

Screening of rhizobacteria from rhizosphere of healthy chili to control bacterial wilt disease and to promote growth and yield of chili

YULMIRA YANTI^{1*}, FUJI FEBRIA ASTUTI², TRIMURTI HABAZAR¹, CHAINUR RAHMAN NASUTION³

¹Faculty of Agriculture, Universitas Andalas. Kampus Unand Limau Manih, Padang 25163, West Sumatra, Indonesia. Tel. +62-751-72773, Fax.: +62-751-72702, *email: yy.anthie79@gmail.com

²Faculty of Mathematics and Natural Sciences, Universitas Andalas. Padang 25163, West Sumatra, Indonesia

³Post-graduated Program, Faculty of Agriculture, Universitas Andalas. Padang 25163, West Sumatra, Indonesia

Manuscript received: 12 April 2016. Revision accepted: 1 November 2016.

Abstract. Yanti Y, Astuti FF, Habazar T, Nasution CR. 2016. Screening of rhizobacteria from rhizosphere of healthy chili to control bacterial wilt disease and to promote growth and yield of chili. *Biodiversitas* 17: 1-9. Bacterial wilt on chili cause by *Ralstonia solanacearum*. This disease is important, causing plant death and significant yield losses. Biological control is desirable because control with other methods gives variable results. One of the important group of biological agent is the plant growth promoting rhizobacteria (PGPR). This group can control plant pathogens and enhance the growth and yield through direct and indirect mechanisms. The aim of this research was to obtain indigenous rhizobacterial isolates from rhizosphere of healthy chili, which are effective to control bacterial wilt disease and to promote plant growth as well as to increase plant yields. The rhizobacterial isolates were collected from healthy chili rhizosphere in endemic area of bacterial wilt at Banuhampu Sub-district, Agam District, West Sumatra, Indonesia. Screening method of rhizobacterial isolates to control bacterial wilt was based on in planta technique. This technique was consist of three steps, as follow: (i) Screening of 43 rhizobacterial isolates to induce the hypersensitive reaction on *Mirabilis jalapa*. (ii) Screening of 42 rhizobacterial isolates (from first step) to increase growth of chili seedlings and (iii) Screening of 20 selected rhizobacterial isolates (from second step) to control bacterial wilt on chili. *R. solanacearum* were inoculated on the 6 weeks chili plants using Two strains of rhizobacterial isolates from chili rhizosphere (RZ.2.1.AG1 and RZ.1.3.AP1) showed high potential for disease suppression and also increased growth and yield of chili.

Keywords: chili, indigenous rhizobacteria, in planta technique, *Ralstonia solanacearum*, screening

Abbreviations: BCA = Biocontrol agent, HR = Hypersensitive reaction, IAA = Indole Acetic Acid, IRB = Indigenous Rhizobacteria, ISR = Induce systemic resistance, NA = Nutrient agar, PGPR = Plant Growth Promoting Rhizobacteria, R = Ralstonia, TZC = triphenyl tetrazolium chloride,

INTRODUCTION

Chili pepper (*Capsicum annum* L.) is an important crop due to its large scale consumption as a seasoning vegetable in Indonesia and many other countries. Bacterial wilt caused by *Ralstonia solanacearum* is one of the important serious vascular diseases of chili crop causing maximum crop losses (Basu 2014), with crop losses between 15% to 55% around the world (El-Argawy and Adss 2016). A devastating disease worldwide, bacterial wilt limits the production of solanaceous crops such as tomato, pepper, eggplant, tobacco and potato as well as other important crops like peanut, banana, ginger and geranium. Approximately 450 crop species have been reported as hosts of this pathogen (Swanson et al. 2005).

Control is difficult due to high variability of the pathogen, limited possibility for chemical control, high capacity of the pathogen to survive in diverse environments and its extremely wide host range. Bacterial wilt management practices like applications of synthetic chemicals, field sanitation, crop rotation and the use of selective cultivars have proven to have limited success making it necessary to consider other control measures (Anith et al. 2004). Biological control strategies may either

help development of alternative management measures. Plant growth promoting rhizobacteria (PGPR) are a group of biological agents, that can control plant pathogens and enhance the growth and productivity by exerting beneficial effects through direct and indirect various mechanisms, such as providing the N source for plant through the N fixation, exerting a biological control of soil-borne pathogens as well as producing the plant-stimulating growth substances (phytohormone) (Piromyou et al. 2011). They are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. Most biocontrol research has focused on a limited number of bacterial (*Bacillus*, *Burkholderia*, *Lysobacter*, *Pantoea*, *Pseudomonas*, and *Streptomyces*) (Pal and McSpadden Gardener 2006).

Unfortunately, the interaction between associative PGPR and plants can be unstable. The good results obtained in vitro cannot always be dependably reproduced under field conditions. The lack of correlation between in vitro and in vivo effectiveness of biological control had already been observed by Ran et al. (2005), who reported that fluorescent *Pseudomonas* sometimes succeeded as a biocontrol agent in vitro or under controlled conditions but

failed under pot experiments and field conditions. The same result has been reported by Nguyen and Ranamukhaarachchi (2010) on bacterial wilt disease incidence in capsicum was higher with antagonist strain TR15 (43.8%) and in tomato with strain TR2 (66.7%), and 52.1 and 56.3% respectively, with TR10 and TR7). Thus, these antagonists showed high antagonism in vitro, but were not effective in vivo under greenhouse conditions. To achieve the maximum growth promoting interaction between PGPR and plant it is important to discover how the rhizobacteria exerting their effects on plant and whether the effects are to control plant pathogens or to increase growth and yield. Therefore, it is necessary to develop efficient strains in greenhouse and field conditions. One possible approach is to explore soil microbial diversity for PGPR having combination of PGPR activities and well adapted to particular soil environment (Pal and McSpadden Gardener 2006), as well as rhizosphere of plant or indigenous rhizobacteria (IRB).

Generally to characterize the biological control agents, it has been done primarily through isolation, characterization, and application of individual organisms. The most common step to screen PGPR as biocontrol agents (BCA) to control plant pathogen are in vitro test, molecular identification, and in vivo (in planta) assays, such as toward *Rhizoctonia solani* on groundnut (Ganesan and Sekar 2011); *Xanthomonas oryzae* pv. *oryzae* on rice (Velusamy et al. 2013); *Ralstonia solanacearum* on tomato (Lwini and Ranamukhaarachchi 2006) and on tomato and pepper (Nguyen and Ranamukhaarachchi 2010). Base on this reason, look like that this technique is time consuming and laborious, sometimes the potential rhizobacteria will be lost, especially if the mechanism of biocontrol is induce systemic resistance (ISR) of plant.

Since 2008 we have developed the other methods based on in planta selection of enhanced competitive root-colonizing bacteria from rhizosphere of healthy plants in disease endemic area. This approach focuses on general forms of disease suppression, including direct and indirect mechanisms. We characterized only the best rhizobacterial strains which have ability to control bacterial plant pathogens and to increase growth and yield of plants. This technique has the possibility to find the new, easy and cheap biocontrol organisms (Habazar et al. 2010, 2011). We have used these technique to explore PGPR strain to control some bacterial diseases and to increase of growth and yield of plants, such as *Xanthomonas axonopodis* pv. *allii* on onion (Habazar et al. 2010), *R. solanacearum* race 4 on ginger (Habazar et al. 2011), *Xanthomonas axonopodis* pv. *vesicatoria* on tomato, *Xanthomonas axonopodis* pv. *glycines* on soybean (Yanti et al. 2013).

Although the potential to suppress the pathogenic organisms through biological means has been revealed, sufficient information has not been generated so far to fully support the development of biological control measures against *R. solanacearum* on chili. Therefore the aim of this research was to obtain potential indigenous rhizobacterial isolates from rhizosphere of healthy chili, which are effective to control bacterial wilt disease and to promote plant growth as well as to increase plant yields.

MATERIALS AND METHODS

Study area

This research have been done as laboratory and greenhouse experiment at Laboratory of Microbiology, Department of Plant Protection, and greenhouse, Faculty of Agriculture, Universitas Andalas, Padang, Indonesia during February to September 2015. The study included the screening of potential rhizobacteria and their evaluation in planta.

Procedures

The study consisted of three parts: (i) isolation and multiplication of the bacterial wilt pathogens and potential rhizobacterial BCA, (ii) in planta screening through HR on *Mirabilis jalapa* for pathogenicity test, (iii) in planta screening of selected rhizobacterial isolates (from second's step) to increase growth of chili seedlings, and (iv) in planta evaluation of selected rhizobacterial isolates for the control of bacterial wilt disease on chili.

Isolation of potential rhizobacterial BCA and pathogens

Rhizosphere samples were collected from healthy chili's rhizosphere in endemic area of bacterial wilt in Banuhampu, Agam District, West Sumatra Province, Indonesia (Figure 1.A and B). Bacterial wilt diseased chili were collected also at the same location (Figure 1.C). Soil and plant samples were given identification tags indicating the location, date of collection and type of crop, and were brought to the Microbiological Laboratory at Faculty of Agriculture, Universitas Andalas, Padang, West Sumatra, Indonesia. Samples were stored in a refrigerator (at 5°C) until isolation of rhizobacterial and bacterial wilt pathogens, which was done one day after transport to the laboratory. For root suspensions, 0.1 g aliquots of side-roots of chili plants were homogenized with 1 mL of sterilized tap water and shaken with an electric shaker. From this suspension, a dilution series up to 10⁻⁶ was prepared and 1 mL of root suspension was placed in a Petri dish to which 10 mL of Nutrient agar (NA) (37.5 g of NA in 1 liter DW) were added and stirred well. Petri dishes were allowed to stand for 1 h to allow for solidification of the medium. Plates were placed in for 2 days at room temperature (about 30°C). Thereafter, dominant bacterial colonies were purified on NA medium as suspected BCA (Figure 1.D and E). A single colony of bacteria then transferred aseptically to microtube that contain 1 mL of sterilized aquadest as stock and stored in refrigerator (Figure 1.F).

Ralstonia solanacearum was isolated from diseased plant parts using TZC medium, and incubated at 30°C for 48 h. Pathogenicity was confirmed by dipping chili seedlings in a bacterial suspension, followed by transplanting into pots containing sterile soil and examining for typical disease symptoms (Winstead and Kelman 1952). The high virulence isolates were used in this research (Figure 2).

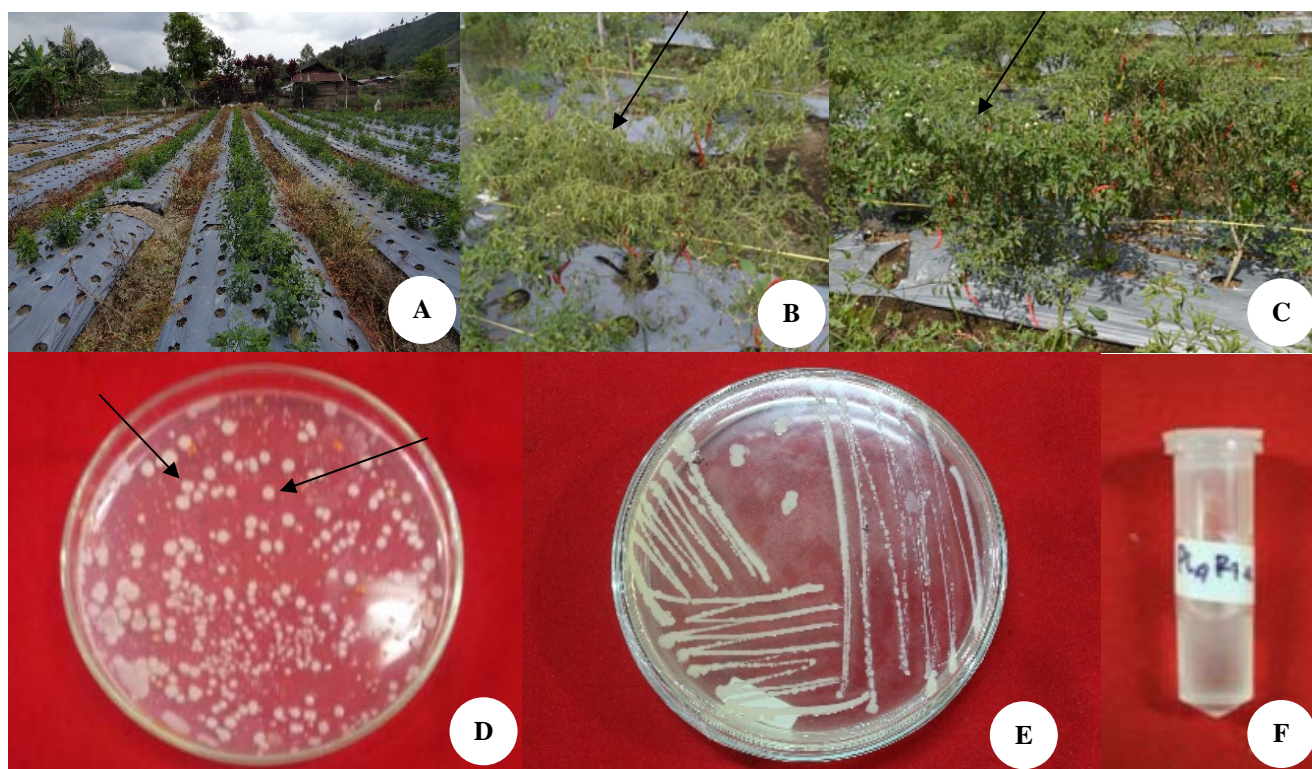


Figure 1. A. Source of rhizobacterial isolate from healthy chili at endemic area of bacterial wilt disease (Banuhampu Sub-district, Agam District, West Sumatra). B. Chili with bacterial wilt symptom. C. Healthy chili. D. Culture of rhizobacterial on NA at 3 days post incubation (dpi). E. Pure culture of rhizobacterial isolates on NA at 3 dpi. F. Stock cultures in aquadest suspension

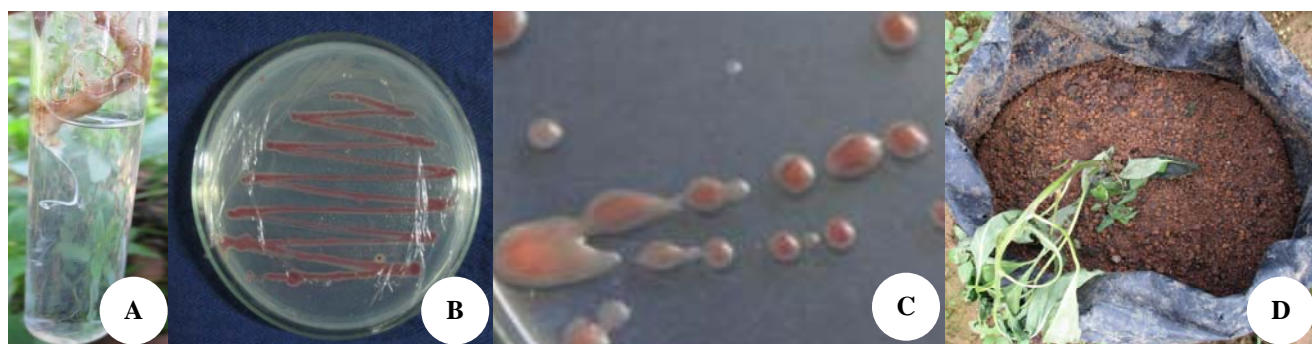


Figure 2. A. Bacterial flux in aquadest from chili root with bacterial wilt symptom. B-C. Pure culture of *R. solanacearum* on TZC (5 dpi). D. Wilted chili after inoculation with *R. solanacearum* (7 dpi)

In planta screening through HR on *Mirabilis jalapa* for pathogenicity test

The rhizobacterial isolates were selected based on pathogenicity test through HR on *Mirabilis jalapa*. The rhizobacterial isolates were cultured on medium NA for two days. The rhizobacterial cultured medium were added 10 mL aquadest and suspended. The bacterial suspensions were determined for bacterial density by comparing with scale 8 of McFarland's solution (approximately 10^8 cell/mL). The bacterial suspensions were infiltrated by injection in leaves tissue of *Mirabilis jalapa*. The HR were observed until 24 hours, the HR positive showing necrotic at infiltrated leaves tissue. Only the HR negative rhizobacterial isolates were used for the next experiment.

In planta screening of selected rhizobacterial isolates to increase growth of chili seedlings

The experiments were conducted in a greenhouse to evaluate the ability of indigenous rhizobacterial isolates to promote growth of chili seedlings. The experiment was used randomized complete design with 43 treatments (42 selected rhizobacterial isolates (from second's step) and control) and six replicates.

Multiplication of selected rhizobacterial isolates

The selected rhizobacterial isolates were cells from stocks were first grown on NA medium to verify their purity. The inoculum was produced by transferring one loopful from the culture to 100 mL of NB in a 250 mL

Erlenmeyer flask and incubating at room temperature ($28\pm 2^{\circ}\text{C}$) on a shaker at 150 rpm for 48 h (preculture). For mainculture was produced by transferring 1 mL from preculture to 50 mL of coconut water in a 250 mL flask and incubating at the same manner as preculture. After 48 h incubation, the broth containing approximate 9×10^8 cfu/mL. The bacterial suspensions were determined for bacterial density by comparing with scale 8 of McFarland's solution (approximately 10^8 cell/mL) (Habazar et al. 2011).

Inoculation of selected rhizobacterial isolates on chili's seed

Seeds of chili were obtained from selected fruit of the best performance of chili plant in the field at Banuhampu Sub-district, Agam District, West Sumatra. Germination test of chili seeds were carried out by the paper towel method. Rhizobacterial isolates were used as seed treatments. For seed treatment, the seeds were initially surface-sterilized with 1% sodium hypochlorite and soaked in a double volume of sterile distilled water and then in bacterial suspension for 15 minutes. The bacterial suspensions were drained off and the seeds were dried under shade for 30 min and planted (Habazar et al. 2014). All the recommended agronomical practices such as irrigation and weeding were conducted to raise the crop. Five plants were selected at random and tagged in each plot for growth and yield observation. Seed germination was estimated 15 days after sowing. The seedlings height and number of leaves were measured at 21 days-old plants.

In planta evaluation of selected rhizobacterial isolates for the control of bacterial wilt disease on chili

The experiments were conducted in a greenhouse to evaluate the ability of selected rhizobacterial isolates to control bacterial wilt on chili. The experiment was used randomized complete design with 22 treatments (20 selected rhizobacterial isolates (from third step), antibiotic and control (without rhizobacterial) and six replicates. The rhizobacterial isolates were inoculated on three weeks old chili seedlings by root dipping technique before transplanting. Plants were grown in greenhouse with humidity and temperature depends on the natural conditions. Suspension of *R. solanacearum* were inoculated on one-month old chili by stem injection (10^8 CFU/mL).

Disease assessments were incubation period, disease incidence, disease severity. To examine the effect of rhizobacteria on the plant growth characteristics were counted plant height, number of leaves, time of the first flower, and fruit weight.

RESULTS AND DISCUSSION

Isolation of potential rhizobacterial BCA

The bacterial diversity in healthy chili rhizosphere showed heterogenous with regard to colony morphology and Gram reaction (Table 1). Isolations acquired 43 different colonies of bacteria. Chili rhizosphere was dominated by Gram negative bacterial population with white, irregular, flat colonies. Only one rhizobacterial isolate RZ.2.2.AG3 showed positive by hypersensitive

reaction. It is mean that this rhizobacterial isolate as plant pathogen, also this isolate was not tested for the next experiment.

In planta screening of selected rhizobacterial isolates to increase growth of chili seedlings

Not all of indigenous rhizobacterial isolates can increase germination rate of chili seeds and seedling's height. Only 16 of indigenous rhizobacterial isolates could increase germination rate of chili seeds increase from 96-100 % compare to control (92 %) (Table 2). The other 9 indigenous rhizobacterial isolates have the same germination rate with control plant (Figure 3) and the rest 18 indigenous rhizobacterial isolates showed lower than control plant. Seedling's height of chili also had increased after inoculation by 18 indigenous rhizobacterial isolates from 4,87-7,13 cm compare to control 4,82 cm. Most of these indigenous rhizobacterial isolates reduced the seedling's height. The best indigenous rhizobacterial isolates to promote growth of chili seedlings were RZ.2.2.AG2, RZ.2.2.AG4, RZ.2.1.AG2 and RZ.1.3.AP1.

In planta evaluation of selected rhizobacterial isolates for the control of bacterial wilt disease and to promote growth and yield of chili

All selected rhizobacterial isolates (from second step experiment) caused a reduction of wilt disease (Table 3). The incubation period of *R. solanacearum*-infested chili treated with rhizobacterial isolates were longer (19.67-27.33 dpi) or without wilted until the end of experiment, 42 dpi) than control (12.67 dpi) (Table 3 and Figure 4). Bacterial wilt disease incidence varied between 0.00-33.00 %, especially on plants, which were introduced with 13 rhizobacterial isolates no wilt (disease incidence and disease severity 0 %). If we compare with plants without rhizobacteria (control) had a disease incidence 100.00 %.

The height of plant after 90 days of transplanting increased significantly with 5 rhizobacterial isolates RZ.2.2.AG2, RZ.2.1.AG1, RZ.1.3.AP1, and RZ.2.1.AP4 as compared to control set whereas isolates RZ.2.1.AG1, RZ.2.1.AP4, RZ.1.2.AP1, RZ.2.2.AG2, and RZ.1.3.AG4 significantly enhanced the number of leaves (Table 4 and Fig 5). The other rhizobacterial isolates showed lower of plant height and number of leaves compare than control.

Not all of selected rhizobacterial isolate accelerated flower phase and enhanced fruit yield of chili. The flower phase was earlier on two rhizobacterial introduced chili (47.00-47.33 dat). Fruit yield increased on seven rhizobacterial introduced chili (34.33-89.67 g) compare than control (32.00 g) (Table 5). The highest fruit yield exposed to RZ.2.1.AG1 and increased 180.21 % compare than control.

Discussion

This study revealed that the chili seedlings that were grown with twenty rhizobial isolates had greater value in all the growth parameters monitored such as seed germination's rate and plant, than the control which was not treated with any biofertilizer had the lowest value (Table 2). These results were similar with the findings of

previous studies by some authors. It was determined that the PGPR applications could be able to increase plant growth, germination rate of seed, improve transplant emergence, response to stress conditions and protect from disease (Elkinci et al. 2014), such as *Azospirillum*, *Pseudomonas* and *Azotobacter* have significant impact on seed germination and transplant growth (Shaukat et al. 2006a, b, Nezarat and Gholami 2009). Application of strains BSCBE4 and PA23 at the rate of 20 g kg⁻¹ of seed significantly increased the growth of hot pepper seedling (Nakkeeran et al. 2006). According to Kokalis-Burelle et al. (2003) PGPR could be used to obtain standard sized transplant in less time and a more vigorous transplant for transplant production. Besides, it was stated that PGPR can be applied at the sowing and transplanting stage wherefore used to control harmful microorganisms and can be increased

growth in stress conditions as well as healthy plants also (Gül et al. 2008). The highest fresh shoot and dry root weight of cabbage transplants were obtained from application of *Bacillus megaterium* KBA-10 and root diameter, root length and fresh root weight were obtained from application of *B. megaterium* TV-91C (Elkinci et al. 2014).

Introduction of IRB isolates shown decrease of incubation time, incidence and severity of diseases (Table 3). 13 isolates IRB shown decrease of incidence up to 100% and showed no symptoms until the end of observations. Introduction IRB isolates also showed plant growth promotion after inoculation of pathogen. Isolates RZ.2.2AG2 and Isolates RZ.2.1AG1 have highest ability to promote growth rate of chili and also have zero incidence and shown no symptoms of diseases.

Table 1. Diversity of morphological colony, physiological characters and pathogenicity/hypersensitive reaction of indigenous rhizobacterial isolates from rhizosphere of healthy chili

Rhizo-bacterial isolate	Morphology character of colony (2 dpi)					HR	Gram reaction
	Form	Elevation	Margin	Diameter (cm)	Color		
RZ.1.1.AP1	Circular	Flat	Flat	0.2	White	-	+
RZ.1.2.AP1	Circular	Convex	Flat	0.2	White	-	-
RZ.1.3.AP1	Rhizoid	Flat	Rhizoid	0.9	White	-	-
RZ.1.4.AP1	Circular	Convex	Flat	0.5	White	-	-
RZ.2.1.AP1	Irregular	Flat	Irregular	0.5	White	-	+
RZ.2.2.AP1	Rhizoid	Flat	Rhizoid	1.0	White	-	-
RZ.2.3.AP1	Irregular	Flat	Lobate	5.1	White	-	+
RZ.1.1.AP2	Irregular	Flat	Filiform	2.3	White	-	+
RZ.1.2.AP2	Rhizoid	Flat	Rhizoid	1.1	White	-	+
RZ.1.3.AP2	Irregular	Flat	Lobate	3.2	White	-	+
RZ.2.1.AP2	Irregular	Flat	Lobate	4.1	White	-	+
RZ.2.2.AP2	Irregular	Unbonate	Irregular	2.1	White	-	+
RZ.2.3.AP2	Irregular	Flat	Lobate	3.1	White	-	+
RZ.1.1.AP3	Rhizoid	Flat	Rhizoid	1.2	White	-	-
RZ.1.2.AP3	Rhizoid	Flat	Rhizoid	1.1	White	-	-
RZ.1.3.AP3	Irregular	Flat	Undulate	0.7	White	-	-
RZ.2.1.AP3	Circular	Convex	Flat	0.2	White	-	-
RZ.1.1.AP4	Rhizoid	Flat	Rhizoid	0.9	White	-	-
RZ.1.2.AP4	Rhizoid	Flat	Rhizoid	1.3	White	-	-
RZ.1.3.AP4	Circular	Flat	Raised	0.8	White	-	+
RZ.1.4.AP4	Rhizoid	Flat	Rhizoid	1.2	White	-	-
RZ.1.5.AP4	Circular	Flat	Convex	0.1	White	-	-
RZ.2.1.AP4	Irregular	Raised	Flat	0.6	White	-	-
RZ.2.2.AP4	Rhizoid	Flat	Rhizoid	1.2	White	-	-
RZ.2.3.AP4	Irregular	Flat	Flat	0.7	White	-	+
RZ.2.4.AP4	Rhizoid	Flat	Rhizoid	1.5	White	-	+
RZ.2.5.AP4	Rhizoid	Flat	Rhizoid	1.4	White	-	-
RZ.1.1.AG1	Irregular	Flat	Lobate	4.0	White	-	-
RZ.1.2.AG1	Irregular	Flat	Filiform	1.2	White	-	+
RZ.2.1.AG1	Irregular	Flat	Lobate	5.0	White	-	+
RZ.2.2.AG1	Circular	Convex	Flat	0.3	yellow	-	-
RZ.2.1.AG2	Circular	Flat	Flat	0.8	White	-	+
RZ.2.2.AG2	Circular	Convex	Flat	0.2	yellow	-	-
RZ.1.1.AG3	Circular	Raised	Filiform	1.1	White	-	+
RZ.2.1.AG3	Rhizoid	Flat	Rhizoid	1.1	White	-	-
RZ.2.2.AG3	Circular	Convex	Flat	0.5	Yellow	+	-
RZ.1.1.AG4	Irregular	Flat	Filiform	2.0	White	-	-
RZ.1.2.AG4	Irregular	Flat	Lobate	0.9	White	-	+
RZ.1.3.AG4	Circular	Convex	Flat	0.8	White	-	-
RZ.1.4.AG4	Circular	Convex	Flat	0.6	Yellow	-	-
RZ.2.1.AG4	Irregular	Flat	Filiform	1.4	White	-	-
RZ.2.2.AG4	Irregular	Flat	Lobate	7.0	Transparent white	-	+
RZ.2.3.AG4	Irregular	Flat	Lobate	3.5	White	-	+

Note: HR = Hypersensitive Reaction as pathogenicity test

Table 2. Germination rate and seedling's height of rhizobacterial introduced chili (21 days after inoculation)

Rhizobacterial isolates	Germination rate		Seedling's height			
	(%)	Increase (%)	cm	\pm SD		Increase (%)
RZ.2.2.AG2	100	8.70	7.13	\pm 0.15	a	47.59
RZ.2.2.AG4	100	8.70	6.93	\pm 0.23	b	43.45
RZ.2.1.AG2	96	4.35	6.77	\pm 0.05	bc	40.00
RZ.1.3.AP1	100	8.70	6.70	\pm 0.00	cd	38.62
RZ.1.5.AP4	96	4.35	6.57	\pm 0.15	de	35.86
RZ.1.4.AP4	92	0.00	6.47	\pm 0.05	ef	33.79
RZ.2.1.AG3	96	4.35	6.47	\pm 0.05	ef	33.79
RZ.2.1.AG1	96	4.35	6.37	\pm 0.05	fg	31.73
RZ.2.3.AP1	96	4.35	6.27	\pm 0.11	g	29.66
RZ.1.4.AG4	100	8.70	6.00	\pm 0.10	h	24.14
RZ.1.3.AG4	96	4.35	5.87	\pm 0.05	hi	21.38
RZ.2.1.AP1	92	0.00	5.83	\pm 0.05	hi	20.69
RZ.1.2.AP1	92	0.00	5.73	\pm 0.95	ij	18.62
RZ.1.1.AG4	96	4.35	5.70	\pm 0.00	ij	17.93
RZ.2.1.AP4	100	8.70	5.57	\pm 0.05	j	15.17
RZ.2.5.AP4	96	4.35	5.37	\pm 0.05	k	11.04
RZ.2.1.AP3	92	0.00	4.93	\pm 0.11	l	2.07
RZ.2.3.AG4	92	0.00	4.87	\pm 0.05	l	0.69
Kontrol	92	0.00	4.83	\pm 0.05	lm	0.00
RZ.2.1.AP2	96	4.35	4.67	\pm 0.15	m	-3.45
RZ.1.1.AP1	96	4.35	4.43	\pm 0.05	n	-8.28
RZ.1.1.AG1	88	-4.35	4.20	\pm 0.10	o	-13.10
RZ.1.1.AP2	84	-8.70	4.20	\pm 0.10	o	-13.10
RZ.1.1.AP3	80	-13.04	4.20	\pm 0.10	o	-13.10
RZ.1.1.AG3	84	-8.70	4.10	\pm 0.10	op	-15.17
RZ.2.2.AG1	88	-4.35	4.07	\pm 0.15	op	-15.86
RZ.2.2.AP1	88	-4.35	4.07	\pm 0.15	op	-15.86
RZ.2.2.AP2	84	-8.70	4.07	\pm 0.05	op	-15.86
RZ.2.4.AP4	80	-13.04	4.07	\pm 0.05	op	-15.86
RZ.2.1.AG4	88	-4.35	4.00	\pm 0.10	pq	-17.24
RZ.1.1.AP4	88	-4.35	3.97	\pm 0.21	pqr	-17.93
RZ.2.2.AP4	92	0.00	3.93	\pm 0.15	pqr	-18.62
RZ.1.2.AG1	96	4.35	3.87	\pm 0.20	qrs	-20.00
RZ.1.2.AG4	88	-4.35	3.87	\pm 0.25	qrs	-20.00
RZ.1.3.AP2	92	0.00	3.83	\pm 0.06	qrs	-20.69
RZ.2.3.AP2	80	-13.04	3.83	\pm 0.05	qrs	-20.69
RZ.1.2.AP2	88	-4.35	3.80	\pm 0.10	rst	-21.38
RZ.2.3.AP4	88	-4.35	3.80	\pm 0.10	rst	-21.38
RZ.1.2.AP4	92	0.00	3.73	\pm 0.11	st	-22.76
RZ.1.3.AP3	88	-4.35	3.63	\pm 0.20	tu	-24.83
RZ.1.4.AP1	88	-4.35	3.53	\pm 0.15	uv	-26.90
RZ.1.2.AP3	88	-4.35	3.43	\pm 0.05	vw	-28.97
RZ.1.3.AP4	84	-8.70	3.33	\pm 0.15	w	-31.03

Note: Means with the same letter are not significantly different by Duncan multiple range test at $p < 0.05$

PGPR treatments increased shoot weight, shoot length and stem diameter of muskmelon and watermelon transplant (Kokalis-Burelle et al. 2003). Also, the researchers indicated that root weight of transplant was increased by PGPR. In another study, PGPR isolates increased shoot length, root length and dry matter production of shoot and root of *Cicer arietinum* transplant (Misra et al. 2010). Turan et al (2014) reported that seed inoculation of the PGPR strains improved growth and quality of the cabbage transplants.

Table 3. Effect of rhizobacterial isolates on bacterial wilt disease of chili

Rhizobacterial Isolates	Incubation period		Disease incidence		Disease severity		
	dpi	Prolonged (%)	%	Reduced (%)	%	Reduced (%)	
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	27.33	b	116.93	67	33	2 b	33
RZ.2.3.AG4	26.33	b	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
Control-	12.67**	cd		100	0	3 a	

Note: Means with the same letter are not significantly different by Duncan multiple range test at $p < 0.05$. *Plants were still live until the end of observation.

Table 4. Effect of all rhizobacterial isolates on growth of chili in pot experiment

Rhizo-bacterial isolates	Plant height		Number of leaves			
	cm	\pm SD	Increased (%)	Pieces \pm SD	Increased (%)	
RZ.2.2.AG2	68.00 \pm 7.21	a	60.64	173.67 \pm 28.04	ab	82.17
RZ.2.1.AG1	63.33 \pm 2.08	a	49.62	210.00 \pm 2.08	a	120.29
RZ.1.3 .AP1	53.33 \pm 1.53	ab	25.99	118.00 \pm 23.58	bcd	23.78
RZ.1.3.AG4	45.67 \pm 4.04	bc	7.88	170.00 \pm 103.18	bc	78.33
RZ.2.1.AP4	43.33 \pm 1.53	bcd	2.37	191.67 \pm 17.01	ab	101.06
Control +	42.33 \pm 1.15	bcd		95.33 \pm 7.09	cde	
RZ.1.4.AP4	37.67 \pm 4.04	bcd	-11.02	92.67 \pm 39.31	de	-2.79
RZ.1.4.AG4	35.67 \pm 1.15	cd	-15.74	81.00 \pm 8.54	de	-15.03
RZ.2.1.AP2	33.67 \pm 5.13	cde	-20.47	127.33 \pm 62.74	bcd	33.57
RZ.2.1.AP1	33.33 \pm 1.52	cdef	-21.25	72.67 \pm 5.03	de	-23.77
RZ.1.1.AP1	32.00 \pm 6.08	cdef	-24.40	57.67 \pm 8.50	de	-39.51
RZ.1.2.AP1	30.00 \pm 2.00	cdef	-29.13	191.00 \pm 37.32	ab	100.36
RZ.2.1.AP3	29.33 \pm 3.21	def	-30.70	86.00 \pm 8.89	de	-9.79
RZ.1.1.AG4	26.33 \pm 3.05	ef	-37.79	66.67 \pm 6.43	de	-30.07
RZ.2.3.AG4	16.33 \pm 28.29	ef	-61.41	28.67 \pm 49.65	e	-69.33
RZ.1.5.AP4	16.00 \pm 27.71	f	-62.20	60.00 \pm 103.92	de	-37.06
RZ.2.1.AG3	*		*			
RZ.2.2.AG4	*		*			
RZ.2.5.AP4	*		*			
RZ.2.1.AG2	*		*			
RZ.2.3.AP1	*		*			

Note: Means with the same letter are not significantly different by Duncan multiple range test at $p < 0.05$. *Plants were died

Table 5. Effect of rhizobacterial isolates on flower phase and yield of chili in pot experiment

Rhizobacterial isolate	Flower phase		Fruit Weight	
	Day after transplanting	Effectivity (%)	g	Effectivity (%)
RZ.2.1.AP4	47.00 a	12.96	57.33 c	79.17
RZ.2.1.AG1	47.33 a	12.35	89.67 a	180.21
RZ.1.4.AG4	53.67 ab	0.62	40.33 d	26.04
Control +	54.00 ab	0.00	32.00 d	0.00
RZ.2.1.AG2	54.00 ab	0.00	*	
RZ.2.2.AG2	55.33 abc	-2.47	72.67 b	127.08
RZ.2.2.AG4	55.67 abc	-3.09	*	
RZ.2.5.AP4	62.00 bcd	-14.81	*	
RZ.1.3.AP1	64.67 cd	-19.75	74.33 ab	132.29
RZ.2.3.AG4	64.67 cd	-19.75	*	
RZ.2.1.AP1	66.67 d	-23.46	34.33 d	7.29
RZ.2.1.AP2	67.67 d	-25.31	47.00 d	46.88
RZ.2.1.AP3	68.33 d	-26.54	30.00 d	-6.25
RZ.1.1.AG4	70.00* d	-29.63	*	
RZ.1.1.AP1	70.00* d	-29.63	*	
RZ.1.2.AP1	70.00* d	-29.63	*	
RZ.1.3.AG4	70.00* d	-29.63	*	
RZ.1.4.AP4	70.00* d	-29.63	*	
RZ.1.5.AP4	70.00* d	-29.63	*	

Note: Means with the same letter are not significantly different by Duncan multiple range test at $p < 0.05$. *no fruit; *no flower until 70 days after transplanting

**Figure 3.** Growth performance of chili seedlings, control (left) and rhizobacterial inoculated chili seedling (right)**Figure 4.** Healthy chili after rhizobacterial inoculation (8 dpi of *R. solanacearum*) (left). Bacterial wilt disease on control chili plant (right).**Figure 5.** Growth of control plant (without rhizobacterial isolates) (left), rhizobacterial isolates inoculated chili and *R. solanacearum* (right)

This study demonstrated that 13 selected rhizobacterial isolates from healthy chili rhizosphere at endemic area of bacterial wilt disease (indigenous) reduced disease incidence caused by *R. solanacearum* on chili (0 %) compare with control plant (100 %). This result confirmed to our previous research, that 41 rhizobacterial isolates from healthy ginger rhizosphere at endemic area of bacterial wilt disease showed no bacterial wilt disease on

chili (0 %) compare than control plant (100%) (Habazar et al. 2011). Wydra and Semrau (2005) also reported comparable *R. solanacearum* wilt disease reduction associated with biocontrol agents *Bacillus* spp. and fluorescent *Pseudomonas*.

The effectivity of those rhizobacterial isolates reduced bacterial wilt disease incidence and provided disease suppression equal or superior compare with the other experiment, such as by Nguyen and Ranamukhaarachchi (2010) tested bio-control agents caused a significant reduction of bacterial wilt disease compared to the control. Biocontrol agents TR6 and LR10 showed the highest disease suppression for both pepper (8.3 and 29.2 %) and tomato (18.8 and 29.2 %). Lang et al. (2007) used the conventional copper hydroxide-mancozeb treatment to control *Xanthomonas* leaf blight on onion under field conditions. Under field conditions at one location, biweekly or weekly applications of bacteriophages reduced disease severity by 26 to 50%, which was equal to or better than weekly applications of copper hydroxide plus mancozeb. Acibenzolar-S-methyl also successfully reduced disease severity by up to 50% when used alone preventively or followed by biweekly bacteriophage applications. The protection afforded on rhizobacteria-treated plants resulted no bacterial wilt symptom on chili. This suggested that rhizobacteria treatment for some extend able to induced plant systemic resistance to overcome bacterial wilt infection on ginger. Beneficial effects of PGPR and bioprotectants on plants have been reviewed. Some other mechanism such as hydrocyanic acid, siderophores and induction of resistance may also play a role in the action of PGPR. So that rhizobacterial agents will probably be one of the most significant strategies for disease management (Compant et al. 2005).

Not all rhizobacterial isolates showed the ability to enhance growth and yield of chili. Five of rhizobacterial introduced chili showed higher height (43.33-68.00 cm) which the effectivity to increase plant height varied from 2.37-60.64 % compare than control (42.33 cm) at 42 days after transplanting (dat). Assessment of average number of the leaves showed seven of rhizobacterial introduced chili more leaves (118-120 pieces) than control (95.33 pieces), which the effectivity to increase plant height varied from 23.78-120.29 %. The flower phase was earlier on two rhizobacterial introduced chili (47.00-47.33 dat). Fruit yield increased on seven rhizobacterial introduced chili (34.33-89.67 g) compare than control (32.00 g). These result confirmed to our previous research, that all rhizobacterial isolates from healthy ginger rhizosphere at endemic area of bacterial wilt disease increased growth and yield of ginger compare than control plant (Habazar et al. 2011). Our results are in agreement with the findings of earlier research. study with *Bacillus subtilis* and *B. amyloliquefaciens* have been increased of plant growth and yield on different plants (Kokalis-Burelle et al. 2003). According to Nguyen and Ranamukhaarachchi (2010) rhizobacterial isolates TR6 treatments resulted in the highest pepper and tomato fruit weight compare than control plant. The ability to increase growth and yield of chili by rhizobacterial isolates were presumable, that PGPR

are effective root colonizers that survive and proliferate along with plant roots, resulting in enhanced plant growth (Whipps 2001). Egamberdieva (2008) observed the positive effect of PGPR on the growth of wheat and pea.

In conclusion, among 43 rhizobacteria isolated, we have found two rhizobacterial isolates, RZ.2.1.AG1 and RZ.1.3.AP1 as the most effective against *R. solanacearum* and had greater fruit weight, earlier flower's phase, number of leaves and plant height, compared with the untreated control. Control of *R. solanacearum* by these isolated rhizobacteria may help reducing chemical applications and their environmental impacts in agricultural systems where solanaceous crops are grown and will lower the cost of disease management. The use of rhizobacterial for increasing the yield and crop protection is a promising approach in modern systems of sustainable agriculture. Future research should be directed towards detailing the mode of action of these strains and field studies need to be conducted to confirm the effectiveness of these isolated rhizobacterial under natural conditions.

ACKNOWLEDGEMENTS

This research was funded by Hibah Guru Besar Universitas Andalas, Padang, West Sumatra, Indonesia, Contract No. 524/XIV/A/UNAND-2016 at May 9th 2016. The authors would like to sincerely thanks to Rector of Universitas Andalas.

REFERENCES

- Anith KN, Momol MT, Kloepper JW, Marios J, Olson SM, Jones JB. 2004. Efficacy of plant growth-promoting rhizobacteria, acibenzolar-S-methyl and soil amendment for integrated management of bacterial wilt on tomato. *Plant Dis* 88: 669-673.
- Basu A. 2014. Bio-efficacy of *Pseudomonas fluorescens* (7% WP and 5% SC formulations) against bacterial wilt disease of chili. *Asia Pac J Sustain Agric Food Energ* 2 (2): 36-40.
- Compant S, Duffy B, Nowak J, Clement C, Barka EA. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Appl Environ Microbiol* 71 (9): 4951-4959.
- Egamberdieva D. 2008. Plant growth promoting properties of Rhizobacterail isolates from what and pea grown in loamy sand soil. *Turk J Biol* 32: 9-15.
- El-Argawy E, Adss IA. 2016. Quantitative gene expression of peroxidase, polyphenoloxidase and catalase as molecular markers for resistance against *Ralstonia solanacearum*. *Amer J Mol Biol* 6 (2): 88.
- Ekinci M, Turan M, Yildirim E, Güneş A, Kotan R, Dursun A. 2014. Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* L. var. *Botrytis*) transplants. *Acta Sci Pol Hortorum Cultus* 13 (6): 71-85.
- Ganesan S and Sekar R. 2011. Screening of biocontrol agents against *Rhizoctonia solani* causing web blight disease of groundnut (*Arachis hypogaea* L.). In: Stoytcheva M (eds.). *Pesticides in the Modern World-Pests Control and Pesticides Exposure and Toxicity Assessment*, Intech, Rijeka, Croatia.
- Gül A, Kidoğlu F, Tüzel Y, Tüzel HI. 2008. Effects of nutrition and *Bacillus amyloliquefaciens* on tomato (*Solanum lycopersicum* L.) growing in perlite. *Spanish J Agri Res* 6 (3): 422-429.
- Habazar, T, Jamsari, Nasrun, Yanti, Y. 2010. In planta technique, for screening rhizobacteria as biocontrol agents against bacterial plant pathogens. Paper presented in: International Seminar of Indonesian Society of Microbiology (ISISM), Bogor. 4-7 October

- Habazar T, Nasrun, Dachryanus, Suharti N and Yanti Y. 2012. In planta technique, for screening rhizobacteria as biocontrol agents of bacterial wilt on ginger. 1st SIB International Conference on Biodiversity, Solo 23-24 July
- Kokalis-Burelle N, Vavrina CS, Reddy MS, Kloepper JW. 2003. Amendment of muskmelon and watermelon transplant media with plant growth promoting rhizobacteria: Effects on transplant quality, disease, and nematode resistance. HortTech 13 (3): 476-482.
- Lang JM, Gent DH, Schwartz HF. 2007. Management of *Xanthomonas* leaf blight of onion with bacteriophages and a plant activator. Plant Dis 91: 871-878.
- Lwini M and Ranamukhaarachchi SL. 2006. Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. Intl J Agric Biol 8 (5): 657-660.
- Misra M, Kumar U, Misra PK, Prakash V. 2010. Efficiency of plant growth promoting rhizobacteria for the enhancement of *Cicer arietinum* L. growth and germination under salinity. Adv Biol Res 4 (2): 92-96.
- Nakkeeran S, Kavitha K, Chandrasekar G, Renukadevi P, Fernando WGD. 2006. Induction of plant defence compounds by *Pseudomonas chlororaphis* PA23 and *Bacillus subtilis* BSCBE4 in controlling damping-off of hot pepper caused by *Pythium aphanidermatum*. Biocontrol Sci Technol 16 (4): 403-416
- Nezarat S, Gholami A. 2009. Screening plant growth promoting rhizobacteria for improving seed germination, transplant growth and yield of maize. Pakistan J Biol Sci 12 (1): 26-32.
- Nguyen MT and Ranamukhaarachchi SL. 2010. Soil-borne antagonists for biological control of bacterial wilt disease caused by *Ralstonia solanacearum* in tomato and pepper. J Plant Pathol 92 (2): 395-406.
- Pal KK and McSpadden Gardener BB. 2006. Biological Control of Plant Pathogens. The Plant Health Instructor DOI: 10.1094/PHI-A-2006-1117-02.
- Piromyou P, Buranabanyat B, Tantasawat P, Tittabutr P, Boonkerd N, Teamroong N. 2011. Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. European J Soil Biol 47 (1): 44-54.
- Ran LX, Liu CY, Wu GJ, Van Loon LC, Bakker PAHM. 2005. Suppression of bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. in China. Biol Control 32: 111-120.
- Shaukat K, Affrasayab S, Hasnain S. 2006a. Growth responses of *Heliantus annuus* to plant growth promoting rhizobacteria used as a biofertilizer. J Agric Res 1: 573-581.
- Shaukat K, Affrasayab S, Hasnain S. 2006b. Growth responses of *Triticum aestivum* to plant growth promoting rhizobacteria used as a biofertilizer. Res J Microbiol 1: 330-338.
- Swanson JK, Yao J, Tans-Kersten J, Allen C. 2005. Behavior of *Ralstonia solanacearum* race 3 biovar 2 during latent and active infection of geranium. Phytopathology 95: 136-143.
- Turan M, Ekinci M, Yildirim E, Güneş A, Karagöz K, Kotan R, Dursun A. 2014. Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. Turkish J Agric For 38: 327-333.
- Velusamy P, Immanuel JE, Gnanamanickam SS. 2013. Rhizosphere bacteria for biocontrol of bacterial blight and growth promotion of rice. Rice Sci 20 (5): 356-362.
- Winstead NN, Kelman A. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology 42: 628-634.
- Wydra K, Semrau J. 2005. Phenotypic and molecular characterization of the interaction of antagonistic bacteria with *Ralstonia solanacearum* causing tomato bacterial wilt. In: Zeller W, Ulrich C. (eds). 1st International Symposium on Biological Control of Bacterial Plant Diseases, Darmstadt, Germany.
- Yanti Y, Habazar T, Resti Z, Suhailita D. 2013. Screening of isolates rhizobacteria of soybean plants healthy rooting for disease control of pustules bacteria (*Xanthomonas axonopodis* pv. *glycines*). Jurnal HPT Tropika 13 (1): 24-34. [Indonesian]