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ORIGINAL RESEARCH

The effect of plant growth promoting Rhizobacteria on germination and seedlings growth of chili

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Abstract. Rhizobacteria are bacteria that live in symbiosis with plant roots in the rhizosphere. Some rhizobacteria play rules as plant growth promoting rhizobacteria. The aim of this study was to investigate the effects of seeds on seed germination and seedling growth. An additional aim was to figure out the competence of rhizobacteria isolates to dissolve phosphate, produce acetic indole acid (IAA) and siderophore. Eighteen rhizobacteria isolates were used in this research, and each isolate was analyzed in vitro. Then, the seeds were inoculated with rhizobacteria isolates in suspension then germinated. Seedlings were transplanted into a plastic pot filled with medium soil and compost (in a 2:1 ratio). The germination was observed every day for 14 days, and the seedlings were observed at 4 and 6 weeks after transplanting. The results showed that all the rhizobacteria isolates produced IAA; 13 rhizobacteria isolates were capable of dissolving phosphate; and 12 rhizobacteria isolates produced siderophore. Seedtreatment using RBNA 14, RBNA 13, RBKB 5, and RBSPA 14 was adequate to increase the germination in seed viability and vigor also increase seedling growth.

Keywords: isolate, IAA, phospate, siderophore, transplanting

INTRODUCTION

In total, red chili commodities in Aceh province in 2019 produced 13.09 tons ha^{-1} of product and the potential yield of these commodities was around 20.00 tons ha^{-1} [1]. The productivity cannot reach the average of the potential yield because of the diversity of plant growth in the fields. The growth and production have not yet been optimized, so it is worth exploring efforts to increase plant growth, by using plant growth promoting rhizobacteria (PGPR) and inducing plant systemic resistance [2].

Plant growth promoting rhizobacteria (PGPR) serve many useful roles, such as producing growth hormones (auxins, gibberellins, and cytokinins), nitrogen fixation, solubilizing phosphates, and developing a plant's systemic resistance [2,3]. The genus of rhizobacteria have been shown to increase plant growth, including these various rhizobacteria: *Achromobacter xylosoxidans* [4], *Bacillus* sp. [5], *B. aquimaris* [6], *Burkholderia cenocepacia* [7], *Pseudomonas aeruginosa* [8], *Klebsiella* sp. [9], *Variovorax paradoxus* [10], *Halobacillus dabanensis* [11], *Serratia liquefaciens* [12], *Rhizobium tropici* [13], *Staphylococcus sciuri* [14]. Genus *Bacillus* sp., and *Pseudomonas* sp., are the most frequently used rhizobacteria in the process of optimizing plant growth [15]. Rhizobacteria actively colonize roots and utilize exudates and lysates released by the plant roots as nutrients [2].

Previous studies showed that treatment using rhizobacteria on the seeds before they were planted actually accelerated seed germination, increased seedling growth, and increased vegetative and reproductive plant growth [16]. Rhizobacteria *P. flurescens, B. substillis*, and *S. marsescens* are able to solubilize phosphate

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[17]. The genus *P. putida* and *P. aeruginosa* able to synthesize IAA [18,19].

The application of *B. subtilis, B. pumilus, B. amyloliquefaciens* and *P. fluorescens* effectively increase the germination growth of the seed, vegetative, and reproductive growth of tomato plants [20]. Two rhizobacteria isolates increased the vegetative growth of chili including qualities like plant height, and number of leaves, flowers, and fruit [21]. The utilization of rhizobacteria is capable of increasing plant height, stem diameter, number of leaves, and fruit production [22]. This study aimed to determine the effect of seed treatment using rhizobacteria on the germination and vegetative growth phases of chili seedlings, and to evaluate the ability of rhizobacteria isolates to dissolve phosphate, produce IAA, and enhance siderophores.

METHODOLOGY

Rhizobacteria mechanisms as PGPR agents

Rhizobacteria isolates used in this experiment are the private collection isolates from the results of previous studies that were isolated from the chili rhizospheres. Evaluation was conducted on 18 rhizobacteria isolates to evaluate their ability to produce indole acetic acid (IAA), dissolve phosphate and siderophore, and increase viability and vigor. The growth of the chili seedlings were measured 4 and 6 weeks after transplanting.

The ability of rhizobacteria isolates to produce IAA was analyzed using the Glickman and Dessaux methods [23]. Rhizobacteria isolate was grown for 24 hours, then added to the medium of the amino acid tryptophan 0.5 gL^{-1}. The bacteria was centrifuged (at 10,000 rpm) for 10 minutes, then the supernatant was taken and filtered with a porous nitrocellulose membrane (0.2 μm), afterward determined to be IAA. The growing regulatory substance (IAA) in the pure bacterial filtrate was determined using FeCl3 12 gL^{-1} reagent in 7.9 M H2SO4. FeCl reagent (1 ml) and the bacterial filtrate (1 ml) was inserted into the Eppendorf tube, then the mixture was cached in a dark room at a temperature of 27°C for 30 minutes. Thereafter, the IAA was quantitatively analyzed by spectrophotometer (λ550 nm).

The activity of rhizobacteria in dissolving phosphate was determined in Pikovskaya's medium agar, by adding tricalcium phosphate (TCP) as an insoluble phosphate source. Next, the media was poured into a petri dish, then several holes were made in the media using a cork borer and each was filled with as much as 0.2 ml bacterial suspension. Based on the formation of a clear zone *(halo zone)* around the hole containing the bacterial suspension, it was determined that the isolate has the ability to dissolve phosphate [24].

Siderophore production of rhizobacteria isolates was determined by using media (1 L aquadest, 20 g sucrose, 2 g Lasparagin, 1 g K_2HPO_4, and 0.5 g $MgSO_4$) for 24 hours at room temperature. A suspension of rhizobacteria was centrifuged (at 11,000 rpm) for 30 minutes. The supernatant was separated by a nitrocellulose membrane of 0.2 μm. The siderophore content was determined by adding 1 ml of FeCl 0.01 M to 3 ml of supernatant and as a comparison of the supernatant, one test was carried out without the addition of FeCl. Siderophore detection was measured by spectrophotometer (Novaspec II model) (λ410 nm) [25].

Seed treatment with Rhizobacteria

Rhizobacteria isolates were grown in nutrient agar medium and incubated for 48 hours. Bacteria that were grown were diluted with the aquadest to obtain a population density of 10^9 cfu ml^{-1}, which is equivalent to OD_{600}=0.164 using a spectrophotometer [26]. PM 999 chili cultivar seeds were sterilized by soaking in 50 ml 90% alcohol for five minutes, then rinsed three times using aquadest, and the seeds then laid in laminar air flow cabinet (LAFC) for an hour. After this, a total of 1 g of seeds was soaked in a solution of rhizobacteria isolate each (50 ml) at 27°C in 24 hours. The seeds were then ready to be used as planting material.

Viability and vigor chili seeds examination

The treated seeds were planted in a plastic tray (27 length x 56 width x 5 cm height) with medium soil and compost (2:1). The planting media was first sieved using a mesh sieve, size 5. One hundred seeds were planted in each experimental unit and the process was repeated three times. Furthermore, measurements of the viability and vigor parameters of the seeds were carried out. The total viability variable was observed based on the maximum growth potential (MGP) variable. Observations were made on the last day, 14 days after planting (DAP) by counting the number of seeds that showed growth symptoms. The germination rate was calculated based on the percentage of normal germination; the first count was 7 DAP and the second was 14 DAP. Simultaneously, growth was observed on day 10, based on the criteria for normal germination. The vigor index was calculated based on the number of normal sprouts at the first observation (7 DAP). The growth rate was calculated based on the

Table 1. The ability of rhizobacteria isolates to produce IAA, dissolve phosphates, and produce siderophores

Type of Rhizobacteria Isolate	IAA compound (µ/ml filtration)**	Dissolve phosphate*	Siderophore production (Unit)
RB2NA 1	4.17 bc	+	0.000
RB3SPA 1	2.38 a	+	0.000
RBNA 14	21.15 h	+	0.115
RB1NA 1	2.32 a	-	0.000
RBSPA 2	5.00 cde	-	0.013
RBNA 13	25.58 j	+	0.117
RB2SPA 1	3.92 b	-	0.024
RBSPA 5	6.40 ef	-	0.018
RBNA 4	4.49 bcd	-	0.016
RBKB I	4.05 bc	+	0.000
RBKB 3	5.81 ef	+	0.058
RBKB 5	12.24 g	+	0.143
RBSPA 19	2.52 a	+	0.077
RBNA 9	5.78 ef	+	0.038
RBSPA 12	2.63 a	+	0.000
RBSPA 14	22.12 i	+	0.173
RBNA 8	4.14 bc	+	0.000
RBNA 3	5.14 de	+	0.033

Information: * for the activity of phosphate solvents: + positive reactions, formed halo, - negative reactions, no halo formed. ** The numbers in columns with the same letter are no different from the DMRT test at $\alpha = 0.05$.

total additional normal germinations each day following this. Observations were made every day for 14 days of germination. Time to 50% of total germination (T_{50}), was determined based on the number of seeds germinating each day, up to 50% of total germination [27].

The examination of growth of chili seedlings

At four weeks after planting, seeds that germinated were moved to planting media in plastic pot containers with 500 g of a mixture of soil and compost. To observe the growth of the seedlings, 10 chili plants were separated inito each experimental unit, and the experiment was repeated three times. The farming took place in a greenhouse. Evaluation of the role of PGPR in the growth of chili seedlings was observed up to 8 weeks after transplanting (WAT), the observations of the height and the number of leaves were taken 4 and 6 WAT and the biomass weight of seedlings only at the age of 6 WAT [27].

The data from the observations were analyzed using ANOVA, by SPSS 25.0 program. If the results of the analysis of variance (Test F) showed a significant effect, the data analysis continued with the DMRT test procedure to compare the difference in the average treatment.

RESULTS AND DISCUSSION

Rhizobacteria isolate production of IAA, dissolve phosphates, and siderophores

Table 1 indicates that rhizobacteria isolates were able to produce IAA in different concentrations. Among the 18 rhizobacteria isolates, four rhizobacteria isolates (RBNA 13, RBNA 14, RBSPA 14, RBKB 5) were able to produce a significant amount of IAA compared to other rhizobacteria isolates. RBNA 13 rhizobacteria isolate produced the IAA in the highest concentration (25.573 μml^{-1} filtrate). Three other rhizobacteria isolates of the group also produced high concentrations of IAA: RBSPA 14 (22.132 $\mu ml^{-1)}$, RBNA 14 (21.142 μml^{-1}), and RBSPA 19 isolates (12.242 $\mu ml^{-1)}$. Among the 18 rhizobacteria isolates, 13 isolates showed an ability to dissolve phosphate (Table 1). Twelve rhizobacteria isolates were capable of producing siderophore, while the other isolates were unable to produce siderophore.

Several studies showed that isolates from the rhizobacteria *Pseudomonas* spp. have a significantly higher average IAA production than isolates from the *Bacillus* spp or *Serratia* spp.. The difference in the ability to produce IAA from various rhizobacteria isolates was

Table 2. Average vigor and viability of chili seeds and biomass weight from pre-planted seed treatment using rhizobacteria isolates

Rhizobacteria Treatment	MGP (%)	GC (%)	SG (%)	GR-R (%)	VI (%)	T_{50} (days)	Biomass weight (g)
Control	96.67	95.33 a	60.00 a	61.76 a	60.00 a	7.34 a	0.27 a
RB2NA 1	100.00	100.00 c	85.00 c-i	78.04 cde	95.00cde	7.63 bc	0.59 bc
RB3SPA 1	99.00	98.00 bc	76.00 a-e	71.48 bc	76.00 de	7.80 c	0.41 bc
RBNA 14	100.00	100.00 c	98.00 i	76.49 cde	98.00 e	8.85 hi	0.59 bc
RB1NA 1	100.00	99.33 bc	71.00 abc	72.31 bcd	71.00a-d	7.77 c	0.36 a-c
RBSPA 2	100.00	100.00 c	92.00 e-i	81.37 de	92.00b-e	7.63 bc	0.49 ab
RBNA 13	100.00	100.00 c	95.00 ghi	78.09 cde	95.00cde	10.24 i	0.59 bc
RB2SPA 1	100.00	100.00 c	96.00 hi	75.77 cde	97.00 de	8.35 ef	0.45 bc
RBSPA 5	100.00	98.67 bc	67.00 ab	64.19 ab	69.33abc	7.36 a	0.46 a-c
RBNA 4	100.00	98.67 bc	78.00 b-f	71.09 abc	68.00 ab	8.08 d	0.27a-c
RBKB I	100.00	100.00 c	74.00 a-d	74.38 cde	74.00 a-e	8.30 e	0.48 a-c
RBKB 3	100.00	100.00 c	100.00 i	78.33 cde	99.33 e	8.73 gh	0.43a-c
RBKB 5	100.00	100.00 c	93.00 f-i	79.75 cde	99.33 e	9.29 i	0.60 bc
RBSPA 19	100.00	99.33 bc	79.00 b-g	75.40 cde	79.00 a-e	8.53 fg	0.42a-c
RBNA 9	100.00	100.00 c	89.33 d-i	76.13 cde	89.33b-e	7.63 bc	0.33 ab
RBSPA12	100.00	100.00 c	96.67 hi	79.02 cde	97.33 de	7.59 bc	0.45a-c
RBSPA14	100.00	100.00 c	100.00 i	82.14 e	96.00 de	9.07 i	0.68 c
RBNA 8	100.00	100.00 c	80.00 b-h	73.79 b-e	76.00 a-e	8.31 e	0.56 bc
RBNA 3	100.00	100.00 c	63.33 ab	71.24 bc	75.33a-e	7.44 ab	0.34 ab

Note: The numbers followed by the same letter in the same column are not significantly different at the 0.05 level (DMRT). MGP (maximum growth potential), GC (germination capacity), VI (vigor index), SG (simultaneous growth), GR-R (relative growth rate), and T_{50} (time needed to reach 50% relative total

determined by the isolates examination [28]. *P. fluorescens* has the ability to colonize plant roots more quickly and easily metabolize organic compounds produced by plant roots [29,30]. The ability to colonize plant roots has implications for the amount of tryptophan amino acids obtained from plant root exudates for IAA production by rhizobacteria. The capability of rhizobacteria isolates to act as PGPR is determined by their role in dissolving phosphate [18]. The effectiveness of phosphate solubilizing rhizobacteria is valuable if the phosphate needed by plants is not available in the soil [28]. The PGPR group of bacteria is able to produce siderophore compounds, colonize roots and produce Fe nutrients to be available to plants [31,32], and it is antagonistic to pathogens [33].

Effect of seed treatment with Rhizobacteria on viability and vigor of chili seeds

Seed treatment with rhizobacteria isolates was able to increase the viability and vigor of chili growth compared with the control (Table 2). In the total viability, there was no significant difference between the control and the seed treatment with rhizobacteria. Overall, the chili seeds reached the maximum growth potential (96.67–100.00%). Almost all rhizobacteria isolates were able to increase germination (100%) compared to the control treatment. In the experimental group, growth of 13 isolates was significantly higher (85–100%) than the control treatment. Thirteen isolates were able to increase the relative growth rate (76.13-82.14%) compared to the control treatment, 15 isolates of rhizobacteria were able to increase the vigor index of up to 99.33% compared to the control. Four rhizobacteria isolates were able to accelerate the germination day by 50% (T50), specifically isolates RBNA 14, RBNA 13, RBKB 5, and RBSPA 14. In the dry biomass weight variable of normal germination, eight rhizobacteria isolates had a weight of biomass that is heavier than other treatments.

The increase in viability and vigor of chili seeds from pre-planting seed treatment using PGPR bacteria was closely related to the ability of these bacteria to produce growth regulators and the ability to dissolve phosphate.

Table 3. The effect of the utilization of pre planted rhizobacteria on the growth of chili seedlings

Rhizobacteria Isolates	Plant Height (cm)		Leaves Per Plant (unit)		Biomass weight (g) 6 WAT
	4 WAT	6 WAT	4 WAT	6 WAT	
Rhizobacteria					
Control	17.30 a	21.67 a	9.40 a	11.73 a	7.33 a
RB2NA 1	22.63 bc	26.67 cd	10.77 ab	12.47 ab	9.67 ab
RB3SPA 1	19.47 ab	23.33 bc	10.50 ab	16.10 cd	9.67 ab
RBNA 14	23.60 c	27.33 d	13.10 c	17.10 d	13.00 c
RB1NA 1	22.40 bc	26.67 cd	12.53 bc	13.20 bc	11.00 ab
RBSPA 2	20.10 ab	23.70 bc	10.53 ab	11.08 ab	11.00 ab
RBNA 13	23.43 c	28.27 d	13.33 c	17.40 d	13.00 c
RB2SPA 1	21.97 bc	26.17 cd	11.30 ab	14.90 bc	10.33 ab
RBSPA 5	22.63 bc	27.70 cd	12.40 bc	12.70 bc	12.00 bc
RBNA 4	20.40 ab	23.70 bc	10.70 ab	13.67 bc	11.00 ab
RBKB I	22.20 bc	27.80 cd	12.53 bc	14.00 c	12.00 bc
RBKB 3	21.20 ab	27.33 cd	10.77 ab	12.47 ab	9.67 ab
RBKB 5	23.43 c	27.70 d	12.53 bc	17.10 d	13.00 c
RBSPA 19	21.13 ab	26.13 cd	12.08 bc	15.30 bc	12.00 bc
RBNA 9	20.87 ab	26.50 cd	11.43 ab	13.57 bc	12.67 bc
RBSPA 12	21.03 ab	23.70 bc	10.65 ab	13.08 bc	11.33 ab
RBSPA 14	23.60 c	28.27 d	13.10 c	17.20 d	13.67 c
RBNA 8	22.50 bc	25.13 cd	11.97 ab	13.87 bc	12.00 bc
RBNA 3	22.40 bc	26.67 cd	11.33 ab	13.20 bc	11.00 ab

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

All rhizobacteria showed the ability to produce IAA. The use of PGPR rhizobacteria in the pre-planting treatment of chili seeds plays an important role, especially in the process of seed germination under stressful environmental conditions [34].

The presence of groups of rhizobacteria in the root system of plants causes plants to resist abiotic stresses, increase the absorption of plant nutrients, and utilize nutrients more efficiently [34,35]. Six rhizobacteria isolates (*P. putida, P. libanensis, P. aeruginosa, Bacillus subtilis, B. megaterium,* and *B. cereus*) were able to increase seed germination by 75.60-91.10% compared to controls [36].

The use of rhizobacteria isolates increased germination, maximum growth potential, growth speed, vigor index, and the simultaneous growth of chili seeds [37]. The utilization of rhizobacteria to increase chili germination was observed through germination and the vigor index [38]. The application of rhizobacteria (*Bacillus* sp.) isolates increased the vigor index and germination of chili peppers [39]. Two rhizobacteria isolates (*B. amyloliquefaciens* and *B. cepacia*) increased the vigor index and normal germination of chili peppers [40].

Effect of seed treatment with Rhizobacteria on chili seedling growth

The treatment of chili seeds before planting with various rhizobacteria isolates was also able to increase the height of the chili plant seedlings and the number of leaves, observed at 4 and 6 weeks after transplanting (WAP) (Table 3). Among the 18 rhizobacteria isolates tested, 4 rhizobacteria isolates (RBNA 14, RBNA 13, RBKB 5, and RBSPA 14) significantly increased the height of chili seeds aged 4 DAP (23.60 cm) and 6 DAP (28.17 cm), compared to the control seed treatment. In addition to increasing the vertical growth of the plant, four isolates also significantly increased the number of seed leaves than the control group. Four rhizobacteria isolates also have a higher biomass seed weight compared to the control.

Among the 18 rhizobacteria isolates evaluated, seven isolates i.e. RB2NA1, RBNA14, RB1NA1, RBNA13, RBKB1, RBKB3, RBKB5, and RBNA3, were selected as

potential isolates for plant growth promoting rhizobacteria because those isolates showed a better ability to increase the growth of chili seedlings than other rhizobacteria isolates. The role of PGPR on plant growth proved that the bacteria *B. megaterium* strain M3 was effective in increasing growth in mint (*Mentha piperita* L.) [41], *P. fuoresncens* in tomatoes [42,43], and *S. marcescens* EB67 in maize [3]. Treatment of pre-planted wheat and pea seeds using *Pseuomonas* and *Bacillus* strains significantly increased root growth and plant height [44]. Two isolates of rhizobacteria (*B. megaterium* and *P. fluorescence*) were able to increase the growth of chili plants (plant height, root length, number of fruits) [45].

Six rhizobacteria isolates were able to increase the growth of chili seedlings (high plants, root length, wet weight and dry weight). six rhizobacteria produced IAA and siderophores [36]. The utilization of PGPR rhizobacteria from species *B. subtilis* and *P. fluorescens*, is adequate to increase chili plant height and number of leaves [45]. Two rhizobacteria isolates can be applied to increase chili plant seedling height, number of leaves, and stem diameter [37].

Rhizobacteria isolates can be used to increase the growth of chili plants observed through plant height, number of leaves, accelerate flowering and increase the number of fruits compared to the control [21]. Fourteen rhizobacteria isolates were tested and one isolate from species *B. amyloliquefaciens* was able to increase seed vigor and viability up to 84.75%, and able to increase plant height, number of leaves, wet and dry weight of chili plants [40]. Rhizobacteria produces IAA and dissolves phosphate, thereby increasing the growth of chili plants, which was observed through plant height, stem diameter, root length and plant wet weight [38].

Auxin hormone able to increase cell development, stimulate the formation of new roots, stimulate germination and growth of plant [36]. Soil generally contains a lot of phosphorus, but only a small amount is available to plants. Plants are only able to absorb mono or dibasic phosphate, organic phosphate, or insoluble forms of phosphate, so rhizobacteria must be mineralized or dissolved to become available to plants [3]. Siderophores stimulate the biosynthesis of antimicrobial compounds by increasing the availability of Fe minerals, thereby increasing the ability of rhizobacteria to colonize roots and play an important role in the host plant resistance system as PGPR [2].

Two rhizobacteria isolates (*Lysinibacillus varians* and *P. putida*) produced IAA and dissolved phosphate, thereby increasing chili germination, plant height, number of leaves, root length, wet weight, and plant dry weight [46]. Four isolates were able to boost the plant height, stem diameter and number of cocoa leaves; these isolates also produce IAA and peroxidase enzyme as the PGPR activity [47]. Five rhizobacteria isolates i.e *Azotobacter* sp., *B. megaterium, B.coagulants, Flavobacterium* sp., and *P. atmuta,* are relatively effective to increase the plant height, stem diameter, and the number of chili fruit [48].

CONCLUSION

Based on this study, four rhizobacteria isolates (RBNA 14, RBNA 13, RBKB 5, and RBSPA 14) act as PGPR agents for germination and seedling of chili plants. These isolates are also able to dissolve phosphate, produce IAA and siderophore.

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