) in plants, are not symbiotic in N2 fixation and dissolve phosphate. Rhizobacterial isolates can function as growth promoters or plants Plant Growth Promoting Rhizobacteria (PGPR) and as an antagonist against plant pathogens (Timmusk, 2003).

PGPR is a heterogeneous group of bacteria found in the rhizosphere complex on the root surface and associated in the roots, which can improve the quality of plant growth directly or indirectly (Joseph *et al*., 2007). The application of PGPR to the rhizosphere is closely related to its ability to colonize plant roots. Due to the activeness of root colonization, roots absorb microbial products which directly affect root growth and physiology, in addition to influencing pathogen invasion (Soesanto, 2008). Colonization by PGPR can occur through seed cloaking or the addition of a PGPR suspension into the soil at the time of planting (Kloepper and Tuzun, 1996). Rhizobacteria can be applied through seed covering, soaking the seeds in suspension, and watering the PGPR suspension into the soil (Widodo, 2007). The ability of biological agents to control pathogens when applied in the field depends on factors, among others (1) The host plant must provide a suitable environmental niche and dissolved and evaporated nutrients from root, seed, flower, and leaf exudates required for the production of antibiotics for RB, (2) Dry environmental conditions, and (3) The plant surface is not covered by a hydrophobic wax layer (Soesanto, 2008).

Salicylic acid (SA) has an important role in plant defense signaling networks (Pieterse *et al*., 2012). Salicylic acid in general can be known to play a role in plant resistance to disease, including basal resistance, effects trigger immunity and induction of systemic resistance (Induce Systemic Resiatance = ISR) (Vlot *et al*, 2009). In general, it can be stated that biotropic pathogens are more sensitive to the induction of salicylic acid defense, whereas herbivorous pathogens and insects are necrotrophic by the induction of jasmona acid defense. Other growth regulating hormones, such as ethylene, abscisic acid, gibberellin, auxins, cytokinins, and brassinosteroids, are also involved in the regulation of network signaling immune or plant resistance (Pieterse *et al*, 2012), this activity shows that the regulators of plant growth and defense are closely related to signal transduction (Rasmunsoll., *et al* 2013)

This research has analyzed the potential of indigenic rhizobacteria of chili roots to produce salicylic acid from soybean plants. Indigenous rhizobacteria isolates are selected isolates that have been tested for their ability to suppress attacks (*Ralstonia solanacearum*) causes bacterial wilt disease in chili plants. The research objective was to analyze the potential of indigenic rhizobacteria isolates of chili roots in producing salicylic acid in plants.

II. METHOD OF RESEARCH

2.1 Time and Location of Research, and Sampling Method

The research was conducted at the Laboratory of Plant Bacteriology, Department of Plant Pests and Diseases, Greenhouse, Faculty of Agriculture, Andalas University, Laboratory of Natural Material Chemistry, Faculty of Pharmacy, Andalas University. The research took place from July to September 2020.

2.2 Ability Test of Rhizobacteria Isolates to Control Bacterial Wilt Disease in Planta

Selection is carried out in planta using a completely randomized design (CRD). The treatment used was the introduction of 42 RBI isolates on chili seeds with 3 replications. Rhizobacteria isolates introduced to chili and control plants. The data were analyzed by using variance, if they were significantly different, it was followed by testing Least Significance Difference (LSD) at the 5% real level.Root samples of chili plants from endemic areas of bacterial wilt disease (Nagari Sungai Nanam, Lembah Gumanti District, Solok District and Nagari Taluak IV Suku, Banuhampu District Agam Regency), with criteria: 2-3 months old, fruitful and healthy. RBI was isolated by serial dilution, 1 g of roots was washed with distilled water, sterilized the surface with 70% alcohol for 1 minute, rinsed again with sterile distilled water and macerated. The root suspension is diluted to 10- 6, 0.1 ml suspension from a 10- dilution 5 and 10- 6 cultured on Nutrient Agar (NA) media and incubated at room temperature for 48 hours. Indigenous rhizobacteria isolates were selected with the following criteria: dominant colonies with different morphologies, purified on the media and incubated in the same way. Single colonies of indigenous rhizobacterial isolates were transferred inside microtube contains 1 ml of sterile distilled water and stored in the refrigerator.

Indigenus rhizobacteria isolates were rejuvenated on NA medium and incubated for 48 hours. For

introduction to seeds, indigenous rhizobacterial isolates were reproduced on the media in the same way as rejuvenation. Indigenous rhizobacterial cultures were suspended with sterile distilled water, the population density was determined by comparison with the solution McFarland scale 8 (bacterial population 10 8 sel / ml) (Yanti and Resti, 2010). For introduction to plants, indigenous rhizobacteria are reproduced in Nutrient Broth (NB). To preculture, 1 indigenous endophytic bacterial colony was transferred to 25 ml NB medium and incubated 18 hours on rotary shaker horizontal at 150 rpm. To mainculture, 1 ml of rhizobacterial suspension indigenus from preculture transferred into 150 ml NB and incubated for 48 hours in the same way (Trisno, 2010). Indigenous rhizobacteria suspension from mainculture population in the same way as seeds.

The planting medium is a mixture of soil and sterile manure (2: 1 v / v). The chili seeds used are varieties from the same location as the source of the indigenous rhizobacteria isolates. The germination of the seeds was tested by method Standard Germination Test (96%). Chili was introduced with rhizobacteria isolates indigenus twice, namely: 1). In the seeds, the seeds were sterilized with 1% NaOCl, washed with sterile distilled water and dried. The seeds were immersed in a suspension of rhizobacterial isolates indigenus 10 minutes and planted in polybags. 2) For chilies 3 weeks after planting (mst), by pouring 10 ml of rhizobacterial suspension on the soil. Chili plants Inoculation of bacteria was carried out by inserting a bacterial suspension with a bacterial population density of 106 CFU / mL using an inoculation needle at the base of the 35 day old chili seedling. The variables observed were the development of bacterial pustules (incubation period, incidence and severity of leaves). The percentage of disease detection is determined by a formula:

$$E = K - P / K \times 100\%$$

where: E = percentage of emphasis, P = treatment, K = control

2.3 Production of salicylic acid in plants introduced by rhizobacteria

Chili plants (roots) that showed a resistant reaction to bacterial wilt were tracked for theirv salicylic acid levels using the method (Chen et al, 1995) which is modified. Comparators were used as well as controls (without rhizobacteria). 1 g of chili soybean root homogenized with liquid nitrogen in a culture tube measuring 10 x 130 mm, the frozen tissue was washed with 2.5 ml 90% metaol, sonicated for 20 minutes and centrifuged at 2800 g. The pellets were extracted again with 2 ml 100% methanol. The supernatant from the extraction was dried using a liquid nitrogen stream, the residue was suspended in 2 ml 5% Trichlor acetid acid (TCA) and centrifuged at 2500 g for 15 minutes. The supernatant was separated twice with the extraction medium (ethyl acetate: cyclopentane: isopropanol with a ratio of 100: 99: 1). The upper phase is combined and dried with liquid nitrogen. The residue was suspended in 1 ml 20% methanol in 20 mM sodium acetate buffer solution, the solution was filtered using a nylon membrane with a pore of 0.2 μm through a vacuum under pressure of 250 mm Hg. Samples were stored at -20 0 C, and ready to be analyzed by HPLC. The salicylic acid extract was measured by a spectrophotometer and its spectrophotometer was compared with standard salicylic acid (Sigma).

III. INDENTATIONS AND EQUATIONS

46 isolates of ingenus rhizobacteria from chili roots were obtained. Indogenous endophytic bacteria isolates of various colony morphologies. The 46 isolates were tested in planta to see the ability of each isolate in suppressing the attack of bacterial pustules in the greenhouse. Symptoms of an attack *R. solanacearum* characterized by early symptoms in the form of wilting in chili plants starting from the top leaves. Advanced symptoms are characterized by wilting of all plant leaves. Chili plants that were introduced with RBI isolates showed a suppression of the incubation period R. solanacearum which differed significantly among all treatments. The incubation period of bacterial wilt disease in the treatment of each RBI isolate varied between 11.33 days to 27.33 days after inoculation (hsi) compared to negative controls, namely 12.67 his (Table 1). From the observations, there were 13 RBI isolates that did not show any symptoms until the end of the observation (42 dd) with effectiveness 233.33% compared to negative controls. The isolates were RZ.1.1.AG4, RZ.1.1.AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.1.3.AP1, RZ.1.4.AG4, RZ.1.4.AP4, RZ.2.1.AG1 , RZ.2.1.AP1, RZ.2.1.AP2, RZ.2.1.AP3, RZ.2.1.AP4 and RZ.2.2.AG2.

Chili plants introduced with RBI isolates showed a decrease in the incidence of bacterial wilt disease (Table 1). The introduction of RZ.1.5.AP4 isolates and RZ.2.3.AG4 isolates can reduce disease incidence by up to 67% with an effectiveness of 33%. RBI isolates that are able to reduce the incubation period *R. solanacearum* able to reduce the incidence of disease up to 0% with 100% effectiveness (RZ.1.1.AG4, RZ.1.1.AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.1.3.AP1, RZ.1.4.AG4, RZ. 1.4.AP4,

RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP2, RZ.2.1.AP3, RZ.2.1.AP4 and RZ.2.2.AG2). The introduction of RBI isolates can reduce the severity of bacterial wilt disease in chili plants (Table 1). The disease severity level of each isolate can be seen in Table 4. Isolates RZ.2.3.AG4 and RZ.1.5.AP4 showed disease suppression with an effectiveness of 33%. RBI isolates that were able to suppress the incubation period and disease incidence were the best (isolates RZ.1.1.AG4, RZ.1.1.AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.1.3.AP1, RZ.1.4. AG4, RZ.1.4.AP4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP2, RZ.2.1.AP3, RZ.2.1.AP4 and RZ.2.2.AG2) also did not show an emphasis on severity. disease up to a scale of 0 (no symptoms) until the end of the observation with 100% effectiveness and was able to reduce the severity of the disease until the end of the observation (42 dd).

The results of this study are in line with Sutariati's research et al. (2006), that is reported the use of PGPR was able to trigger seed viability and growth of chili plant seeds. The results of the study (Rahni, 2012) also showed an increase in growth in maize and sorghum (Sutariati et al., 2011) and Jatropha (Wibowo, 2010). The ability of rhizobacterial isolates to increase plant growth has been widely reported (Taufik et al., 2005). Currently, rhizobacteria are increasingly being developed, especially in an effort to increase horticultural production and improve the quality of the environment. Rhizobacteria are ideal for use as biocontrol agents because rhizobacteria can serve as the front line defense for plant roots against various pathogens (Pal & Jalali, 1998).

The use of RBI to increase emphasis on pathogen development has been developed. RBI isolates that have been reported to be effective in increasing plant resistance include Isolate P12Rz2.1. and P14Rz1 isolate was able to control bacterial pustules (Yanti *et al*. 2013), ST26c isolate was able to suppress Phytophthora capsici in chili plants (Khaeruni *et al*., 2011), RB.2.4 isolate was able to reduce the incidence and severity of wilt disease fusarium on tomatoes (Khaeruni *et al*. 2013), PKLK5 and P11a isolates were able to suppress Xanthomonas oryzae pv. oryzae in rice plants (Khaeruni *et al*, 2014b), CRb-26, CRb-39, CRb-17, CRb-9 and CRb-14 isolates were able to increase germination, growth and suppress blight in cotton (Mondal *et al*., 1999) and isolates S188, s215 and s288 were able to control R. solanacearum on the tomato plant (Ramadasappa *et al*., 2012).

The ability of 20 rizobacteria isolates to produce salicylic acid and the resulting salicylic acid concentration can be seen in Table 2. nine of the twenty isolates tested were able to produce salicylic acid and the other eleven isolates did not produce salicylic acid Table 2. The isolates were RZ.1.1.AG4, RZ.1.1AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP3, RZ.2.1.AP4, and RZ.2.2.AG2 were shown with electrophoregram peaks at relatively similar migration times with standard salicylic acid. The other four isolates, namely RZ.1.3.AP1, RZ.1.4AG4, RZ1.4.AP4, RZ.2.1.AP2, RZ.1.5.AP4, RZ.2.3AG4, RZ.2.1.AG3, RZ.2.2.AG4, RZ.2.5.AP4, RZ.2.1.AG2 and RZ.2.3.AP1 were unable to produce salicylic acid.

The existence of isolates that were successfully analyzed for their salicylic acid content proved that indigenous rhizobacteria of chili roots were able to produce salicylic acid (Table 2). Isolate RZ.1.1.AG4 at 20,95 ppm followed by RZ.1.1AP1 at 19.27 ppm, RZ.1.2.AP1 at 18.05 ppm, RZ.1.3.AG4 at 16.96 ppm, RZ.2.1.AG1 at 15.45, RZ.2.1.AP1 at 15.25, RZ.2.1.AP3 at 14.28, RZ.2.1.AP4 at 14.09, dan RZ.2.2.AG2 at 13.45 ppm. The high content of salicylic acid in ST4E1.1 isolates was related to the level of plant resistance to bacterial pustules. According to Silverman *et al.* (1995) that plant resistance has a positive correlation with salicylic acid content. Furthermore, Kloepper & Ryu (2006) stated that the colonization of endophytic bacteria Bacillus pumilus SE34 on Arabidopsis triggering the induction of systemic resistance to *Pseudomonas syringae* pv. *Maculicola* that associated with the salicylic acid pathway. Salicylic acid production has an important role in the transduction signal pathway that triggers the induction of systemic resistance and is associated with the accumulation of PR proteins (pathogenesis-related), like PR1 (Lyon 2007). Salicylic acid production by P. aeruginosa 7NSK2 plays an important role in the induction of resistance *Botrytis cinerea*. Mutants of the same strains that do not produce salicylic acid have lost their ability to induce systemic resistance in green beans (De Meyer and Hofte, 1997). *P. fluorescens* Natural CHAO produces salicylic acid under iron-limited conditions and also induces ISR in tobacco against TMV (Tobacco Mosaid Virus).

IV. FIGURES AND TABLES

Table 1. Incubation period of bacterial wilt disease introduced with several isolates RBI

Isolate	Period Incubation (hsi)	Effectiveness (%)	disease Incident	Effectiveness (%)	Severity	Effectiveness (%)
RZ.1.1.AG4	42.00*a	233.33	0	100	0 c	100
RZ.1.1.AP1	42.00*a	233.33	0	100	0 c	100
RZ.1.2.AP1	42.00*a	233.33	0	100	0 c	100
RZ.1.3.AG4	42.00*a	233.33	0	100	0 c	100
RZ.1.3.AP1	42.00*a	233.33	0	100	0 c	100
RZ.1.4.AG4	42.00*a	233.33	0	100	0 c	100
RZ.1.4.AP4	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AG1	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP1	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP2	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP3	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP4	42.00*a	233.33	0	100	0 c	100
RZ.2.2.AG2	42.00*a	233.33	0	100	0 c	100
RZ.1.5.AP4	27.33b	116.93	67	33	2 b	33
RZ.2.3.AG4	26.33b	108.99	67	33	2 b	33
RZ.2.1.AG3	21.67** b	71.96	100	0	3 a	0
RZ.2.2.AG4	21.00**b	66.67	100	0	3 a	0
RZ.2.5.AP4	20.33**bc	61.38	100	0	3 a	0
RZ.2.1.AG2	19.67**bc	56.08	100	0	3 a	0
RZ.2.3.AP1	11.33**d	0.00	100	0	3 a	0
Kontrol-	12.67**cd	100	0		3 a	

* Figures followed by the same lowercase letter in the same row are not significantly different according to LSD at the 5% level.

* The incubation period of 42 days showed no symptoms until the last observation day.

* * * All repetitions in the treatment were off so that data analysis was not continued on the parameters further observations of these isolates.

Table 2: Salicylic acid concentration of selected indigenous rhizobacteria isolates suppress bacterial wilt disease in chili plants

Isolate code	Ability produce acid salicylates	Salicylic acid concentration (ppm)
RZ.1.1.AG4	+	20.95
RZ.1.1.AP1	+	19.27
RZ.1.2.AP1	+	18.05
RZ.1.3.AG4	+	16.96
RZ.1.3.AP1	-	-

RZ.1.4.AG4	-	-
RZ.1.4.AP4	-	-
RZ.2.1.AG1	+	15.45
RZ.2.1.AP1	+	15.25
RZ.2.1.AP2	-	-
RZ.2.1.AP3	+	14.28
RZ.2.1.AP4	+	14.09
RZ.2.2.AG2	+	13.45
RZ.1.5.AP4	-	-
RZ.2.3.AG4	-	-
RZ.2.1.AG3	-	-
RZ.2.2.AG4	-	-
RZ.2.5.AP4	-	-
RZ.2.1.AG2	-	-
RZ.2.3.AP1	-	-
Kontrol-	-	-

V. CONCLUSION

Results of the test twenty chili ingenus rizobakteria isolates were able to suppress the attack of bacterial wilt. The analysis showed that only nine of the twenty isolates contained salicylic RZ.1.1.AG4, RZ.1.1AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP3, RZ.2.1.AP4, dan RZ.2.2.AG2. Eleven other isolates namely namely RZ.1.3.AP1, RZ.1.4AG4, RZ1.4.AP4, RZ.2.1.AP2, RZ.1.5.AP4, RZ.2.3AG4, RZ.2.1.AG3, RZ.2.2.AG4, RZ.2.5.AP4, RZ.2.1.AG2 and RZ.2.3.AP1 do not produce salicylic acid. The highest concentration of salicylic acid from isolate RZ.1.1.AG4 at 20,95 ppm followed by RZ.1.1AP1 at 19.27 ppm, RZ.1.2.AP1 at 18.05 ppm, RZ.1.3.AG4 at 16.96 ppm, RZ.2.1.AG1 at 15.45, RZ.2.1.AP1 at 15.25, RZ.2.1.AP3 at 14.28, RZ.2.1.AP4 at 14.09, dan RZ.2.2.AG2 at 13.45 ppm.

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