Utilization of Phosphate Solubilizing Rhizobacterium Derived from Leguminosae Plants to Stimulating Plant Growth and Induce Systemic Resistance of Soybean (*Glycine max* L. Merrill) to Mosaic Diseases

Made Sudana*, I.G.N. Alit Susanta Wirya and Gusti Ngurah Raka

Faculty of Agriculture, University of Udayana, Bali, Indonesia

*Corresponding author

**A B S T R A C T**

In Indonesia, soybeans are grown in paddy fields after rice harvesting, the production is much lower compared to soybean planted on dry land it is caused by the growth of roots and plants, is not good caused by former land of rice plants is often flooded, a result the soil structure becomes solid, less oxygen, microorganism population is low, and most of the nutrients are still bound in soil granular and poorly absorbed by plant roots. Additionally soybean planting in paddy fields, susceptible to pests and diseases, because of lack of nutrients for plants produce secondary metabolites that can protect plants from pathogens. Therefore conducted research in order to obtain phosphate solubilizing rhizobacterium (PSR) from Rhizosfera of the Leguminosae plants capable of inducing growth of Rhizobium on soybean root. So expect the soybean plants will get enough Nitrogen from the Rhizobium and Phosphate from PSR activity. Isolation of PSR will be isolated from the roots of Leguminosae plants besides soybean in the all districts of Bali. From the results of isolation was obtained 147 isolates Rhizobakteria, after study its ability to stimulate the growth of soybean roots, obtained 58 isolates were able to stimulate root growth. Of the 58 rhizobacteria isolates was tested his ability as a Phosphate solubilizing, was only obtained 14 PSR. Isolates as phosphate solubilizing highest were produced by Rhi 36 and Rhi 35 isolates, both PSR was isolated from roots undis (Cajanus *cajan*) with dilutions index respectively 8.80 and 7.75, while the lows phosphate dilution index was produced by isolates Rhi 52, from *Solanum nigrum*, with 0.06 dissolution Index. Rhi 36 isolates, is the best isolates to increase vegetative soybean plant growth. To improve the generative growth is best to Rhizobacteria isolate of Rhi 9 from *Leucaena glauca* roots. Phosphate solubilizing rhizobacterium as a potential inducer of systemic resistance against virus pathogen of soybean such as Potyvirus and Cucumber Mosaic Virus (CMV) is Rhi 6 isolate from agathi root plants (*Sesbania grandiflora*), Rhi 53 from Pigeon pea plant (*Cajanus cajan*) and Rhi 3 from Sword Jack Bean plants (*Stylosanthes guianensis*). The all three isolates induces systemic plant resistance against the virus by stimulating the plant to produced chemical compounds such as phenol, salicylic acid and peroxidase.

**Keywords**

Phosphate solubilizing rhizobacterium, root of leguminosae, inducer of systemic resistance against virus pathogen.

**Article Info**

Accepted: 20 August 2016
Available Online: 10 September 2016
**Introduction**

In Indonesia, soybeans are grown in paddy fields after rice harvesting, the production is much lower than soybeans planted on dry land. This is caused by the growth of roots and plants, which is not good because the former land of rice plants is often flooded, resulting in the soil structure becoming solid, with less oxygen, microorganism population, and most of the nutrients still bound in granular soil and poorly absorbed by plant roots. Additionally, soybean planting in paddy fields is susceptible to pests and diseases because of the lack of nutrients for plants to produce secondary metabolites that can protect plants from pathogens.

Soybean plants can meet their nitrogen needs by conducting bacterial symbiosis with nitrogen-fixing bacteria from the air, i.e., Rhizobium; but the mechanism of symbiosis between soybean plants and Rhizobium is often compromised by the physical, chemical, and biological soil (Sprent, 1976). In the state of the environment which meets the requirements of growing, symbiosis that occurs is able to meet 50% or even entire crop nitrogen requirement by tying up free nitrogen (Saono, 1981). In addition, the Rhizobium bacteria have a positive impact both directly and indirectly on the physical and chemical properties of the soil, thus increasing soil fertility (Alexander, 1977).

Phosphate in paddy soil is generally available to low for plant, then to make sufficient availability of phosphate in the soil, phosphate solubilizing rhizobacterium from root of Leguminosae plant is needed, which is capable of dissolving phosphate bound to granules of soil organic matter, but it is also expected that microbes are capable of inducing Rhizobium growth.

Hence to increase the growth of Rhizobium in the soil, we need to be sought for the bacteria that live on the surface of plant roots (Rhizobacteria) and able to stimulate the growth of Rhizobium, likewise Rhizobium increasingly forms nodules and plants are getting a lot of intake of nitrogen from the air so that plant growth is lush and healthy. With a good growth of plants, the plant will produce exudates on the surface of plant roots; the exudate is rich in protein, carbohydrates, and vitamins needed for the survival of Rhizobacteria on soybean roots (Zhang et al., 2002). Several types of Rhizobacteria besides being capable of serving as phosphate solvent, also act as inducers of systemic plant resistance to pathogens, e.g., *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Bacillus subtilis* (Munees and Kibret, 2014), and *B. subtilis* strain QST713, can improve soil fertility and acts as a fungicide for some plant pathogens (Ambreen Akhtar et al., 2012), *Brevibacterium iodinum* KUDC1716, promotes plant growth and increase systemic resistance of chili to brown spots disease on plant leaves (Jin-Soo Son et al., 2014). Phosphate solubilizing rhizobacterium, *Enterobacter intermedium* 60-2G can stimulate cucumber plant growth and serves as an inducer of systemic resistance of plants to Scabies disease which is caused by *Cladosporium cucumerinum* (Kim, Young-Cheol et al., 2002).

**Materials and Methods**

Isolation of phosphate solubilizing rhizobacterium from Leguminosae to stimulate the Rhizobium in the form of nodules on soybean plants

Leguminosae plant roots are washed with water until free of soil. Then dried with tissue paper in a sterile petri, when dry, the roots inserted a tube containing sterile water and contains 10% MgCl2, while in the shake shake to release the layers of root exudates into the water, then water containing root
exudates taken to get microbes. 1 ml dissolve in evenly spread on a petri containing NA medium and incubated for 2 days, and isolated the growing bacterial colonies. Further to test the bacteria as phosphate solubilizing, then bacteria were grown back on the media Pikovskaya + PCNB. Rhizobakteria growing by establishing a clear zone around the colony was phosphate solubilizing rhizobacterium being searched (Jin-Soo Son, et al., 2014; Hefdiyah and Maya Shovitri, 2014). While the efficiency index dissolving phosphate (IEP) by Rhizobacteria can be measured by using the following formula, (figure 1)

**Selection of Rhizobium bacteria forming nodules on Soybean Plant Roots**

Rhizobium bacteria were isolated from root nodules of soybean plants from all regencies in Bali. In the laboratory, the roots were washed clean in order to clear the soil attached to them and plant nodule became noticeable. The roots were dried in sterile tissue paper and then the root nodule was put into the test tubes containing 75% alcohol for 5 minutes, in order to sterilize the surface of the root nodules of microbes, then root nodules were re-dried on tissue paper. Next the root nodules were cut using a sterilized scalpel knife, and inoculated into petri containing special media for Rhizobium i.e. YEMA medium (Yeast Extract Mannitol Agar) (Vincent, 1970) consisting of: K₂HPO₄ 0.5 g, MgSO₄·7H₂O 0.2 g, 0.1 g NaCl, CaCO₃ g, Mannitol 10 g, Yeast extract 3 g, PCNB 2 g, Agar 20 g, 1.000 ml of distilled water with a pH of 6.8.

Cultures were incubated for 2-4 days at room temperature and in a dark room; the bacteria that grow were isolated and cultured back in media YEMA without PCNB. To get the Rhizobium bacteria forming nodules on soybean plants were tested with Koch's postulates, bacteria that formed the most root nodules were used in the study.

**Effect of phosphate solubilizing rhizobacterium on the growth of soybean plants**

Rhizobacteria microbes isolated from Leguminosae plants were tested for their ability to stimulate the formation of compounds inducer of systemic resistance in plants soybean, and soybean crop growth and yield of soybean crops in greenhouses were done in the following way:

Preparing phosphate solubilizing rhizobacterium isolates, respectively cultured on Potato peptone glucose (PPG) media and incubated for 2 days until the media looked cloudy and overgrown with bacteria, then the bacteria solution was diluted three times to get a bacterial concentration of 10⁶ cfu / ml.

**Preparing inoculum sources of Rhizobium bacteria**

Rhizobium isolates Btl 8 were cultured on YEM and incubated for 2 days. Further the bacterial suspension was diluted three times to get a concentration of Rhizobium bacteria of 10⁶ cfu / ml.

Seed treatment: Preparing humus media whose raw materials were derived from cow manure waste, humus media were packed in plastic bags 150 g respectively and sterilized using autoclave. After a humus media was cold, the media were inoculated respectively with 1 ml Rhi 11 (*Psophocarpus tetragonolobus*) of leguninosae plants, this treatment was repeated 3 times in order to obtain 39 inoculum bags and one bag as a control. Then the media that already contained phosphate solubilizing rhizobacterium were inoculated back with 1
ml culture of bacteria Rhizobium. Then humus media that had been inoculated with Rhizobium bacteria Rhizobacteria incubated for 30 days, while every day culture was shaken. Soybean seeds before being planted were first imbibed in humus media for 24 hours, 50 soybean seeds in one bag containing Rhizobakteria humus.

Planting seeds: After soybean seeds got imbibition treatment from phosphate solubilizing rhizobacterium, soybean seeds were planted in 2 kg of mix soil media, soil: cow manure (2: 1) in the pot, and humus of former imbibition media was mixed evenly into the planting medium. Every pot was planted with 4 soybean seed would be limited to two plants, which had respectively received seed treatment with Rhizobacteria phosphate solvent treatment in accordance with the treatment tested. The treatments tested were 14 treatments consisting of 13 phosphate solubilizing rhizobacterium isolates from leguminosae plants, and one control without bacteria treatment, i.e. with water treatment alone. The design used was completely randomized design (RAK). At 2 weeks after planting, the plants were inoculated with the virus that causes mosaic disease; the inoculation was done mechanically using the juice of soybean plants, infected by Mosaic virus.

The content of Total Phenol performed according to the method Singleton and Rossi (1965) salicylic acid were analyzed using a modified method Tenhaken and Rubel (1997) and Martinez et al., (2000); Peroxidase activity by measuring levels of a protein using the method of Lowry (1959) in Loebenstein and Lindsey (1961)

During maintenance, the following observation was made:

Plant height, number of leaves, and number of chlorophyl;

Content of N, P, and K of the soil;

Mosaic Symptoms that appeared on plants, tested by Elisa method;

The number of pods, number of seeds and seed weight, planting;

Then also observed were Total Phenol compounds, salicylic acid, and a peroxidase is done in the Biochemistry laboratory.

Phosphate solubilizing rhizobacterium which are capable of producing root nodules of Rhizobium with the largest number and weight and able to provide the best soybean plant growth and protect soybean plants against mosaic virus disease are the best phosphate solubilizing rhizobacterium

**Results and Discussion**

**Phosphate solubilizing rhizobacterium**

From the results of isolation, 247 Rhizobacterial isolates were obtained from Leguminosae plants, after doing testing its ability to stimulate growth in soybean roots, 58 isolates were obtained, which were able to stimulate root growth. The 58 Rhizobacteria isolates were tested of their ability as Phosphate solvent, indeed only 17 Rhizobacteria were obtained Phosphate solubilizing rhizobacterium (Table 1). In table 1, it can be seen that the best Rhizobakteria were isolates Rhi 36 and Rhi 35 (Rhizobakteri pigeonpea plant roots) followed by Rhi 51 and Rhi 53 which are also derived from pigeonpea plants (*Cajanus cajan*).

**Selection of Rhizobium bacteria forming nodules on Soybean Plant Roots**

This research was conducted in order to obtain *Rhizobium* Bacterial isolates that can
have symbiosis with soybean crop in terms of supply of Nitrogen, hence collection of nodules was carried out on soybean crops were from various regions which are the locations of soybean crops, and the results can be seen in Table 2.

In table 2, it can be seen that all Rhizobium isolates capable of forming nodules on the plants of soybean, but isolate Btl 8 has a higher ability in the formation of nodules in comparison to other isolates, isolate Btl 8 was also able to increase soybean plant growth compared to other isolates, isolates btl 8 will be used for further research

Test of Effects of Phosphate solubilizing rhizobacterium on growth and yield of soybean plants

This study is a greenhouse study while the treatment is 13 Phosphate solubilizing rhizobacterium as a result of the isolation of various leguminosae plants, and the application was mixed with Rhizobium bacteria Isolates Btl 8. The observation of the effect of Rhizobacteria on soybean plant growth can be seen in Table 3

In Table 3, it appears that Rhizobacteria isolates Rhi 46 derived from Solanum torvum can improve the highest plant growth in comparison with other treatments followed by Rhi 36 (Cajanus cajan) and Rhi 48 isolates also derived from Cajanus cajan, While the highest number of leaves produced in the treatment of Rhi 26 (Solanum nigrum) but the lowest number of leaves produced by treatment Rhi 46 (Solanum torvum).

The highest amount of chlorophyll in the leaves of soybean plants was produced by treatment of Rh 48 (Cajanus cajan) and the lowest number of chlorophyl in the treatment Rhi 6 (Sesbania rostrata).

In Table 3, although the treatment of Rhi 46, generating the highest plants, yet dry weight of plants at treatment turned out to be quite low, indicating the content of water in plants by Rhi 46 treatment (Solanum torvum) is quite high, in Table 3, it seems that Rhi 9 treatment (Leucaena glauca) and Rhi 53 of Cajanus cajan was able to stimulate the soybean plant roots and is followed by treatment of the Rhi 26. However Phosphate solubilizing rhizobacterium influence on the growth of bacteria forming nodules (Rhizobium) on soybean plants seem that treatment Rhi 26 (of Solanum nigrum) and Rhi 46 (of Solanum torvum) spur the growth of the largest nodule on soybean plant roots.

Rhizobakteria application influence on soybean crop production, shown in Table 4, that the control treatment (plant not given Rhizobakteria) produced the lowest crop production in comparison with plants that got the application Rhizobacteria Phosphate solvent.

Treatment of Rhi 6 (Sesbania rostrata) produced the highest number pods ever, the highest number of seeds the and the largest seed weight in comparison with the other treatments, so here it seems clear that rhizobacteria solvent phosphate originating from Sesbania rostrata plants had a potential for use as biological fertilizer to soybean plants, in Table 4. It appears that Isolate Rhi 6, stimulated the growth of generative phase and Rhi 9 (Leucaena glauca) stimulated vegetative growth of soybean plants.

Effect of Phosphate solubilizing rhizobacterium against induction of systemic resistance of soybean plant to mosaic virus

In Table 5, it appears the treatment of Rhi 36 (of Cajanus cajan) and Rhi 48 (of Cajanus
Cajanus cajan plants) produce the highest peroxidase, Salicylic acid and phenolic compound indicating the plant will be susceptible to mosaic disease, followed by treatment of Rhi 9 (of *Leucaena glauca*) and Rhi 11 (of *Psophocarpus tetragonolobus*). In this study, it appears that the treatment of Rhi 7 (*Vigna sinensis*), did not produce peroxidase, but salicylic acid and high phenolic compounds resistant to Potyvirus but in this study it also appears that the control also has enough production of peroxidase compounds, phenol compounds, and salicylic acid but susceptible to mosaic disease. This might be due to the fact that the plant roots are not disturbed by bacteria, compared with Rhi 53 (*Cajanus cajan*), Rhi 36 (*Cajanus cajan*) and Rhi 7 (*Vigna sinensis*).

If observed the effect of the compounds formed during the reaction inducer of systemic resistance, it turns out that phenolic compounds, salicylic acid and peroxidase do not affect the plant resistant to the virus, it can be seen in Table 5, that the treatment of Rhi 9 (of *Leucaena glauca*) and Rhi 48 (from *Cajanus cajan*) produce all these compounds in high enough quantities, but the plant infected with CMV and Potyvirus virus (fig. 2), while in treatment of Rhi 6 (from *Sesbania rostrata*) produce high amount of peroxidase as well as phenols and salicylic acid, resistant to both viruses, so from this observation it can be said that there are some other compounds formed by plants that are induced by Rhizobacteria, and these compounds need to be investigated further.

### Table 1: Efficiency Index of Rhizobacteria Phosphate Solvent of Leguminosae plant roots after being cultured in media Pikovskaya + PCNB (Rhizobacteria phosphate solvent)

| No | Rhizobacteria phosphate solvent type | Phosphate solvent type | Efficiency Index |
|----|------------------------------------|------------------------|------------------|
| 1  | Rhi 53 (*Cajanus cajan*)            |                        | 4.71             |
| 2  | Rhi 26 (*Solanum nigrum*)          |                        | 1.07             |
| 3  | Rhi 11 (*Psophocarpus tetragonolobus*) |                        | 4.56             |
| 4  | Rhi 52 (*Solanum nigrum*)          |                        | 0.64             |
| 5  | Rhi 55 (*Vigna sinensis*)          |                        | 4.73             |
| 6  | Rhi 46 (*Solanum torvum*)          |                        | 0.70             |
| 7  | Rhi 38 (*Cajanus cajan*)           |                        | 0.11             |
| 8  | Rhi 7 (*Vigna sinensis*)           |                        | 8.42             |
| 9  | Rhi 36 (*Cajanus cajan*)           |                        | 8.80             |
| 10 | Rhi 35 (*Cajanus cajan*)           |                        | 7.75             |
| 11 | Rhi 51 (*Cajanus cajan*)           |                        | 4.85             |
| 12 | Rhi 5 (*Sesbania grandiflora*)     |                        | 1.44             |
| 13 | Rhi 58 (*Vigna sinensis*)          |                        | 0.38             |
| 14 | Rhi 9 (*Leucaena glauca*)          |                        | 0.27             |
| 15 | Rhi 6 (*Sesbania rostrata*)        |                        | 0.29             |
| 16 | Rhi 8 (*Gliricidia sepium*)        |                        | 0.45             |
| 17 | Rhi 48 (*Cajanus cajan*)           |                        | 1.52             |
Table 2 Growth of soybean plants and Rhizobium ability to form root nodules (three-week-old plants)

| Rhizobium type | root | Stalk | leaf | Root nodule | Total | Wet weight (g) | Dry weight (g) | Wet weight (g) | Dry weight (g) |
|----------------|------|-------|------|-------------|-------|----------------|----------------|----------------|----------------|
| Btl 1          | 0.79 | 0.11  | 2.80 | 0.45        | 3.44  | 0.54           |                | 26             | 0.22           | 0.05           |
| Btl 2          | 0.77 | 0.10  | 2.36 | 0.34        | 3.35  | 0.45           |                | 24             | 0.18           | 0.04           |
| Btl 3          | 0.66 | 0.11  | 2.73 | 0.42        | 3.44  | 0.53           |                | 22             | 0.18           | 0.04           |
| Btl 4          | 1.09 | 0.12  | 2.87 | 0.45        | 3.68  | 0.55           |                | 32             | 0.21           | 0.04           |
| Btl 5          | 0.48 | 0.08  | 2.68 | 0.39        | 2.82  | 0.43           |                | 26             | 0.11           | 0.02           |
| Btl 6          | 1.19 | 0.14  | 3.30 | 0.51        | 4.45  | 0.64           |                | 31             | 0.21           | 0.05           |
| Btl 7          | 0.96 | 0.11  | 2.76 | 0.43        | 3.61  | 0.51           |                | 26             | 0.17           | 0.04           |
| Btl 8          | 1.50 | 0.17  | 2.58 | 0.40        | 4.34  | 0.64           |                | 26             | 0.24           | 0.05           |
| Btl 9          | 1.19 | 0.12  | 2.68 | 0.38        | 4.46  | 0.61           |                | 27             | 0.27           | 0.06           |
| Btl 10         | 1.10 | 0.15  | 2.67 | 0.38        | 4.34  | 0.61           |                | 34             | 0.20           | 0.05           |
| Btl 11         | 1.30 | 0.12  | 2.69 | 0.38        | 4.08  | 0.57           |                | 36             | 0.19           | 0.04           |

Table 4 Phenol compound content, Salicylic acid, Peroxidase and plants infected by Virus CMV and Potyvirus in soybean plants after application of Phosphate solubilizing rhizobacterium

| No  | Phosphate solubilizing rhizobacterium | phenol (ppm) | Salicylic acid (ppm) | Peroxidase µm/g/hour | Elisa Test Plants infected with virus |
|-----|--------------------------------------|--------------|----------------------|----------------------|---------------------------------------|
| 1   | Rhi 53 (Cajanus cajan)                | 9            | 24                   | 0.22                 | -                                     |
| 2   | Rhi 36 (Cajanus cajan)                | 14           | 27                   | 0.29                 | +                                     |
| 3   | Rhi 11 (Psophocarpus tetragonolobus)  | 28           | 26                   | 0.56                 | +                                     |
| 4   | Rhi 51 (Cajanus cajan)                | 14           | 13                   | 0.41                 | -                                     |
| 5   | Rhi 6 (Sesbania rostrata)             | 17           | 24                   | 0.89                 | -                                     |
| 6   | Rhi 3 (Stylosanthes guianensis)       | 26           | 29                   | 0.61                 | -                                     |
| 7   | Rhi 26 (Solanum nigrum)               | 19           | 21                   | 0.44                 | -                                     |
| 8   | Rhi 48 (Cajanus cajan)                | 26           | 36                   | 0.92                 | +                                     |
| 9   | Rhi 7 (Vigna sinensis)                | 21           | 17                   | 0.0                  | +                                     |
| 10  | Rhi 9 (Leucaena glauca)               | 28           | 39                   | 0.77                 | +                                     |
| 11  | Rhi 10 (Erythrina variegata)          | 11           | 13                   | 0.0                  | -                                     |
| 12  | Rhi 55 (Vigna sinensis)               | 29           | 28                   | 0.02                 | +                                     |
| 13  | Rhi 46 (Solanum torvum)               | 18           | 16                   | 0.11                 | +                                     |
| 14  | Water (Kontrol)                       | 17           | 15                   | 0.43                 | +                                     |
Table 3 Effect of Phosphate solubilizing rhizobacterium on the soybean plant growth (average / per plant)

| No | Phosphate solubilizing rhizobacterium | Height (cm) | Number of leaves | Chloropyl content SPAD | Number of pods | Number of seeds | Seed Weight (gr) | Plant dry weight (gr) | Number of nodules | Root dry weight (gr) |
|----|--------------------------------------|-------------|-----------------|------------------------|--------------|----------------|-----------------|---------------------|------------------|-------------------|
| 1  | Rhi 53 (*Cajanus cajan*)              | 40.13bc     | 5.25c           | 26.12ab                | 26.665cd     | 54.83cd        | 10.42de         | 3.48cd              | 47.08d           | 1.98cd            |
| 2  | Rhi 36 (*Cajanus cajan*)             | 45.32cd     | 4.83bc          | 26.93ab                | 22.50ab      | 51.16bc        | 10.16cd         | 3.68cd              | 52.5e            | 2.04d             |
| 3  | Rhi 11 (*Psophocarpus tetragonolobus*)| 44.25c      | 4.66bc          | 28.05ab                | 24.50cd      | 52.50cd        | 10.86de         | 3.12c               | 42.91c           | 1.92c             |
| 4  | Rhi 51 (*Cajanus cajan*)             | 39.88ab     | 6.00cd          | 27.20ab                | 23.83b       | 46.33ab        | 9.98cd          | 2.66a               | 50.00de          | 1.63a             |
| 5  | Rhi 6 (*Sesbania rostrata*)          | 38.42ab     | 5.16c           | 25.48a                 | 28.00d       | 59.16d         | 10.81de         | 3.03bc              | 37.08b           | 1.77b             |
| 6  | Rhi 3 (*Stylosanthes guianensis*)    | 42.50bc     | 6.08cd          | 32.12bc                | 22.33ab      | 48.66bc        | 10.10cd         | 3.04bc              | 39.16bc          | 1.85bc            |
| 7  | Rhi 26 (*Solanum nigrum*)            | 39.20ab     | 6.50d           | 29.55bc                | 25.50cd      | 56.50cd        | 11.42e          | 3.14c               | 52.08e           | 1.96cd            |
| 8  | Rhi 48 (*Cajanus cajan*)             | 45.83cd     | 4.50b           | 34.17c                 | 21.16a       | 44.50ab        | 9.83abc         | 2.98abc             | 44.16e           | 2.03d             |
| 9  | Rhi 7 (*Vigna sinensis*)             | 43.30bc     | 4.83bc          | 30.42bc                | 24.16bcd     | 47.83abc       | 9.79abc         | 2.97abc             | 45.83cd          | 1.86bc            |
| 10 | Rhi 9 (*Leucaena glauca*)            | 32.38a      | 4.91bc          | 29.93bc                | 27.16cd      | 47.00ab        | 8.74a           | 4.03d               | 46.25d           | 2.34e             |
| 11 | Rhi 10 (*Erythrina variegata*)       | 43.32bc     | 4.83bc          | 29.45b                 | 24.50cd      | 51.33bc        | 10.66de         | 3.19c               | 35.83b           | 1.53a             |
| 12 | Rhi 55 (*Vigna sinensis*)            | 44.73cd     | 4.75bc          | 33.20bc                | 24.33bcd     | 44.83ab        | 9.46a           | 3.48cd              | 41.66c           | 1.59a             |
| 13 | Rhi 46 (*Solanum torvum*)            | 45.90d      | 3.66a           | 27.32ab                | 23.66b       | 50.00bc        | 10.02cd         | 2.84ab              | 49.00de          | 1.81b             |
| 14 | Water (Kontrol)                      | 43.62bc     | 4.08ab          | 26.50ab                | 23.33ab      | 40.16a         | 9.69abc         | 2.23a               | 25.83a           | 1.64ab            |
Fig. 1 Clear zone of phosphate solubilizing by microbe

IEP = \frac{\text{Diameter of clear zone} (B - A)}{\text{Diameter of colonies} (A)}

IEP = Phosphate dilution index

Fig. 2 Plants Disease to Mosaic Virus

Potyvirus Virus

Virus CMV Virus
In conclusion, from the results of this study, some conclusions can be arrived at, which are useful for cultivating soybeans in paddy fields namely; Rhizobakteria obtained from various Leguminosae plants contained 13 isolates of Phosphate solubilizing rhizobacterium in soil. Rhizobakteria isolated from Cajanus cajan plant roots has a high ability as phosphate solubilizing in comparison with other isolates. Bacteria able to form root nodules and living in symbiosis with soybean crop are isolate Rhizobium Btl 8, this isolates are derived from soybean plant roots that grow in Pedungan area Denpasar. To encourage the best isolate vegetative growth was Rhi 6 of Sesbania rostrata to stimulate the best generative growth is isolate Rhi 9 from Leucaena glauca. Phosphate solubilizing rhizobacterium having a potential as inducer of systemic resistance of soybean plants to the pathogen of CMV and Potyvirus virus is Rhi 6 of Sesbania rostrata roots, Rhi 53 of Cajanus cajan roots and Rhi 3 of the Stylosanthes guianensis roots. It is supposed that the three isolates induce the plant by forming a chemical compound that is not only a phenol, salicylic acid and peroxidase but other compounds which are synergistic with phenolic compound

Acknowledgement

I would like to thank the Direktorat Riset dan Pengabdian kepada Masyarakat Direktorat Jendral Penguatan Riset dan Pengembangan Kementerian Riset Teknologi dan Pendidikan Tinggi for providing research funds so that research can work well.

References

Alexander, M. 1977. *Introduction to Soil Microbiology*. John Willey and Son.New York.
Adisarwanto, T., dan R., Wudianto. 1999. Meningkatkan Hasil Panen Kedelai di Lahan Sawah-Kering-Pasang Surut. Penebar Swadaya. Bogor. 86 hal.
Etha Marista, S., Khotimah, R., Linda. 2013 Bakteri Pelarut Fosfat Hasil Isolasi dari Tiga Jenis Tanah Rizosfer Tanaman Pisang Nipah (*Musa paradisiaca* var. nipah) di Kota Singkawang. Protobiont, 2013. Vol 2(2): 93 – 101.
Doke, N., K. Tomiyama and N. Furuichi. 1982. *Elicitation and supression of hypersensitive response in host-parasite specificity*. pp 79-96. Dalam Yasuji Asada, W.R. Bushnell, Seiji Ouchi, and C.P. Vance (Eds.) *Plant infection, The Physiological and biochemical basis*. Japan Scientific Societies Press, Tokyo
Hanuddin, W., Nuryani, E., Sifilia, I., Jadhika dan, B., Marwoto. 2010. Formulasi biopestisida berbahan aktif Bacillus subtilis dan Pseudomonas florescens dan Corynebacterium sp nonpatogenik untuk mengendalikan penyakit karat pada krisan. *J. Hort.*, 20(3): 247-261.
Hanuddin dan, B., Marwoto. 2003. Pengendalian penyakit layu bakteri dan akar gada pada tomat dan Caisim menggunakan Pseudomonas florescens. *J. Hort.*, 13 (2): 58-66.
Hapsoh, 2008. Pidato pengukuhan Guru Besar, Universitas Sumatra Utara, 14 Juni.
Hidayat, A., Mulyani, A. 2002. Lahan Kering untuk Pertanian. Di dalam: Adimihardja A, Mappaona, Saleh A (Penyunting). Teknologi. Pengelolaan Lahan Kering Menuju Pertanian Produktif dan Ramah Lingkungan. Bogor: Puslitbangtanak. hlm 1-34.
Hoerussalam, Aziz Purwantoro, dan Andi Khaeruni. 2013. Ketahanan tanaman jagung (*zea mays l.*) terhadap penyakit bulai melalui seed treatment serta pewarisannya pada generasi S1. *Ilmu Pertanian*, Vol. 16 No.2, 42-59.
Gaur, A.C. 1981. Phosphomicroorganism and Varians Transformation in Compost Technology. *FAO Project Field Document*, 13: 106-111.
Good, R.N., Z. Kiraly and K.R. Wood. 1986. The biochemistry and physiology of plant disease. University of Missouri, Press. Columbus

Kuc, J. 1983. Induced systemic resistance in plant caused by fungi and bacteria, pp: 192-221 dalam B.J. Deveral (Eds.), The dynamics host defence. Acad. Press, Sydney, New York, London

Kloeper, J.W., Wei, L., Tuzun, S. 2004. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. Phytopathol., 86: 221-224.

Loebenstein, G. and N.N. Lindsey. 1961. Peroxidase activity in virus infected potatoes. Phytopathol., 51: 533-537.

Martinez, C., J.C. Baccou, E. Bresson, Y. Baissac, J.F. Daniel, A. Jalloul, J.L. Montilet, J.P. Geiger, K. Assigbetse, & M. Nicole. 2000. Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic response of cotton challenged by a virulent race of Xanthomonas campestris pv. malvacearum. Plant Physiol., 22: 756 - 766

Purwaningsih, 2003. Pengaruh mikroba tanah terhadap pertumbuhan dan hasil panen kedelai (Glycine max L). Berita Biologi, 5: 373-378.

Rachman, S. 2002. Penerapan Pertanian Organik, Penerbit Kanisius, Yogyakarta.

Rao, N.S. 1994. Mikroorganisme Tanah dan Pertumbuhan Tanaman. Edisi Kedua. Jakarta: UI-Press.

Rukmana, S.K., dan Y. Yuniarsih. 1996. Kedelai, Budidaya Pasca Panen. Penerbit Kanisius. Yogyakarta. 92 hal

Singleton, V.L. and J.A. Rossi. 1965. Colorimetry of Total Phenolic with Phosphomolybdic Phosphotungstic Acid Reagent. American J. Enol. Viticulture, 16: 147.

Susanto, R. 2002. Penerapan Pertanian Organik. Kanisius. Yogyakarta

Surtiningsih, T., Farida dan, T., Nurhayati. 2009. Biofertilisasi Rhizobium pada tanaman kedelai (Glycine max (L). MERR). Berk.Penel. Hayati. 15: 1-5.

Taufik. M., A. Rahman, A. Wahab, dan S.H. Hidayat. 2010. Mekanisme ketahanan terinduksi oleh plant growth promoting rhizobacteria (PGPR) pada tanaman cabai terinfeksi cucumber mosaic virus (CMV). J. Hort., 20(3): 274-283.

Tenhaken, R. & C. Rubel. 1997. Salicylic acid needed in hypersensitif cell death in soybean but does not act a catalase inhibitor. Plant Physiol., 115: 291-298.

Tomiyama, K. 1982. Hypersensitive cell death. Its significane and physiology, pp. 329-344 dalam Yasuji Asada, W.R. Bushnell, Seiji Ouchi, and C.P. Vance (Eds.) Plant infection, the physiological and biochemical basis. Japan Scientific Societies Press, Tokyo

Waluyo, L. 2008. Teknik Metode Dasar Mikrobiologi, Universitas Muhamadiyah Malang Press, Malang.

Widawati, S. dan Suliasih, 2006, Populasi Bakteri. Populasi Bakteri.

Zhang, S., Reddy M.S., Klopper J.W. 2002. Development of assay for assessing induced systemic resistance by plant growth-promoting rhizobacteria against blue mold of tobacco. Biol. Control, 23: 79-86.

How to cite this article:

Made Sudana, I.G.N. Alit Susanta Wirya and Gusti Ngurah Raka. 2016. Utilization of Phosphate Solubilizing Rhizobacterium Derived from Leguminosae Plants to Stimulating Plant Growth and Induce Systemic Resistance of Soybean (Glycine max L. Merrill) to Mosaic Diseases. Int.J.Curr.Microbiol.App.Sci. 5(9): 600-610.
doi: http://dx.doi.org/10.20546/ijcmas.2016.509.068