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Original Article

IAA-producing and phosphate solubilizer of rhizosphere actinobacteria consortium to promote plant growth in soybean (*Glycine max* L.)

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Abstract

Plant Growth-Promoting Rhizobacteria (PGPR) are microbes that inhabit the rhizosphere and rhizoplanes environment and can enhance plant growth. The application of PGPR in agriculture can reduce the use of chemical fertilizers on soil. The purpose of this study was to determine the ability of the actinobacteria consortium to produce Indole-3-Acetic Acid (IAA), solubilize phosphate, and improve soybean growth in the greenhouse. The results of this study showed that the actinobacteria (*Streptomyces* sp. ASR58, *Streptomyces* sp. ASR67) and *Rhizobium* sp. which are used in this study are mutually compatible and tolerant to several types of fungicides and bactericides. Meanwhile, the consortium of *Streptomyces* sp. ASR58 and *Streptomyces* sp. ASR67 produces the highest concentration of IAA i.e. 25.11 ppm compared to each isolate and other bacterial consortia. Accordance to the quantitative phosphate solubilization assay, *Streptomyces* sp. ASR67 resulted in the highest dissolved inorganic phosphate i.e. 179.7 $\pm$ 13.3 mg / L. Inoculation of *Streptomyces* sp. ASR58 and *Streptomyces* sp. ASR67 consortium into soybean seeds can significantly increase 54.6% in stem length, 29% root growth, and 20.4% in plant dry weight. This research indicated that *Streptomyces* sp. ASR58 and *Streptomyces* sp. ASR67 consortium resulted in the best growth toward soybean plants compared to other bacterial consortia.

Keywords: Actinomycetes, IAA, Phosphate solubilization, Rhizosphere

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Introduction

High-input chemical fertilizer in the soil is usually added to increase the quantity and quality of crop production. Using chemical fertilizers for a long time can alter the soil fauna ecology, reduce soil fertility, contaminate water, lead to heavy metal contamination, and affect human health (Savci, 2012); (Chandini et al., 2019). To decrease the negative effects of chemical fertilizers and create sustainable agriculture in the future, we should apply environmentally friendly ways by using biofertilizers

and microbes-based-product. Therefore, the use of Plant Growth-Promoting Rhizobacteria (PGPR) plays an important role in agriculture due to their potency as a plant growth promoter and biocontrol agent (Basu et al., 2021). In addition, the PGPR application can also improve the quantity and quality of crop yield.

The role of PGPR in improving plant growth has been extensively studied and commercialized in the market as a bacterial inoculant (Santoyo et al., 2021). At present, the use of bacterial consortia from several bacterial strains has been widely investigated to obtain more beneficial characteristics compared to the application of single bacterial strains. Some studies have also showed that the use of the PGPR consortium can increase nodulation and growth of several types of plants (Shaharoona et al., 2006); (Tilak et al., 2006). This is due to bacterial consortia consisting of several appropriate bacterial species which interact synergistically (Ju et al., 2019). In addition, the use of bacterial consortia can accommodate several mechanisms of plant growth promotion through nutrients uptake (Rana et al., 2012), pest and plant pathogen inhibition (Villa-Rodríguez et al., 2019), nitrogen fixation, phytohormone production and iron chelation (Gosal and Kaur, 2017).

Actinobacteria, an important rhizospheric bacterial group commonly interact with plants by producing growth-promoter and plant pathogens inhibitor compounds. Many studies reported that soil and rhizospheric actinobacteria are capable to increase plant growth and inhibit the spread of pathogens. These Gram-positive bacterial groups have been widely utilized as biofertilizers and biopesticides to combat plant pathogens in several developed countries like the United States, Canada, Finland, and New Zealand (Palaniyandi et al., 2013). Meanwhile, in Indonesia, the use of actinobacteria as plant growth-promoter and plant pathogen biocontrol in agriculture is not common yet. Considering that Indonesia is a tropical country with the second highest level of biodiversity in the world, it should be easy to explore various potential actinobacteria with certain characteristics to develop an effective and environmentally friendly actinobacteria-based biofertilizer as well as biopesticides.

Streptomyces sp. ASR67, *Streptomyces* sp. ASR58, and *Rhizobium sp.* are potential PGPR isolated from the soybean plant rhizosphere. In previous studies, it was known that actinobacteria species i.e. *Streptomyces* sp. ASR67 and *Streptomyces* sp. ASR58 have the ability to increase soybean growth *in vitro* and inhibit the growth of *Rhizoctonia solani*, respectively (Fatmawati et al., 2020). In addition, each actinobacteria species is also known as a phosphate solubilizer, IAA hormone, siderophore, and HCN producer (Fatmawati et al., 2019). However, the effect of these actinobacteria consortia towards soybean plants growth is unknown. Therefore, the effect of actinobacteria and rhizobium bacteria consortium in promoting the growth of soybean plants have been analyzed in this study. Specifically, the purpose of this study was to determine the ability of the actinobacteria consortium to produce IAA, solubilize phosphate and improve soybean growth in the greenhouse. The application of PGPR to soybean plants is expected to predispose an optimal effect in improving the growth of soybean plants. Furthermore, the results of this study can be developed as effective and environmentally friendly plant growth-promoting agents to create sustainable agriculture in the future.

Material and Methods

Reculturing of Actinobacteria and *Rhizobium* sp. isolates

Two actinobacteria isolates, *Streptomyces* sp. ASR67 and *Streptomyces* sp. ASR58 which are isolated from soybean rhizosphere from Sukabumi, West Java were recultivated in ISP2 agar medium (4 g yeast extract; 10 g malt extract; 4 g dextrose; 20 g agar; pH 7.3) and incubated for 14 days at room temperature. *Rhizobium* sp. isolate is also recultured on LB agar medium (10 g tryptone; 5 g yeast extract; 10 g NaCl; 20 g agar) and incubated for 4 days at room temperature.

Compatibility test between Actinobacteria isolates and *Rhizobium* sp.

The compatibility test aims to determine the interaction between actinobacteria isolates and *Rhizobium* sp. The compatibility test was carried out using cross streak method on modified Luria Agar (LA) medium with the following composition (1 L distilled water): 5 g yeast extract, 10 g casein, 0.5 g NaCl, and 20 g agar (Retnowati et al., 2019). Afterward, the bacterial cultures were incubated for 7 days at 28 $\pm$ 2 °C of temperature. Antagonistic interactions were detected by the presence of a clear zone around the actinobacteria culture. The treatment of each isolate was repeated three times.

Fungicides and bactericides species tolerance examination

Actinobacteria and Rhizobium isolates were streaked onto ISP2 agar and LB agar medium containing 2000 ppm of each fungicide and bactericide i.e. mancozeb, benomyl, chloramphenicol, and rifampicin. The bacterial cultures were then incubated at 28 ° C for 5 days. Actinobacteria growth was measured on a scale of 0-3 where 0 = no growth, 1 = thin growth, 2 = moderate growth, and 3 = very well grown.

Measurement of IAA production by bacterial consortium

Production of indol-3-Acetic Acid (IAA) was measured according to (Khamna et al., 2010) with modification in the duration of incubation time. IAA production was measured from each bacterial isolate (*Streptomyces* sp. ASR67, *Streptomyces* sp. ASR58, and *Rhizobium* sp.), actinobacteria + *Rhizobium* sp. consortium, and all bacterial consortia. Two agar plugs (8 mm diameter) of actinobacteria and *Rhizobium* sp. colonies were firstly grown on ISP 2 solid medium for 7 days and then transferred to 100 mL of ISP2 liquid medium containing 100 ppm of L-tryptophan (L-trp) for 10 days incubation at 28° C of incubator shaker. After 10 days, the bacterial cultures were centrifuged for 15 minutes at 11.000 rpm; 4° C. For IAA detection, 2 mL of supernatant was mixed with 2 mL of Salkowski's reagent (150 mL H_2SO_4; 250 mL distilled water; 7.5 mL 0.5M $FeCl_2 \cdot 6H_2O$) and incubated in a dark room for 20 minutes. The formed red or pink color intensity was measured at 530 nm using a UV VIS spectrophotometer and then calculated using the IAA standard curve to determine the IAA concentration.

Measurement of dissolved inorganic phosphate production bacterial consortium

To determine the dissolved inorganic phosphate, 1 plug (1x1 cm) of actinobacteria isolate was inoculated on 25 mL of Pikovskaya Broth medium and incubated in the shaker incubator at 150 rpm for 7 days. Simultaneously, 1 mL sterile 0.85% NaCl was added to the Pikovskaya Broth medium and used as a control. After 7 days of incubation, 10 mL of dissolved inorganic phosphate were filtered using filter paper (Whatman no.1) to separate the remaining $Ca_3(PO_4)_2$. The filtrate was then centrifuged at 10.000 rpm for 20 minutes. A total of 1 mL of supernatant was transferred to a flask and homogenized with 10 mL of Chloromolybdic acid and 100 µl of Chlorostannous reagent, sequentially. The distilled water was then added to the flask until the volume reaches 50 mL. After incubating for 10 minutes, the absorbance of the sample was measured at 600 nm. The dissolved inorganic phosphate concentration was determined using a phosphate standard curve (Thakur and Parikh, 2016).

Greenhouse assay for the consortium growth promoter

The ability of actinobacteria and rhizobium isolates to stimulate soybean growth was tested on the Wilis soybean cultivar in the greenhouse. This experiment was carried out in 7 experimental groups which consist of *Streptomyces* sp. ASR 58; *Streptomyces* sp. ASR 67; *Rhizobium* sp.; *Streptomyces* sp. ASR 58 + *Streptomyces* sp. ASR 67; *Streptomyces* sp. ASR 58 + *Rhizobium* sp.; *Streptomyces* sp. ASR 67 + *Rhizobium* sp. and *Streptomyces* sp. ASR 58 + *Streptomyces* sp. ASR 67 + *Rhizobium* sp. Meanwhile, the ISP2 liquid medium was used as a control. Each treatment used 10 soybean seeds with three replications. Initially, each bacterial treatment was cultivated in an ISP2 liquid medium for 7 days. The soybean seeds were sequentially surface sterilized using 96% alcohol for 5 minutes, 2.5% sodium hypochlorite solution for 1 minute, and washed using sterile distilled water 10 times. Sterilized seeds were then soaked in each 7 day old bacterial culture (10^7 CFU ml^{-1}) for 60 minutes and planted on the planting media containing soil: sand (3: 1). After 14 days of planting, the shoot length, root length, and dry weight were measured. To determine the effect of each treatment, the growth parameters data were analyzed statistically using SPSS software by one-way analysis of variance (ANOVA) and Tukey's test at the 95% confidence level.

Results

Compatibility between Actinobacteria and *Rhizobium* sp.

Compatibility test between two actinobacteria strains (*Streptomyces* sp. ASR 67 and *Streptomyces* sp. ASR 58) and *Rhizobium* sp. using the dual culture method showed no inhibition zone at the intersection streak of three bacterial isolates. According to this, the actinobacteria isolates and *Rhizobium* sp. can grow synergistically. The compatibility test result was displayed in Table 1.

Table-1. Compatibility of *Streptomyces sp.* ASR 58, Streptomyces *sp* ASR 67, and *Rhizobium* sp.

No	Strain	*Streptomyces sp.* ASR58	*Streptomyces sp* SR67	*Rhizobium sp.*
1	*Streptomyces sp.* ASR 58	+	+	+
2	*Streptomyces sp* ASR 67	+	+	+
3	*Rhizobium sp.*	+	+	+

Note:
+ = compatible
= not compatible

Bactericides and fungicides tolerant in Actinobacteria and *Rhizobium* sp.

In *in vitro* conditions, the three tested bacteria showed different levels of tolerance to Chloramphenicol, Rifampicin, Benomyl, and Mancozeb. All bacteria are still capable to grow slightly into thickly on LA medium supplemented with each bactericide and fungicide. The bacterial growth on a medium containing bactericides and fungicides are shown in Table 2.

Indole Acetic-Acid (IAA) production by actinobacteria, *Rhizobium* sp., and bacterial consortia

Actinobacteria and *Rhizobium* sp., the rhizospheric bacteria were tested for their ability to produce IAA phytohormones which play a role in increasing plant growth. The IAA production assay results were obtained from bacterial single isolate and consortium (a combination of two and three isolates) (Figure 1). According to the results, *Streptomyces* ASR 67 and *Streptomyces* AR58 consortium produced the highest IAA concentration i.e. 25 ppm, while *Rhizobium* sp. produced the lowest IAA concentration i.e. 4 ppm. In addition, the consortium of *Streptomyces* sp. and *Rhizobium* sp. resulted in lower IAA concentration compared to single actinobacteria isolate.

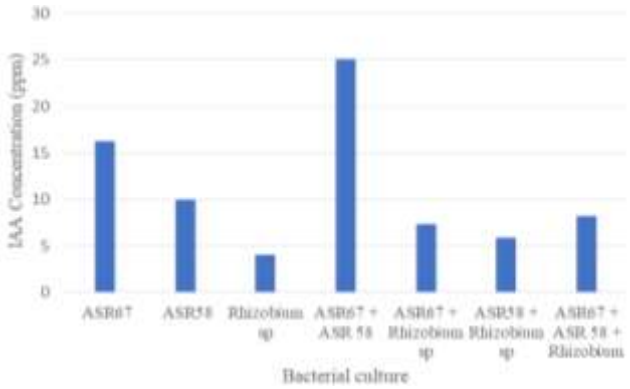

Figure-1. IAA Production by Actinobacteria: *Streptomyces* sp. ASR67, *Streptomyces* sp. ASR58, *Rhizobium* sp., and Actinobacteria-*Rhizobium* sp. consortium

According to the qualitative phosphate solubilization assay, *Streptomyces* sp. ASR58, *Streptomyces* sp. ASR67 and *Rhizobium* sp. can solubilize phosphate. Phosphate solubilization by these bacteria was indicated by the presence of a clear zone around the bacterial colonies on the Pikosvkaya Agar medium. In addition, the quantitative phosphate solubilization test showed that each bacterial isolate has different activity in solubilizing phosphate (Figure 3). The highest solubilized phosphate was resulted by *Streptomyces* ASR67 with 180 mg/L of phosphate concentration. Meanwhile, *Streptomyces* ASR58 and *Rhizobium* sp. consortium resulted in the lowest solubilized phosphate i.e. 10 mg/L. generally, actinobacteria and *Rhizobium* sp. consortium displayed lower activity in solubilizing phosphate than single bacterial isolate (Figure 2).

Table-2. Tolerance of bacteria to several types of bactericides and fungicides

Strain	Mancozeb	Benomyl	Rifampicin	Chloramphenicol	Control LA
Streptomyces sp. ASR 58	3	3	2	2	3
Streptomyces sp. ASR 67	3	3	2	2	3
Rhizobium sp	3	3	2	1	3

Note: 0 = no growth
1 = slight growth
2 = moderate growth
3 = thick growth

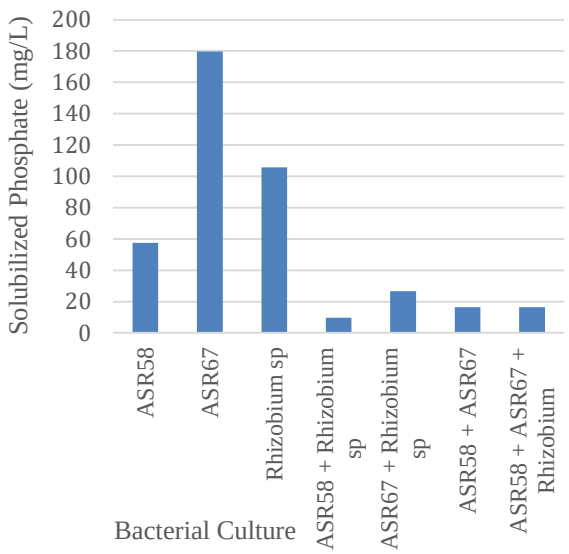

Figure-2. Quantitative phosphate solubilization by Actinobacteria: *Streptomyces* sp. ASR67 and *Streptomyces* sp. ASR58, *Rhizobium* sp., and Actinobacteria and Rhizobium sp. consortium on the liquid Pikovkaya medium

Effect of bacterial consortia on the growth of soybeans in the greenhouse assay.

According to the greenhouse experiment, single actinobacteria, *Streptomyces* sp ASR 58 + *Rhizobium* sp. consortium, *Streptomyces* sp ASR 58 + *Streptomyces* sp ASR 67 consortium, and three bacterial consortium treatments significantly increased the soybean growth compared to control treatment (Table 3). Specifically, *Streptomyces* sp. ASR 58 + *Streptomyces* sp. ASR 67 consortium treatment resulted in the longest shoot length i.e. 19.33 cm which indicated that this treatment significantly increased the shoot length compared to other treatments. In addition, *Streptomyces* sp. ASR 58 + *Streptomyces* sp. ASR 67 consortium treatment also showed the longest root length i.e. 8.90 cm but it is not significantly different compared to *Streptomyces* sp. ASR 67 and *Streptomyces* sp ASR 58 + *Streptomyces* sp ASR 67 + Rhizobium sp. treatments.

Discussion

Interaction between microbes and plant in the rhizosphere environment contribute to beneficial effect for soil fertility and plant fitness. Beneficial soil bacteria isolated from the rhizosphere are usually termed plant growth-promoting bacteria or rhizobacteria (PGPB/ PGPR) due to their prosperity in improving plant growth and yield especially in nutrient-poor environments. The application of PGPR could enhance plant growth by increasing the availability of important plant nutrients such as nitrogen, phosphorus, and iron (El-Tarabily et al., 2021). PGPR also synthesizes siderophore, phytohormone (auxin and gibberellin), ACC deaminase as well as several volatile organic compounds which can introduce plant endurance toward plant pathogens (Riaz et al., 2021). In this study, *Streptomyces* sp. species from soybean rhizosphere which are proven as IAA producers and phosphate solubilizers were further studied to determine the ability of *Streptomyces* sp. and *Rhizobium* sp. consortium to enhance the soybean plant growth.

Table-3. The growth of soybean plants treated with various bacterial treatments in the greenhouse assay

Treatment	Growth Parameter		
	Shoot length (cm)	Root length (cm)	Dry weight (mg)
Streptomyces sp. ASR 58	16.37 $_{bc}$	8.30 $_{bc}$	141 $_{ab}$
Streptomyces sp. ASR 67	18.47 $_{cd}$	8.70 $_{c}$	144 $_{b}$
Rhizobium sp.	14.63 $_{ab}$	7.33 $_{ab}$	128 $_{ab}$
Streptomyces sp. ASR 58 + *Rhizobium* sp.	16.53 $_{bc}$	7.73 $_{abc}$	144 $_{b}$
Streptomyces sp. ASR 67 + *Rhizobium* sp.	14.47 $_{ab}$	7.97 $_{abc}$	135 $_{ab}$
Streptomyces sp. ASR 58 + *Streptomyces* sp. ASR 67	19.33 $_{d}$	8.90 $_{c}$	147 $_{b}$
Streptomyces sp. ASR 58 + *Streptomyces* sp. ASR 67 + *Rhizobium* sp.	17.03 $_{bcd}$	8.73 $_{c}$	147 $_{b}$
Control	12.5 $_{a}$	6.90 $_{a}$	122 $_{a}$

Note: The same letter at the end of the number in the same column shows that the values are not significantly different in the Tukey test (P <0.05).

On the initial compatibility test, we obtained that the two *Streptomyces* sp. isolates and *Rhizobium* sp. can grow synergistically on the growth agar medium. It can be primary result to further develop the bacterial isolates as plant growth promoter agents in the term of PGPR consortium design. The bacterial consortium commonly consists of two or more different species with positive interaction (Sarma et al., 2015) as well as favorable traits for plants. Several studies have reported that bacterial consortia provide more beneficial features in plants than single bacteria through various mechanisms of plant growth promotion and plant pathogen management (Ju et al., 2019). Moreover, pesticides tolerant PGPR are also important in the application of PGPR on the conventional agricultural system which usually uses huge number of pesticides. According to the pesticide tolerance test results, *Streptomyces* sp. ASR 58, *Streptomyces* sp. ASR 67 and *Rhizobium* sp. are tolerant to the mancozeb and benomyl because their main ingredients of them are antifungal compounds that specifically inhibit the growth of pathogenic fungi in soil and plants. Meanwhile, the three bacterial isolates showed less tolerance to the rifampicin and chloramphenicol which indicated that the main ingredients of these bactericides strongly inhibit the growth of the tested bacteria. This result informs us that *Streptomyces* sp. ASR 58, *Streptomyces* sp. ASR 67 and *Rhizobium* sp. are the potential to apply as PGPR on the conventional agricultural system which has been applied bactericides and fungicides massively in the field (Sreevidya et al., 2016).

The plant growth promoter bacteria are usually characterized by the ability to produce plant growth hormones like IAA or nutrient solubilization like phosphate. The previous study proved each *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 isolates were able to produce IAA and solubilize phosphate (Fatmawati et al., 2019). However, this current study demonstrated that the *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 consortium resulted in the highest IAA concentration compared to single isolate as well as other bacterial consortium. Interestingly, the actinobacteria and *Rhizobium* sp. consortium exactly decrease the ability of each *Streptomyces* sp. isolate in producing IAA. It is suspected that the existence of *Rhizobium* sp. was inhibited to produce IAA in microbial consortia. This finding is also supported by the study from (Imada et al., 2017) which state that the IAA production by *Rhizobium* sp. was strongly inhibited by the presence of NH_4^+ in the culture medium. In contrast to the IAA production, every single bacterial isolate showed higher ability in solubilizing phosphate than bacterial consortium. The highest solubilized phosphate resulted from *Streptomyces* sp. ASR67 with 179.7 $\pm$ 13.3 mg/L of concentration. The low phosphate solubilization activity by bacterial consortium might be caused by pH decline on the medium which further affected the bacterial growth. According to (Rashid et al., 2004), the influencing factors of phosphate solubilization by microbes are the excretion of organic acids which can decrease the pH medium. The ability of PGPR to solubilize phosphate plays important role in supplying available phosphate to stimulate plants root and stem growth (Hamdali et al., 2008). Meanwhile, the phosphate deficiency will cause the plants to crumple more easily and slower the fruit ripening process (Aziz et al., 2013).

Streptomyces sp. ASR58 and *Streptomyces* sp. ASR67 is a Gram-positive bacterium isolated from the soybean rhizosphere. *Rhizobium* sp. is also a bacteria that is often found in the roots of leguminous plants. In the previous studies, *Streptomyces* sp. ASR58, *Streptomyces* sp. ASR67 and *Rhizobium* sp. be able to increase the growth of soybean plants and reduce sprouts in soybean (Fatmawati et al., 2020). Therefore, in this study, a greenhouse experiment was further conducted to prove the competence of *Streptomyces* sp. and *Rhizobium* sp. in improving the growth of soybean plants. According to the greenhouse experiment result, the application of *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 consortium displayed the finest impact on soybean plant growth compared to a single bacterial isolate as well as another bacterial consortium. The stem and root length of the soybean plant treated with *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 consortiums are 54.6% and 29% longer than soybean plants treated with control treatment. The *Streptomyces* sp. consortium also increased plant dry weight in an amount of 20.4% higher than the control treatment. This finding indicated that the improvement of soybean plant growth is more influenced by IAA production than phosphate solubilization by *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 consortium. It is also suspected that the two *Streptomyces* sp. consortium can grow synergistically to gain the capability in producing secondary metabolites related to plant growth promoters.

The utilization of actinobacteria, rhizobium, and rhizobacter consortia has been studied extensively by many researchers. Bacterial consortium can increase soybean germination, NPK absorption, soil pH, and organic matter content by 34.8%, 83.6%, 0.7%, and 0.68%, respectively (Amule et al., 2018). (Solans et al., 2016) also revealed that the inoculation of four actinobacterial isolates, namely *Streptomyces* MM40, *Actinoplanes* ME3, *Micromonospora* MM18, and *Frankia* sp. in a single culture did not show any enhancement in the growth of the test plants. Whereas, the use of these actinobacterial consortiums showed an increase in nodulation, shoot dry weight, and root dry weight of *Ochetophila trinervis*. This is presumably because *Frankia* sp. is known as a symbiotic N-fixing actinobacteria that contributes to stimulating nodulation in plants.

The use of a microbial consortium is considered to be more effective in increasing plant growth compared to a single microbial isolate (Shaharoona et al., 2006); (Tilak et al., 2006). The existence of several microbial species combinations will lead to creating beneficial interaction among microbes. The formed synergy interaction will generate more appropriate conditions for microbes to produce metabolite compounds, provide nutrients, degrade inhibitor compounds, and complete the function as a plant growth promoter that will increase the plant growth. According to (Tabacchioni et al., 2021) with the use of a microbe consortium, a unique ecosystem and an ideal environment for microbial growth will be formed, so their ability to produce bioactive compounds also increases.

Conclusion

Based on the results of this study, we can conclude that *Streptomyces* sp. ASR 58, *Streptomyces* sp. ASR 67 and *Rhizobium* sp. are mutually compatible and tolerant to several types of fungicides and bactericides. Furthermore, *Streptomyces* sp. ASR 67 showed the highest solubilized phosphate, while the *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 consortium resulted in the highest IAA concentration. The ability of *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 consortium in producing IAA was also confirmed in a greenhouse experiment where this actinobacteria consortium generated the finest effect on shoot and root length as well as dry weight of soybean plant. This result is predicted to be more influenced by IAA production than phosphate solubilization. Compared with other treatments, giving a combination of *Streptomyces* sp. ASR58 and *Streptomyces* sp. ASR67 in soybean can be more stimulate the growth of soybean plants. This finding indicated that the application of actinobacterial consortium is considered to be more beneficial than single bacterial. Moreover, *Streptomyces* sp. ASR 58 and *Streptomyces* sp. ASR 67 consortium is promising to be developed as a biofertilizer for sustainable agriculture in the future.

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Fatmawati U: Planned and conducted the research, collected & analyzed the data and wrote the manuscript

Sari DP: Collected, analyzed and interpreted the data

Santosa S: Provide the material, supervised the research and interpreted the data

Wiraswati SM: Designed research methodology and wrote the manuscript