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Phosphate solubilizing bacteria inducing systemic resistance with a potential for use as biofertilizer for rice

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ABSTRACT

Induced systemic resistance (ISR) and biofertilizer are two activities in plant protection from pathogens as well as an alternative to the use of traditional fertilizers. The purpose of the study was to determine other features of 4 bacteria that have the ability as phosphate solvents and produce indole and siderophore compounds that determine them as inducers of systemic resistance and indicate their possibility to be applied as biofertilizers in rice plants. This study is experimental with four (4) bacteria that have been identified and characterized molecularly: *Paenibacillus alvei* APR, *Paenibacillus alvei* AP6SR, *Bacillus cereus* RH8SR, and *Bacillus cereus* RH10SR. The results showed that the characteristics of the four bacteria could be applied as biofertilizers. The ability to fix nitrogen was shown by *Paenibacillus alvei* AP4SR, *Paenibacillus alvei* AP6SR and *Bacillus cereus* RH10SR, while the ability to dissolve potassium was shown by *Paenibacillus alvei* AP4SR, *Bacillus cereus* RH8SR, and *Bacillus cereus* RH10SR. Only three isolates were able to increase plant metabolite levels, namely *Paenibacillus alvei* AP4SR, *Bacillus cereus* RH10SR and *Bacillus cereus* RH8SR. Bacterial inoculation had a significant effect on plant height and the number of tillers at the age of 40 days, the number of tillers/plant increased to 56.52%.

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INTRODUCTION

Population growth and increasing demand for food are global problems. Rice is the staple food of the Indonesian people which always gets more attention than other crops. Intensification and extensification of rice crops are always pursued with the aim that the people do not lack food, and are even expected to be self-sufficient in rice. The use of superior seeds followed by the use of

chemical fertilizers resulted in a higher quantity of grain obtained in a single application of chemical fertilizers (Singh et al., 2013).

Currently, sustainable cultivation of rice plants should be carried out, the amount of land that has been degraded and the high number of pathogens attack, causing plant productivity to decrease. Efforts that can be made include the use of safe fertilizers such as organic fertilizers and biological fertilizers which can also overcome plant pathogens. Constantly, soil organic matter undergoes oxidative depolymerization organic compounds increase the reactivity of organic molecules to the mineral phase in the soil aggregation process (de Tombeur et al., 2018). Soil organic matter is the main source of C, H, O, N, P, and S nutrients, their cycle and availability are constantly dependent on the rate of microbial immobilization and mineralization (Liu et al., 2016; Adiaha, 2017).

Plant pathogens are a threat to the productivity of agricultural crops. The use of chemical pesticides is very effective and convenient to use but has the potential to threaten the environment and all types of life on earth. Therefore, the use of biological agents or biofertilizer can be a safer and more sustainable strategy. Biofertilizer help in increasing crop productivity by way of increased BNF, increased availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth through hormonal action or antibiosis, or by decomposition of organic residues. Biofertilizers help increase plant productivity by increasing BNF, increasing the availability or uptake of nutrients through removal, or increasing the stimulation of plant growth through hormonal or antibiotic action, or breaking down organic residues (Mohammadi & Sohrabi, 2012).

The results of previous studies obtained 4 functional bacteria, 2 bacteria from the rhizosphere, and 2 other bacteria from the roots of rice plants that thrived among other rice plants in acid sulphate soils. The characterization and the results of the sequencing and phylogenetic bacteria obtained are bacteria of the genus Bacillus similar to the bacteria *Paenibacillus alvei* strain NBRC 334, *Paenibacillus alvei* strain DSM29, *Bacillus cereus* ATCC 14579, and *Bacillus cereus* strain ATCC 14579, these four bacteria have the ability to dissolve phosphates and can produce indole compounds and siderophores (Dewi & Pujiasmanto, 2019). The test results of the four bacteria had similarities below 97%, suspected to be novelty bacteria, so in this study *Paenibacillus alvei* strain NBRC 3343 was coded as *Paenibacillus alvei* AP4SR, *Paenibacillus alvei* strain DSM29 (*Paenibacillus alvei* AP6SR), *Bacillus cereus* ATCC 14579 (*Bacillus cereus*) and RH8SR. *Bacillus cereus* strain ATCC 14579 (*Bacillus cereus* RH10SR),

Many types of microbes have shikimate pathways that play an important role in inducing the formation of phenol-derived compounds produced by the host plant. Other phenolic components that also play an important role in inducing systemic resistance (ISR) are t-chlorogenic acid, shikimic acid, myricetin, ferulic acid, syringed acid, and quercetin which increased in number (1.5-2 times) in Chickpea leaves after the application of. Pseudomonas aeruginosa PHU094 (Sarma et al., 2015). Potential microbial applications in increasing systemic resistance of plants to pests and diseases mostly use microbial groups that act as biofertilizers.

The mechanism of rhizobacteria in controlling pathogens and diseases is carried out in several ways, including producing antibiosis, competition for space or nutrients, competition for the use of Fe elements through the production of siderophores, as well as by inducing systemic resistance in plants (Van Loon, 2007). Siderophores are one form of the compound with low molecular weight, usually below 1 kDa, which can chelate iron (Fe), this compound increases the competitive potential because siderophores have antibiotic activity and increase iron (Fe) nutrients for plants (Glick, 1995). Siderophores produced by rhizobacteria improve plant health at various levels, improve Fe nutrients, inhibit the growth of microorganisms by releasing antibiotic molecules, and inhibit the growth of pathogens by inhibiting the availability of Fe nutrients for

pathogens (Shen et al., 2013). Siderophores are also stated as compounds that can induce systemic resistance in plants.

Bacillus is a biological agent as efficient biological control and is a very friendly way of dealing with pests, even now a large number of Bacillus strains are effective for controlling pests and diseases (Goswani et al., 2016). Bacteria from the genus Bacillus can also be used as biofertilizers because of their ability to provide nutrients through phosphate dissolution, nitrogen fixation, and phytohormone production (Dobbelaere et al., 2002).

The development of pathogen control using systemically plant pathogen control methods needs to be supported by appropriate research studies with defence systems in production plants. Rice plants are known to produce various kinds of secondary metabolite compounds that play an important role as pest antifeedant, antimicrobial, and defence against abiotic environmental stress. One of the productions of secondary metabolites is phenolic compounds. In addition, responses to changes in plant anatomy morphology are also formed to control pest attacks.

The objective of the study was to analyze other abilities of indigenous bacteria resulting from research to be used as effective biological fertilizers (fixing nitrogen, dissolving potassium) as well as inducing systemic plant resistance (ISR) such as producing phenolic compounds, flavonoids, and tannins as an effort to defend plants against pests, diseases, and abiotic stress.

RESEARCH METHODS

Research Design

The effect of bacteria on growth was only carried out on plant height and number of tillers/clumps at 56 days, using a completely randomized design with 5 treatments consisting of no bacterial isolates, *Paenibacillus alvei* AP4SR, *Paenibacillus alvei* AP6SR, *Bacillus cereus* RH8SR and *Bacillus cereus* RH10SR.with 4 replications, and 3 samples per experimental unit. The location of rice planting was carried out in Sungai Rengas village, with a study duration of 8 months (May – December 2019).

Population and Samples

The sample of research are Bacteria from the 2015 experiment obtained from the roots and rhizosphere of rice grown in acid sulfate soils which have been identified as *Paenibacillus alvei* AP4SR, *Paenibacillus alvei* AP6SR, *Bacillus cereus* RH8SR and *Bacillus cereus* RH10SR.

Tools and Materials

The tools used in this research were autoclave, test tube, petri dish, writing utensil, polybag, buncen, ose, cotton, aluminum foil, rap plastic. While the materials used were; TSA (Tryptone soya agar), TSB (Tryptone soya broth), 70% alcohol, Asbhy media, Aleksandrov media, cotton and chitin colloid, spiritus, rice seeds, and aquadest.

Procedures

The Ability of Bacteria Test

Rejuvenation and reproduction of bacteria

The bacteria used are a collection of research results in 2015 and have been identified molecularly in 2016, so they need to be rejuvenated first. Bacterial rejuvenation was carried out using TSA (Tryptone Soya Agar) media until there was a lot of bacterial growth in the petri dish. Propagation of bacteria by growing bacteria on an inclined agar medium containing TSA was carried out for 7 days.

Test the Ability of Nitrogen Fixing Bacteria

The media used to test the ability of bacteria to fix nitrogen was Ashby media with media composition: 20 g Mannitol., 0.2 g Dipotassium phosphate., 0.2 g Magnesium sulphate, 0.2 g

sodium chloride, 0.1 g Potassium sulphate, 5 g of Calcium Carbonate, 15 g of agar, and 1 litter of aquadest. Ashby's solution was sterilized for 15 minutes. The bacteria to be tested were grown on a petri dish containing 1 ml of Ashby media. Placed in an ambient room (temperature 25°C) for 7-14 days. Bacteria that have the ability to fix nitrogen are characterized by a clear zone around the colony.

The Ability of Bacteria to Dissolve of Potassium

The medium used to test the ability of bacteria as a K solvent was Aleksandrov's medium with a media composition of 5 g Glucose, 0.5 g $MgSO_47H_2O$, 0.006 g $FeCl_2$., 0.1 g $CaCO_3$, 2 g Ca_2PO_4, 3 g Feldspar and 1 litter of aquadest. Aleksandrov media was sterilized for 15 minutes. Bacteria are grown in media that is placed in a petri dish. then placed at room temperature (ambient room) for 7-14 days. Bacteria that have the ability as K solvent are characterized by the formation of a seed zone around the bacterial colony.

Maceration Extraction of Rice Samples (Ridlo et al., 2017)

Rice plants that will be macerated are 56 days after planting, are rice plants that Rice have been inoculated by bacteria by soaking the seeds in a solution containing bacteria for 1 x 24 hours, then planted. Extraction is done by maceration using ethanol (Setha et al., 2013). A sample of 150 g was cut ± 5 mm, macerated in 400 mL of ethanol for 24 hours, and vacuum filtered, then the filtrate was evaporated using a rotary evaporator at a temperature of 60°C to obtain the extract yield.

The effect of bacteria on growth

Bacterial inoculation, nursery and planting

Before sowing, the seeds were inoculated for 1 x 24 hours in TSB media with the appropriate bacteria for treatment. Immersion using an infusion bottle. then sown in plastic boxes measuring 15 cm x 25 cm, until the seedlings are ready to be moved at the age of 7 days after sowing. he planting medium used was 8 kg/polybag consisting of a mixture of acid sulphate soil that had been air-dried and sieved as much as 7.5 kg, then 500 g of banana peel compost was added.

Data Analysis

Total phenolic analysis of rice plants

Analysis of the phenolic content of the extract was carried out based on the modified method of Farhan et al., (2012). Rice extract with a concentration of 300 ppm was dissolved in 5 mL of methanol. 0.2 mL, the extract was then added with 1 mL of Folin-Ciocalteu reagent (1:10 v/v) and 3 mL of 2% (w/v) Na2CO3 solution. The solution was homogenized and incubated at room temperature for 1 hour in no light (dark). The absorbance of the solution was measured at 765 nm with a UV-VIS Spectrophotometer. With the same procedure, standard curves of gallic acid were made with concentrations of 0, 20, 40, 60, 80, 100 ppm. Determination of the total phenolic content of rice used the standard curve equation for gallic acid and calculated using the following formula:

Total phenol = Phenolic content x (extract dilution volume measured extract volume) weight extract.

Analysis of total flavonoids in rice plants (Sultana et al., 2009)

Total flavonoid was performed by using the colorimetric method with minor modification. In brief, 0.5 g rice sample extracts of plant material were mixed with 10 mL aqubidestilata. Furthermore, 1 mL of the extracted sample was taken and put in 4 ml of water in a test tube, followed by adding 0.3 ml 5% $NaNO_2$ solution. After 5 minutes, 0.3 ml of 10% $AlCl_3$ were added and let it stand for 6 minutes before the addition of 2 ml of 1M NaOH. The solution was

then diluted with 2.4 ml water and mixed well. The absorbance was measured at 510 nm using a spectrophotometer. Quercetin standard solution (20 – 140 µg/ml) was prepared with the same procedure to obtain the calibration curve and the blank solution was using distilled as a sample. Total flavonoid content was presented as percentage of total quercetin equivalent per 1g extract (mg QE /g).

Analysis of total tannins in rice plants (Mukhriani et al., 2014)

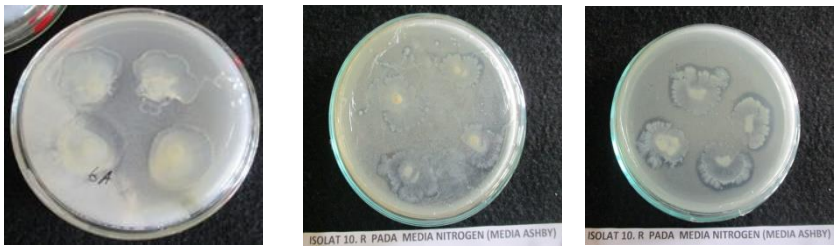
The tannin content test of rice samples was carried out by first making a standard tannic acid curve using 0.1 g of tannic acid dissolved in 100 mL of distilled water, and making a series of 20, 40, 60, 80, and 100 ppm dilutions. Measurement of the tannins of rice samples was carried out by taking 0.5 g of extract and diluting it with distilled water to 10 ml. then 1 ml of the sample was pipetted, then put into a 10 mL tube containing 7.5 mL of distilled water. Then 0.5 mL of Folin Ciocalteau reagent was added and incubated for 3 minutes, then 1 mL of saturated Na_2CO_3 solution was added. Then it was incubated for 15 minutes, and the wave absorption was measured on a 740 nm UV-Vis spectrophotometer. The absorbance obtained was measured for its concentration using the standard curve regression equation that had been made.

RESULTS

The results of the nitrogen-fixing ability test of the four bacteria tested showed that *Bacillus cereus* RH8SR did not have the ability to fix nitrogen, while those with nitrogen-fixing ability were *Paenibacillus alvei* AP4SR, *Paenibacillus alvei* AP6SR, and *Bacillus cereus* RH10SR (Figure 1). Bacteria *Paenibacillus alvei* AP6SR has an ability that exceeds the bacteria *Paenibacillus alvei* AP4SR and *Bacillus cereus* RH10SR, which is characterized by the size of the clear zone of bacteria which is wider than other bacteria, quantitatively, it is shown in Table 1.

Table 1. Diameter of the Clear zone of Bacteria in fixing Nitrogen

Bacteria	Diameter of clear zona (cm)
Paenibacillus alvei AP4SR	0.5
Paenibacillus alvei AP6SR	1.8
Bacillus cereus RH10SR	0.5



(a) (b) (c)

Figure 1. Clear zone of *Paenibacillus alvei* AP4SR (a), *Paenibacillus alvei AP6SR (b)* dan *Bacillus cereus* RH10SR (c) in nitrogen fixing

Note: Clear zone of *Paenibacillus alvei* AP4SR (a), *Paenibacillus alvei AP6SR (b)* dan *Bacillus cereus* RH10SR (c) in nitrogen fixing

Testing the four bacteria in dissolving K, there were only 3 bacteria capable of dissolving K, namely *Paenibacillus alvei* AP4SR, *Bacillus cereus* RH8SR and *Bacillus cereus* RH10SR, which were characterized by the presence of a clear zone surrounding the colony (Figure 2), while *Paenibacillus alvei* AP6SR did not have the ability to dissolve K. The clear zone formed on

bacteria that can dissolve K looks like *Paenibacillus alvei* AP4SR has the widest clear zone, followed by *Bacillus cereus* RH8SR and *Bacillus cereus* RH10SR. The clear zone of these three bacteria is quantitatively found in Table 2. *Paenibacillus alvei* AP4SR is a bacterium that forms the highest clear zone of 1.2 cm, this indicates that this bacterium has the ability to dissolve K greater than other bacteria.

Table 2. Diameter of the Clear Zone of Bacteria in Dissolving K

Bacteria	Diameter of clear zona (cm)
Paenibacillus alvei strain AP4SR	1.2
Bacillus cereus RH8SR	1
Bacillus cereus strain RH10SR	0.8

(a) (b) (c)

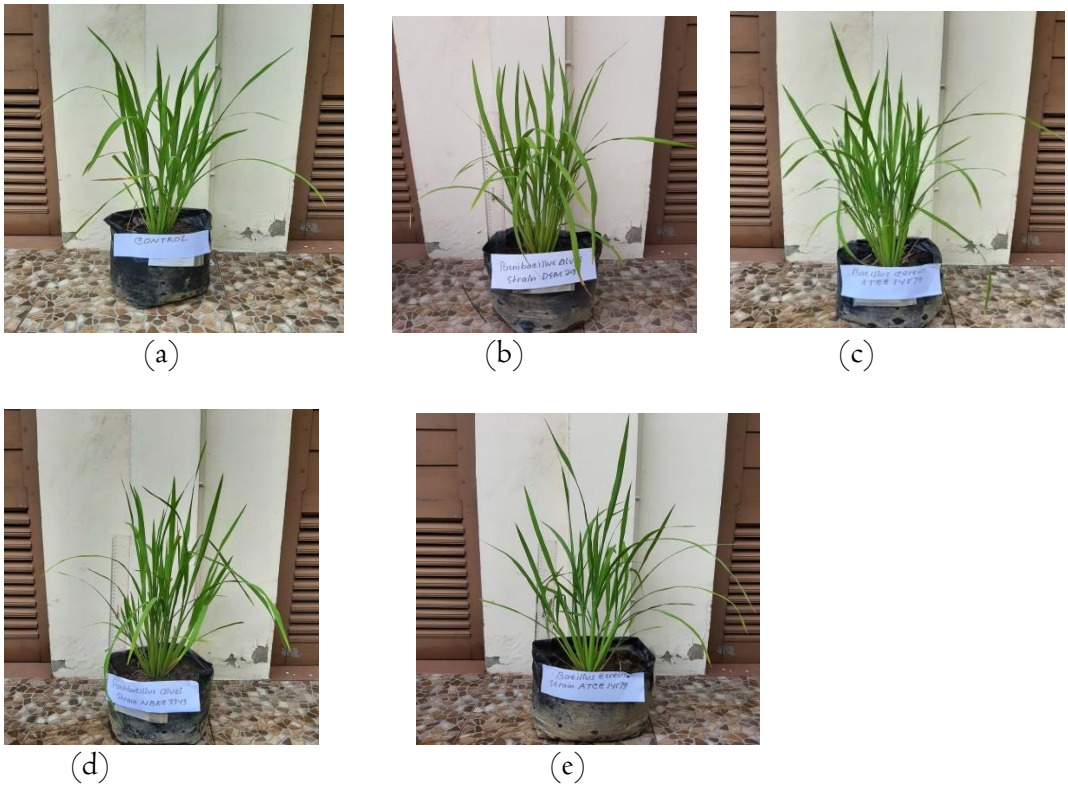
Figure 2. Clear zone *Bacillus cereus* RH10SR (a), *Bacillus c*ereus RH8SR (b) dan *Paenibacillus alvei* AP4SR (c) bakteria in dissolving K

Note: Clear zone *Bacillus cereus* RH10SR (a), *Bacillus c*ereus RH8SR (b) dan *Paenibacillus alvei* AP4SR (c) bakteria in dissolving K

The results of the research showed that the application of bacterial isolates had a potential effect on changes in the phytochemical profile of the plant metabolites produced. Quantitative measurements of phenolic compounds, flavonoids, and tannins carried out on samples of rice roots, stems, and leaves showed significant differences. The four bacterial isolates played more of a role in increasing the production of metabolite compounds in roots and rice stems. Unlike the case with the concentration of compounds in the leaves, which was actually lower than rice that was not inoculated with bacteria. The data showed that the isolate of *Paenibacillus alvei* AP4SR was able to increase the highest flavonoid accumulated in the stem, which was 29.76 (mgQ/g). Meanwhile, *Bacillus cereus* RH10SR isolate was able to increase the tannin content of roots by 55.79 (mgTAE/g), and stems by 70.92 (mgTAE/g). Meanwhile, *Bacillus cereus* RH8SR isolate had an effect on increasing phenol levels of 527.97 (mgGAE/g) in root samples compared to stems and leaves. This isolate was also known to induce an increase in phenolic compounds in stems of 867.03 (mgGAE/g) and 126.51 (mgQ/g) of flavonoids. Unlike the case with the administration of *Paenibacillus alvei* AP6SR isolate which did not affect the increase in levels of metabolites, as evidenced by lower levels of phenols, flavonoids, and tannins compared to control rice that was not inoculated with bacteria (Table 3). This test showed that the simultaneous application of the four bacteria to the rice root samples did not necessarily increase the production of certain compounds in the roots, some data illustrates that the association of microbes in the roots can actually reduce the accumulation of other compounds in the stems and leaves.

Table 3. Levels of secondary metabolites of bacterial inoculated roots, stems and rice samples

Inokulum Bakteri	Root			Stems			Leaves		
	Fenol (mg GAE/g)	Flavonoid (mg Q/g)	Tanin (mg TAE/g)	Fenol (mg GAE/g)	Flavonoid (mg Q/g)	Tanin (mg TAE/g)	Fenol (mg GAE/g)	Flavonoid (mg Q/g)	Tanin (mg TAE/g)
Paenibacillus alvei AP4SR	446.72	29.76	55.01	112.34	0.48	14.28	317.03	43.38	24.52
Paenibacillus alvei AP6SR	498.28	28.66	53.9	193.59	3.73	18.06	201.41	7.55	18.51
Bacillus cereus RH8SR	527.97	28.37	55.01	127.97	0.77	15.73	179.53	8.37	18.17
Bacillus cereus RH10SR	490.47	23.44	55.79	867.03	126.51	70.92	206.09	32.42	19.29
Control/ Without bacteria	502.97	27.38	50.45	284.22	37.93	22.4	771.72	77.87	69.03



(a) (b) (c)

(d) (e)

Figure 3. Without inoculation bacterial (a), *Paenibacillus alvei* AP6SR (b), *Bacillus cereus* RH8SR (c), *Paenibacillus alvei* AP4SR (d) dan *Bacillus cereus* RH10SR (e)

Note: Without inoculation bacterial (a), *Paenibacillus alvei* AP6SR (b), *Bacillus cereus* RH8SR (c), *Paenibacillus alvei* AP4SR (d) dan *Bacillus cereus* RH10SR (e)

The results of the data analysis of bacterial inoculation on seeds gave a significant effect on plant height and number of tillers/clumps planted on acid sulphate soil at the age of 8 weeks (56 days). The average plant height and number of tillers/clumps of different test results are shown in Table 4 and Figure 3. Bacterial inoculation on rice seeds was able to provide significant differences in plant height and number of tillers per clump at 8 weeks (56 days) of age when compared to no bacteria. The best plant height was seen in the inoculation of *Bacillus cereus* RH10SR and *Paenibacillus alvei* AP6SR bacteria. Both of these bacteria were able to exceed the plant height inoculated with *Paenibacillus alvei* AP4SR and *B. cereus* RH8SR.

Table 4. Bacterial inoculation on mean plant height and number of tillers/clumps at 8 weeks (56) days of age.

Bacterial inoculations	Everage plant height	Average number of tillers per clump
Without inoculation	60.5 a	7.75 a
Paenobacillus alvei AP4SR	62.4 b	13.5 ab
Paenibacillus alvei AP6S*R*	63.5 c	11.5 ab
Bacillus cereus RH8SR	62.5 b	15.25 b
Bacillus cereus RH10SR	64.2 c	17.25 b

Numbers followed by the same letter in the same column are not significant difference in HSD 0.05.

DISCUSSION

The results of this study both phosphate solubilizing bacteria *Paenibacillus alvei* AP4SR and *Paenibacillus alvei* AP6SR have the ability to bind N, this is in accordance with the statement of Timmusk and Wagner (2004) which states that *Paenibacillus* bacteria have the ability to bind N, but from these two *Paenibacillus* bacteria, *Paenibacillus alvei* AP6SR has a *greater* ability to bind N. In this study it was also found that of the two *Paenibacillus* bacteria that were able to dissolve K only *Paenibacillus alvei* AP4SR, while *Paenibacillus alvei* AP6SR did not have the ability to dissolve K.

The two phosphate solubilizing bacteria *Bacillus cereus* used in this study have the ability to dissolve K, and between the two, *Bacillus cereus* RH8SR has the ability to dissolve K greater than *Bacillus cereus* RH10SR, indicated by the clear zone area of 1 cm. however, *B. cereus* RH8SR did not have the ability to bind N, so only *Bacillus cereus* AP10SR had the ability to bind N, and dissolve K The four bacteria tested in this study can be declared as biofertilizers in accordance with the statement of Gupta et al., 2012, that bacteria that have the ability to produce indole compounds such as IAA and the presence of siderophore production can be used as biofertilizers, moreover the bacteria tested in addition to producing indole, compounds siderophores, can dissolve phosphates, bind N and can dissolve K.

Phenols, flavonoids, and tannins play an important role in inducing plant resistance. Increased levels of phenol in dicotyledonous and monocot plants correlated with the ability of resistance to several major plant pathogens (Vidhyasekeran, 2001). *Research* shows that one of the factors supporting the high resistance of plants to disease is the increased levels of phenols or polyphenols that can prevent pathogen colonization and development of symptoms (Lyon & McGill 1988). This was also found in samples of rice roots inoculated with *Bacillus cereus* RH8SR bacteria, where the bacteria were able to induce systemic resistance of plants through defense of phenolic compounds. One of them is the resistance of rice to attack by the pathogen *Xanthomonas oryzae* (Xoo) which causes Bacterial Leaf Blight which increases when there is an increase in the levels of phenol, chitinase, -1,3-glucanase, and protein thaumatin in the leaves (Babu et al., 2003). Phenolic compounds are known to contribute dynamically to the growth process, one of which composes the outer tissue components, and plays a role in fighting biotic and abiotic stresses (Awika et al., 2003). Increasing plant resistance to biotic and abiotic stress through the involvement of phenols is carried out based on the ability of compounds to eliminate ROS in cells, through antioxidant mechanisms (Chiappero et al., 2019).

In addition to phenols, flavonoid compounds *were* also increased in plants inoculated with *Paenibacillus alvei* AP4SR, this condition plays an important role in increasing plant resistance against pathogens, herbivore pests, and environmental stress. Flavonoids are very often found to accumulate in cells. Several studies have found that the production of flavonoid components in rice can occur through the OsmiR396-OsGRF8-OsF3H pathway, and is known to play an important role in preventing Brown Planthopper attacks in rice (Dai et al., 2019). The ability of flavonoids as protective compounds is divided into 2 major groups: preformed and induction components. The induction component is the plant response through the production of flavonoids when there is physical injury, infection, and environmental stress (Treutter, 2005). In addition, flavonoids have been known for a long time as UV protectors (Rozema et al., 1997), this happens based on the ability of epidermal flavonoids to absorb UV radiation to protect the inner tissues of leaves and stems. Flavonoids in plants are closely related to associative microorganisms which also play a role in production at the cellular level. The suitability of the association of *Paenibacillus alvei* AP4SR in the tested rice plants was able to indirectly increase the levels of flavonoids which function as antioxidants when there is an excess of light and prevent the accumulation of ROS, as a natural antioxidant function (Tattini et al., 2004).

Tannins are a class of compounds that were also found to increase in rice plants inoculated with *Bacillus cereus* RH10SR. Tannins are known to play an important role in plant defense against herbivorous pests. This is influenced by the smell and taste of tannin which tends to be bitter and is not liked by various pests. The *research* conducted found that the increase in the tannin content of the mango skin was directly proportional to its resistance to the attack of the fruit fly *Bactrocera dorsalis* (Hendel) (Rashmi et al., 2017). Tannins are compounds belonging to the phenol group with a molecular weight of 500-3000 Da, and are commonly found in leaves, bark, fruit, wood, and roots, especially in vacuoles, and play a role in resistance to attack by herbivorous insects (Venisse et al., 2002). The results of the research that has been carried out to see the effectiveness of *Paenibacillus alvei* AP4SR, *Paenibacillus alvei* AP6SR, *Bacillus cereus* RH8SR and *Bacillus cereus* RH10SR in increasing the resistance of rice plants against pests, pathogens, and environmental stress based on levels of phenolic compounds, flavonoids, and tannins, become a real picture. There is an interesting correlation and relationship between potential bacteria on the growth response and biochemistry of rice plants. Through the potential for induction of production of phenolic compounds, flavonoids, and tannins, it is hoped that plant growth plant height and number of tillers per clump The results of the data analysis of bacterial inoculation on seeds gave a significant effect on plant height and number of tillers/clumps planted on acid sulfate soil at the age of 8 weeks (56 days). The average plant height and number of tillers/ clumps of different test results are shown in Table 3. Bacterial inoculation on rice seeds was able to provide significant differences in plant height and number of tillers per clump at 8 weeks (56 days) of age when compared to no bacteria. The best plant height was seen in the inoculation of *Bacillus cereus* RH10SR and *Paenibacillus alvei* AP6SR bacteria. Both of these bacteria were able to exceed the plant height inoculated with *Paenibacillus alvei* AP4SR and *Bacillus. cereus* RH8SR.

The effect of bacteria given on the height of rice plants grown in acid sulfate soil is due to the ability of bacteria as phosphate solvents, N binders and K solvents, which are able to provide sufficient nutrients or nutrients for plant growth, *where* the three elements N, P and K are macronutrients needed for plant growth. It is well known that element P is an essential nutrient needed by rice plants (Yahya et al., 1989; *Kim* et al., 1998), it is absorbed mainly during the vegetative growth and plays a role in plant metabolic processes such as root development, photosynthesis, nutrient transport in plants, meiosis, phospholipids in the wall. cells and plant reproductive parts (Geethalakshmi et al., 2017).

The number of tillers/clumps of rice plants aged 8 weeks (56 days) from seeds inoculated with bacteria produced the most tillers compared to control (without bacteria). The increase in the number of tillers per clump with the addition of bacteria was 34.87% to 56.52%. This increase was due to the ability of bacteria in addition to having the ability to dissolve phosphate, but also to have the ability to bind N and dissolve K.

Another supporting factor is that the bacteria used in this study have the ability to produce indole compounds. Indole compounds are phytohormone compounds produced by microorganisms. Some researchers state that this indole compound can be in the form of auxins, (Aly et al., 2012), cytokinins (Garcia de Salamone et al., 2001), gibberellins (Joo et al., 2004). This compound can be a growth promoter in plants.

CONCLUSION

The four phosphate solubilizing bacteria tested can be declared as biofertilizers, where the bacteria have the ability to dissolve phosphate, fix nitrogen (N), dissolve potassium (K), in addition to producing indole compounds and siderophores. The three phosphate solubilizing bacteria that have the ability to bind N are *Paenibacillus alvei* AP4SR, *Paenibacillus alvei* AP6SR, and *Bacillus cereus* RH10SR, while those that can dissolve K are *Paenibacillus alvei* AP4SR, *Bacillus cereus* RH8SR and *Bacillus cereus* RH10SR. The isolate of *Paenibacillus alvei* AP4SR was known to be the best in increasing the accumulation of flavonoids in the stem, which was 29.76 (mgQ/g). Meanwhile, the induction of phenol metabolites was found in rice roots inoculated with *Bacillus cereus* RH8SR with phenol content of 527.97 (mgGAE/g). In addition to these compounds, inoculation of *Bacillus cereus* RH10SR bacteria was found to increase the highest tannin content in root samples by 55.79 (mgTAE/g).

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