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Isolation and characterization of indigenous Rhizobacteria for control of *Phytophthora* in Red Chili plants and its use as Rhizobacteria management of plant growth

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Abstract

Chili is one of the horticultural commodities that still requires serious handling, especially in the context of increasing production both in quality and quantity. Seed treatment using biological seed treatment agents is an alternative as a substitute for synthetic chemicals in seed treatment. This study aims to determine the inhibitory power of rhizobacteria in the growth of colonies of pathogens carried by chili seeds in vitro and the mechanism of action of rhizobacteria to inhibit the growth of Phytophthora colonies in chili. The research was carried out at the Seed Science and Technology Laboratory, Laboratory of Plant Disease, Biology Laboratory, Teacher Training and Education Faculty, Syiah Kuala University, Inter-University Central Laboratory and Bacteriology Laboratory of IPB Bogor from July 2014 to March 2015. Conducted isolation of carrying pathogens of chili seeds namely pathogenic fungi *P. Capsi* and isolation of rhizobacteria of bio-control agent candidates and rhizobacterial agents promote plant growth candidates. The results showed that the results of detection, exploration and isolation of rhizobacteria from the root system of healthy chili plants obtained 154 rhizobacterial isolates. Antagonistic test results obtained one rhizobacterial isolate, namely *Rbks-5* isolate has a very high inhibitory power on the growth of pathogenic colonies test *S.rolfsii* dan *Phytium sp*. While rhizobacteria *Rbks-6* isolates and *Rbks-7* isolates have very high inhibitory power on the pathogen *S. rolfsii* and *P. capsici*. Rhizobacterial isolates which produced the highest IAA were *Rbkb-2* isolates, followed by 3 isolates capable of producing IAA growth regulators namely *Rbkp-4*, *Rbks-2*, *Rbks-5* rhizobacterial isolates.

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Introduction

Chili is one of the horticultural commodities of high economic value and still requires serious handling, especially in the context of increasing production both in quality and quantity. Statistical data on the area of chili plantations in Indonesia in 2013 reached 2,645,111 ha, ranking the widest first compared to other vegetable crops. However, the productivity level is still below the potential yield, namely the productivity of the new red chili reaches 8.35 tons ha-1 while the potential results can reach 20 tons ha-1.

The use of low quality seeds and infectious diseases is one of the causes of the low productivity of chili in Indonesia. Seed is one source of inoculum for several types of pathogens that cause disease in chili plants. Phytophthora rot is one of the diseases that results in the loss of chili yield throughout the world. In certain conditions even phytophthora rot disease can reduce the yield of chili in the field to 100% (AVRDC, 2004).

The use of high-quality and pathogen-free seeds and protection of seeds through pre-planting seed treatment is one of the strategies to control various disease-causing pathogens in chili plants. Therefore, the availability of pathogenic-free seeds is absolutely necessary. One effort to eliminate seed-borne pathogens and disease events in chili plants, the initial treatment of seeds before planting is very necessary (Maude, 1996; Brandle, 2001).

Seed treatment using biological seed treatment agents is an alternative as a substitute for synthetic chemicals in seed treatment. Biological seed treatment is a seed treatment carried out using one or more organisms other than humans to reduce the number of inoculums or activities to produce disease from a pathogen (Cook and Baker, 1983). Biological control of pathogens in the form of a total or partial reduction of pathogenic populations by other organisms naturally occurs continuously in nature (Agrios, 1997).

Control of seed-borne pathogens in particular through seed treatment with bio-control agents from several recent research results provides very effective results. Inoculation *Serratia plymuthica* strain A21-4 on chili seeds is able to prevent *P. capsici* and *Phytium* infection by up to 86% in seedlings treated (Shen *et al.*, 2002). *B. subtilis* strains, *B. coagulans*, *B. megaterium*, and *S. marcescen* were also shown to have a high inhibitory power on the growth of *P. capsici* colonies of chili plant isolates in vitro and in vivo (Syamsuddin *et al.*, 2007).

The relationship with the role of rhizobacteria both as bio-control agents and rhizobacteria stimulates plant growth towards growth and crop yields, especially red chili plants, both through mechanisms to control disease-causing pathogens and their role as plant growth stimulants and results both in vitro and in vivo. Therefore, this study aims to determine the inhibitory power of rhizobacteria in the growth of colonies carried out by chili seed pathogens in vitro and the mechanism of rhizobacteria in inhibiting the growth of Phytophthora colonies in chili.

Material and methods

The research was carried out at the Seed Science and Technology Laboratory, Laboratory of Plant Disease, Agrotechnology Study Program at the Faculty of Agriculture, Syiah Kuala Darussalam University, Banda Aceh, Biology Laboratory, Teacher Training and Education Faculty, Syiah Kuala University, Inter-University Central Laboratory of IPB Bogor and Bacteriology Laboratory, Faculty of Veterinary Medicine IPB. From July 2014 to March 2015.

The tools used in this study are autoclave, clean band, analytical scales, spectrophotometer, electric oven, binocular microscope, abrasive space, bunsen burner, petri-dish, test tubes, erlenmeyer flask, spacer, gas stove, ose needle, tweezers, growth rack, equipment for gardening and Other equipment needed in this study. The materials used in this study were chili seeds of PM999 and Kiyo F1 varieties, seed pathogenic isolates (*Phytium* sp.), Rhizobacteria, Potato Dextrose Agar (PDA) medium, 96% alcohol, 2% sodium hypochlorite solution, aquades, methylated spirits, soil, manure, SPA media, TSA (tryptic soy agar) media, King'B media, NA media

(nutrient agar), silver black plastic mulch, fertilizer, pesticides and other materials needed in conducting this research.

The research was initiated by isolating pathogens carried by chili seeds, namely pathogenic fungi *P. Capsi* and Isolation of Rhizobacteria Bio-control Agents Candidates and RPPT candidates.

Experiments test the effectiveness of inhibition of rhizobacterial isolates of bio-control agent candidates on the growth of pathogenic colonies carried by chili seeds (*P. capsici*, *C. capsici*, *F. oxysporum*, *S. rolfsii*, *R. solani*, and *Pythium* sp.) was carried out in a completely randomized design trial (RAL) in a non-factorial pattern. 15 selected isolates of bio-control agents (according to the results of the initial potential test in vitro before).

Each is a separate experiment and repeated 3 times. The data obtained were analyzed using SAS analysis program. To test the differences between treatment averages will be continued with Duncan's multiple distance test (DMRT) at the level of $\alpha = 0.05$. Rhizobacteria are selected bio-control agent candidates, testing their antagonistic ability to inhibit the growth of chili seed pathogens colonies using multiple test techniques (dual test).

The characteristics of the rhizobacterial mechanism of biochemical candidate bio-control agents were carried out by analyzing siderophore compounds and the ability of agents to produce hydrogen cyanide (HCN). While to determine the ability of rhizobacteria as candidates for Plant Growth Promoting Rhizobacteria (PGPR) or rhizobacteria stimulates plant growth each of the rhizobacterial isolates in this experiment was evaluated for their ability to produce indole acetic acid (IAA), and the ability to dissolve phosphate (P) and its effects to increase the viability and vigor of the growth of seeds and the growth of chili seedlings. The variables observed included maximum growth potential, germination, vigor index, simultaneous growth, relative growth rate, time needed to reach 50% relative germination, and normal sprout dry weight.

Results and discussion

Results of Exploration, Isolation and Identification of Rhizobacteria of Plant Growth Bio-control Agent Candidates and Rhizobacterial Agent Candidates from Chili Plant Rooting System

The results of rhizobacterial isolation from the root system of chili farmers in Bebesen Subdistrict, Silih Nara Subdistrict and Peugaseng Subdistrict of Central Aceh Regency were obtained by 154 rhizobacterial isolates. All of the rhizobacterial isolates obtained were then tested for their ability to inhibit seed-borne pathogens, using multiple test techniques (Dual Test) in Potato Dextrose Agar (PDA) media as the initial potential test. The test pathogens used were phytophthora blight pathogens on chili plants, namely *Phytophthora capsici*. From the results of the initial potential test obtained 18 isolates that showed the ability to inhibit the growth of the *P. capsici* colonies. While the remaining 136 rhizobacterial isolates did not show significant percentage inhibition. Rhizobacteria which show high inhibitory power to the growth of pathogenic test colonies are: isolates *Rbkp-1*, *Rbkp-2*, *Rbkp-3*, *Rbkp-4*, *Rbkp-5*, *Rbks-1*, *Rbks-2*, *Rbks-3*, *Rbks-4*, *Rbks-5*, *Rbks-6*, *Rbks-7*, *Rbkb-1*, *Rbkb-2*, *Rbkb-3*, *Rbkb-4*, *Rbkt-1*, and *Rbkt-2*. All rhizobacterial isolates that have the ability to inhibit the growth of test pathogenic colonies are then used in further research to be studied both for candidates for bio-control agents and for plant growth-promoting rhizobacterial agents.

Evaluation of the Effectiveness of Rhizobacterial Inhibition on the Growth of Multiple Pathogenic Colonies Carried by Red Chili Seeds in In Vitro

The results of the analysis of variance (Test F) inhibition of rhizobacterial isolates from isolation from the chili root system of Bebesen Subdistrict, Silih Nara Subdistrict, and Peugaseng Subdistrict of Central Aceh District showed that rhizobacteria had significant in vitro growth inhibitory effect on both pathogen *F. oxysporum*, *R. solani*, *S. rolfsii*, *P. capsici*, *C. capsici*, as well as pathogens *Phytium sp*. The average percentage of inhibition of rhizobacterial bio-control agent candidates for each test pathogen is presented in Table 1.

From Table 1 it can be seen that the inhibitory ability of each rhizobacterial isolate differs depending on the test pathogen used. The ability of test pathogens to compete with rhizobacteria of bio-control agent candidates is also dependent on rhizobacterial isolates used.

In general there are several rhizobacterial isolates which have very high inhibitory power and some isolates have high inhibitory power against test pathogens. Rhizobacteria isolate *Rbks-5* was able to inhibit the growth of pathogens colonies of *S. rolfsii* and *Phytium sp*. with very high inhibition (77.45-78.78%), while rhizobacteria isolates *Rbks-6* and *Rbks-7* isolates had very high inhibiting. Ability (75.22-76.33%) on the growth of pathogenic colonies of *S. rolfsii* and *P. capsici*. From Table 1 it can also be seen that there are 2 rhizobacterial isolates that have the ability to inhibit almost all test pathogens except pathogen *R. solani*, with high inhibitory power which ranges from 62.99-69.89%, namely *Rbkp-3* isolates and *Rbkb-1* isolates, 4 rhizobacterial isolates have the ability to inhibit four test pathogens, namely isolates *Rbkp-4*, isolates *Rbkp-1*, isolates *Rbkp-5*, and isolates *Rbks-6*. While 2 isolates inhibited 3 test pathogens, namely isolates *Rbks-5* and *Rbkb-4*. While the other 3 rhizobacterial isolates have the ability to inhibit one test pathogen. The rest have inhibition abilities of less than 60.56% or moderate inhibition categories.

Table 1. Inhibition of Various Rhizobacterial Isolates on the Growth of Carried Pathogen Colonies of Red Chili Seeds (*F. oxysporum*, *R. solani*, *S. rolfsii*, *P. capsici*, *C. capsici*, and *Phytium*, sp) in In Vitro.

Group of Rhizobacteria	Inhibition of Various Rizobacteria on Carried Pathogens of Chili Seeds (%)					
	F. oxysporum	*R. solani*	*S. rolfsii*	*P. capsici*	*C. capsici*	*Phytium*, sp.
Rbkp-1	61.56 ab	10.08 d-g	60.66 cd	69.66 ab	71.77 ab	68.78 abc
Rbkp-2	19.77 bc	6.99 e-h	57.22 de	31.88 g	58.44 bc	69.890abc
Rbkp-3	64.99 a	5.10 gh	62.99 cd	65.25 abc	65.11 abc	69.89 abc
Rbkp-4	60.56 ab	11.00 def	67.45 c	52.99 b-f	66.22 abc	68.78 abc
Rbkp-5	61.67 ab	13.00 cd	64.11 cd	54.11 b-e	62.89 abc	64.33 bcd
Rbks-1	40.77 abc	6.84 fgh	50.73 ef	59.66 a-d	63.99 abc	71.00 ab
Rbks-2	15.43 c	6.55 fgh	43.00 f	36.33 fg	39.55 de	47.67 fg
Rbks-3	15.43 c	10.67 def	45.20 f	36.33 fg	38.44 de	63.22 bcd
Rbks-4	39.55 abc	12.33 cde	57.22 de	56.33 bcd	66.22 abc	69.89 abc
Rbks-5	70.56 a	59.44 b	77.45 a	48.55 c-g	73.10 a	78.78 a
Rbks-6	69.67 a	59.44 b	76.33 ab	67.44 ab	70.66 ab	69.89 abc
Rbks-7	60.56 ab	69.45 a	46.30 f	75.22 a	51.78 cd	57.67 def
Rbkb-1	68.99 a	60.44 b	68.54 bc	65.12 abc	71.77 ab	69.89 abc
Rbkb-2	18.77 bc	16.67 c	46.33 f	65.23 abc	51.77 cd	44.33 g
Rbkb-3	42.88 abc	8.99 d-g	60.67 cd	37.44 efg	40.66 de	58.78 cde
Rbkb-4	47.78 abc	3.66 h	57.33 de	69.66 ab	65.11 abc	66.55 bcd
Rbkt-1	15.43 c	11.00 def	43.00 f	46.33 d-g	35.11 e	62.11 bcd
Rbkt-2	15.43 c	13.00 cd	49.667 ef	53.00 b-f	32.88 e	49.89 efg

Description: The inhibitory activity is very high (> 75%), high inhibitory activity (61-75%), moderate inhibitory activity (51-60%), low inhibitory activity (<50%) and no inhibitory activity (-). with the same letters not significantly different based on the DMRT test at α = 0.05.

Rhizobacterial isolates that have the ability to inhibit the growth of pathogenic colonies tested in vitro on the antagonist test from the results of this study relate to their ability to compete with various pathogens and synthesis of secondary metabolites such as antibiotics, siderophore, hydrogen cyanide (HCN) and synthesis of various enzymes such as chitinase, 1, 3-glucanase, 1, 4-glucanase, cellulase, lipase, protease, and inducil-aminocyclopropane-l-carboxylate (ACC) deaminase (Baharum *et al*., 2003; Sutariati 2006; Kumar *et al*., 2007). The results of the analysis of the ability to produce cyanide acid (HCN) as one of the toxic compounds for pathogens were actually produced by all rhizobacterial isolates that have high healing power on the test pathogens from the results of this study (Table 2). The antagonistic properties of rhizobacteria both in vitro and in vivo against test pathogens indicate that the rhizobacterial group has the potential to be a candidate for bio-control agents.

The results of previous studies have also been reported that some rhizobacteria from both the *Pseudomonas*, *Bacillus*, *Serratia* and other isolates have been shown to be very effective in controlling pathogens that cause disease in plants. *Pseudomonas* spp. rhizobacteria proven effective controlling various pathogens such as *R. solani*, *C. capsici*, *P. infestans*, *F. oxysporum f. sp.* icericeris, and *Pythium aphanidermatum* (Kumar *et al.*, 2002; Sutariati, 2006; Yan *et al.*, 2002; Siddiqui *et al.*, 2005; Ramamoorthy *et al.*, 2002). Bio-control agents from the *Bacillus* spp group effective against fungus control *C. capsici*, *R. solani*, and *Fusarium oxysporum f.* udum (Sutariati, 2006; Szczech and Shoda, 2004; Siddiqui *et al.*, 2005). Bio-control agent of the *Serratia* spp group reported to be effective in controlling several pathogenic fungi such as *C. capsici* and *P. capsici* (Sutariati, 2006; Shen *et al.*, 2002a). Research results of Shen *et al.* (2002b) showed that *S. plymuthica* A21-4 rhizobacteria were very potential as bio-control agents for the control of pathogenic fungi *P.capsici.*

Physiological Characterization of the Mechanism of the Action of Biochemical Growth of Bio-control Agent and Rhizobacterial Agent Candidates for Plant Growth by Biochemistry

The results of the variance analysis (F Test) on some physiological characters of rhizobacterial isolates of bio-control agent candidates and candidates for rhizobacterial agents that stimulate plant growth were obtained from isolation in the root systems of chili farmers in Bebesen Subistrict, Silih Nara Subdistrict, and Peugaseng Subdistrict, Central Aceh District, specifically the ability of rhizobacterial isolates to inhibit the growth of stem rot pathogens in chili plants, the ability to produce Indole Acetic Acid (IAA), the ability of phosphate dissolving rhizobacteria, the production of siderophore compounds, and the ability to produce HCN compounds can be seen in Table 2. Table 2 shows that the ability of various rhizobacterial isolates to inhibit the growth of pathogenic test colonies, the ability to produce IAA growth hormone, the production of siderophore compounds, HCN compounds and the ability to dissolve phosphates variance depending on the rhizobacterial isolates tested.

In vitro rhizobacterial inhibitory test results showed that all rhizobacterial isolates had the ability to inhibit the growth of test pathogenic colonies. It's just that the percentage inhibitory power differs between the tested rhizobacterial isolates.

From Table 2 it can be seen that there is one rhizobacterial isolate which is very high in inhibiting the growth of test pathogenic colonies, namely *Rbks-7* isolates with a percentage of 75.22% inhibition power, then followed by 6 rhizobacterial isolates with high inhibitory power (65.12-69.66%), namely isolates *Rbkp-1*, *Rbkp-3*, *Rbks-6*, *Rbkb-1*, *Rbkb-2*, and *Rbkb-4*. While the rest are 11 rhizobacterial isolates with moderate and low inhibitory activity.

The ability of rhizobacterial isolates to produce growth regulators was also very diverse depending on the rhizobacterial isolates tested. All rhizobacterial isolates produced IAA growth regulators with a range of 3.67 μmL^{-1} filtrate to 19.28 μmL^{-1} filtrate (Table 2). The highest IAA was *Rbkb-2* isolate (19.28 μmL^{-1} filtrate), followed by 3 isolates capable of producing high IAA growth regulators compared to other rhizobacterial isolates, namely *Rbkp-4*, *Rbks-2*, *Rbks-5* rhizobacterial isolates. While the remaining rhizobacterial isolates produce IAA regulators with an amount of less than 7.67 μmL^{-1} filtrate. The ability of phosphate dissolving rhizobacterial isolates turned out that from 18 isolates analyzed 9 isolates had the ability to dissolve phosphate (Table 2), 9 isolates did not have the ability to dissolve phosphate. Whereas the ability to produce siderophore compounds, from 18 rhizobacterial isolates analyzed 11 isolates had the ability to secrete siderophore compounds, 7 isolates did not show their ability to produce siderophore. All rhizobacterial isolates turned out to produce HCN compounds, only the amount was different. Among the 18 rhizobacterial isolates analyzed there was one isolate that produced very high HCN compounds, 6 isolates had the ability to produce HCN compounds in higher amounts than other isolates (Table 2). A total of 7 isolates produced HCN compounds in moderate amounts and 4 rhizobacterial isolates only produced low amounts of HCN compounds. All rhizobacterial

isolates turned out to produce HCN compounds, only the amount was different. Among the 18 rhizobacterial isolates analyzed there was one isolate that produced very high HCN compounds, 6 isolates had the ability to produce HCN compounds in higher amounts than other isolates (Table 2). A total of 7 isolates produced HCN compounds in moderate amounts and 4 rhizobacterial isolates only produced low amounts of HCN compounds.

The role of rhizobacteria as a bio-control agent is related to its ability to produce antibiotic compounds, siderophore production, and production of hydrogen cyanide (HCN). Whereas its ability to act as rhizobacteria is a candidate for plant growth booster agents, competitive rhizobacteria are able to colonize roots and utilize exudates and glycates released by plant roots (Pieterse *et al.*, 2002). The ability of rhizobacteria to colonize roots is an important stage in connection with its role as a plant growth stimulant rhizobacteria (Lugtenberg *et al.*, 2001). The ability to fix nitrogen, dissolve phosphate (P), and produce growth regulators (auxin, gibberellin, and cytokinin) has been widely reported as a mechanism of rhizobacteria in its role as agents driving plant growth and production (Bai *et al.*, 2002; Bae *et al.*, 2007). Indole acetic acid is also produced by *P.aeruginosa*. In addition to synthesizing indole acetic acid (Sutariati, 2006) it is known that isolates of *P.fluorescens* also produced gibberellins and cytokines. Likewise strain of *Bacillus* spp. able to synthesize indole acetic acid (IAA), gibberellins, and cytokines. The ability to synthesize IAA was also found in rhizobacterial isolates from the *Serratia* spp group.

Table 2. Ability of Various Rhizobacterial Isolates to Inhibit the Growth of *P. capsici* Pathogen Colonies In Vitro, Producing Auxin (Indole Acetic Acid or IAA) in Media containing Triptophan Amino Acid, Dissolving Phosphate, Producing Siderophore and HCN Compound Production.

Group of Rhizobacteria	Parameters of the Ability of Various Rhizobacterial Isolates				
	The inhibitory power of Rhizobacteria (%)	IAA content (µ/ml filtrate)**	Ability to Dissolve Phosphates *	Siderophore Production (Abs λ 550 nm)	HCN production **
Rbkp-1	69.66 ab	6.54 cde	-	0.000	+++
Rbkp-2	31.88 g	5.55 def	-	0.037	+
Rbkp-3	65.22 abc	7.10 cd	+	0.194	+++
Rbkp-4	52.99 b-f	12.72 b	+	0.139	++
Rbkp-5	54.11 b-e	5.59 def	+	0.116	++
Rbks-1	59.66 a-d	5.58def	+	0.136	++
Rbks-2	36.33 fg	13.55 b	-	0.137	+
Rbks-3	36.33 fg	3.67g	-	0.000	-
Rbks-4	56.33 bcd	7.67 c	-	0.000	++
Rbks-5	48.55 c-g	13.36 b	-	0.119	++
Rbks-6	67.44 ab	7.23 c	+	0.000	+++
Rbks-7	75.22 a	4.87 cd	+	0.159	++++
Rbkb-1	65.22 abc	5.00efg	+	0.144	+++
Rbkb-2	65.22 abc	19.28 a	+	0.077	+++
Rbkb-3	37.44 efg	4.27fg	-	0.000	+
Rbkb-4	69.66 ab	6.53 cde	+	0.000	+++
Rbkt-1	46.33 d-g	5.36 ef	-	0.015	++
Rbkt-2	53.00 b-f	3.99g	-	0.027	++

Description: * For phosphate solvent activity: + positive reaction, halo-shaped, - negative reaction, not halo-shaped. ** Fig.s in the column with the same letters are not significantly different based on the DMRT test at α = 0.05. ** for HCN production: color of filter paper, +++ brick red, ++ dark brown, + light brown, and - yellow.

The Influence of Seed Treatment using Rhizobacteria Boosting Plant Growth to the Process of Seed Germination and Growth of Chili Plant Seeds

The results of the analysis of the variety of seed treatments before planting using various types of rhizobacteria as a result of isolation from the root chili system of healthy chili plantations showed that the pre-planting seed treatment using various rhizobacterial isolates significantly affected all viability and vigor of growing strength. The average value of the variable viability and vigor of the growth of red chili seeds as a result of the treatment of seeds

using rhizobacteria is presented in Table 3. The treatment of pre-planted chili seeds using rhizobacteria as a result of isolation from the root chili system of healthy chili was shown to be able to increase the viability observed based on the maximum potential growth variable and germination.

While the vigor of seed growth strength, also increased from seeds that have been treated with rhizobacteria before planting, this is evident from the results of evaluation based on variable vigor of growth strengths such as vigor index, relative growth speed, growing simultaneity, time needed to reach 50% total germination relative (T_{50}) and normal sprout dry weight (Table 3). The increase in these two parameters was significantly higher when compared to seeds without treatment, although the results of seed treatment with rhizobacteria in several isolates, some of which have not provided a significant effect of increasing the value of vigor for growth strength.

Table 3 shows that total seed viability was observed based on maximum growth potential, among 18 rhizobacterial isolates tested on seed treatment, it was found that only *Rbkp-3* isolates had a significant effect of increasing viability compared to untreated (control) seeds. While 17 other isolates did not provide a significant increase in the value of viability. Based on the seed germination power variables, none of the rhizobacterial isolates were able to provide an increase in viability based on the significant germination of seeds. Nevertheless, there are relatively few isolates which tend to give an increase in the value of seed germination. In the growth strength measurement variable vigor observed based on the vigor index variable there are 2 isolates that have the potential to be candidates for rhizobacterial agents that promote plant growth. The two isolates, namely *Rbks-6* and *Rbkb-3* isolates significantly gave an increase in the seed vigor index value that was treated before planting.

Table 3. Average Maximum Growth Potential, Germination Power, Simultaneous Growth, Vigor Index, Relative Growing Speed, T_{50} (Time taken to reach 50% relative total germination), Normal Sprout Dry Weight of chili seeds as a result of seed treatment using plant growth stimulant.

Treatment of Rhizobacteria	Total Viability	Potential Viability	Vigor Power Grows				
	Maximum growth potential (%)	Germination (%)	Vigor index (%)	Simultaneity grows (%)	Relative growth speed (%)	T_{50} (day)	Normal sprout dry weight (g)
Control (R_0)	86.33 b	91.00 abc	67.33bc	66.33e	62.76e	7.74 a	0.15 d
Rbkp-1	94.00 ab	91.00 abc	77.00ab	77.00d	80.04abc	5.12 cde	0.66 bc
Rbkp-2	94.00 ab	92.67 ab	71.00abc	80.67bcd	73.48d	6.35 bcd	0.51 bc
Rbkp-3	97.33 a	92.67 ab	76.00abc	89.00abc	78.48abc	5.30 cde	0.64 bc
Rbkp-4	97.00 ab	90.33 abc	64.33c	74.00de	74.31bcd	5.27 cde	0.73 bc
Rbkp-5	95.67 ab	91.67 abc	79.33ab	91.33a	83.3 ab	5.12 cde	0.60 bc
Rbks-1	96.67 ab	91.00 abc	75.67abc	86.00abc	80.08abc	4.84 e	0.60 bc
Rbks-2	96.33 ab	93.33 a	77.67ab	92.67a	77.77abc	5.84 b-e	0.42 cd
Rbks-3	96.33 ab	89.33 a-d	70.67abc	65.67e	66.19de	6.78 ab	0.41 cd
Rbks-4	97.00 ab	89.67 a-d	67.33bc	79.67d	73.09cd	5.58 b-e	0.59 bc
Rbks-5	93.67 ab	86.33 bcd	70.33abc	65.00e	76.38abc	5.80 b-e	0.60 bc
Rbks-6	93.33 ab	89.33 a-d	81.00a	86.67abc	80.32abc	6.23 b-e	0.56 bc
Rbks-7	96.67 ab	85.00 cd	77.33ab	87.67abc	81.75abc	4.86 e	0.47 c
Rbkb-1	93.33 ab	92.00 ab	74.33abc	87.00abc	75.79abc	5.81 b-e	0.75 bc
Rbkb-2	90.33 ab	87.67 a-d	75.33abc	67.33e	77.39abc	6.02 b-e	0.83 b
Rbkb-3	94.33 ab	91.00 abc	80.00a	80.67bcd	78.13abc	5.12 cde	0.70 bc
Rbkb-4	94.67 ab	87.33 a-d	74.67abc	89.33ab	84.140 a	4.94 de	0.48 bc
Rbkt-1	94.00 ab	87.33 a-d	77.00ab	87.67abc	81.02abc	5.08 de	0.63 bc
Rbkt-2	96.33 ab	83.67 d	67.00bc	66.00e	73.24cd	6.56 abc	8.84 a

Description: Numbers followed by the same letters in the same column are not significantly different from the 0.05 test level (DMRT).

The chili seeds that were treated with pre-planting seeds using rhizobacteria turned out to germinate more simultaneously than the seeds without treatment. Among the 18 rhizobacterial isolates used in the pre-planting seed treatment, 10 of them were statistically able to increase the simultaneous value of chili seed growth compared to untreated seeds. Eight rhizobacterial isolates have not given a significant increase in the value of simultaneous growth. The chili seeds that were treated with pre-planting using rhizobacteria showed a higher germination rate. Almost all tested isolates were able to increase seed germination rates. Only one isolate did not give a significant increase in germination rate, namely *Rbks-3* isolates.

Seed treatment using rhizobacteria before planting shortens the time required for 50% germination of relative total germination. Except for *Rbks-3* isolates and *Rbkt-2* isolates which did not give effect to shorten the time of T_{50} seeds that were given pre-planting treatment. Other rhizobacterial isolates have a shortening effect on T_{50} time. The T_{50} time of untreated seed was 7.74 days reduced to 4.8-6.4 days on the seeds that were treated before planting. On the dry weight ratio of normal sprouts from seeds that were treated with pre-planting using rhizobacteria on average higher than the dry weight of normal sprouts from the seeds without treatment. Among the 18 rhizobacterial isolates tested in this study, only 2 rhizobacterial isolates, namely *Rbks-2* isolates and *Rbks-3* isolates that have not shown a significant increase in dry weight of normal chili seedlings.

The increase in the value of viability and vigor of chili seeds that received seed treatment before planting with rhizobacteria was thought to be closely related to the role of the rhizobacteria as PGPR. This is as shown in the results of the analysis of the ability of rhizobacteria to produce IAA growth regulators beforehand. All rhizobacteria show their ability to produce IAA-regulating substances. Besides producing IAA growth regulators, some rhizobacteria have the ability to dissolve phosphate. Seed treatment with PGPR rhizobacteria plays an important role especially beneficial in the germination process of seeds under stressful environmental conditions. Root colonization by rhizobacteria increases root growth and development, is resistant to abiotic stress, absorption and utilization of nutrients more efficiently (Bennett, 2002).

Root colonization by rhizobacteria from pre-planting seed treatment increases root growth and development, is resistant to abiotic stress, absorption and utilization of nutrients more efficiently and plant productivity increases. The use of rhizobacteria as a treatment of seeds can play an important role in many aspects of seed quality and is especially useful in the germination process under stressful environmental conditions (Bennett, 2002). The results of the pre-planting chili seed treatment using *Bacillus polimixa* BG25 + *P.fluorescens* PG01 rhizobacteria to reduce anthracnose caused by *C. capsici* were not only effective in controlling pathogens but also spurring growth and producing high-quality seeds (Sutariati, 2006).

Conclusion

The results of detection, exploration and isolation of rhizobacteria from the root system of healthy chili plants obtained 154 rhizobacterial isolates. The antagonistic test results obtained by one rhizobacterial isolate, namely *Rbks-5* isolate has a very high inhibitory power on the growth of pathogenic colonies test *S.rolfsii* and *Phytium* sp. While rhizobacteria *Rbks-6* isolates and *Rbks-7* isolates have very high inhibitory power on the pathogen *S. rolfsii* and *P. capsici*. Rhizobacterial isolates which produced the highest IAA were *Rbkb-2* isolates (19.28 µ mL^{-1} filtrate), followed by 3 isolates capable of producing IAA growth regulators namely *Rbkp-4*, *Rbks-2*, *Rbks-5* rhizobacterial isolates. The ability of rhizobacterial isolates to dissolve phosphate is 9 isolates, 11 isolates produce siderophore, and all isolates produce HCN compounds. The treatment of pre-planting chili seeds using rhizobacteria was able to increase viability of rhizobacteria isolates *Rbks-6* and *Rbkb-3*.

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