OnLine Journal of Biological Sciences

Original Research Paper

Plant Growth Promoting Activity of Actinomycetes Isolated from Soybean Rhizosphere

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Article history
Received: 21-09-2018
Revised: 26-11-2018
Accepted: 3-01-2019

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Abstract: Plant Growth Promoting Rhizobacteria (PGPR) is considered as the biological agent for improving plant growth. One Group of PGPR that have an important role in growth promoting of plant is Actinomycetes. The objective of this study was to isolate and screen Actinomycetes isolated from soybean rhizosphere as growth promoter of soybean *in vitro*. Fifty-three Actinomycetes isolates have successfully been isolated from soybean rhizosphere using two media, mainly Humic acid Vitamin Agar (HVA) and starch casein agar (SCA). Among 53 isolates, 18 (34%) isolates were able to produce IAA in range of 2.08 ppm to 16.70 ppm. Growth promotion test of soybean *in vitro* using Ragdoll method resulted 7 Actinomycetes isolates that significantly enhanced 3 plant growth parameters, including hypocotyl and radicular length as well as the number of lateral roots. Of those 7 isolates of Actinomycetes, 5 isolates were able to grow on nitrogen-free medium and solubilize phosphate. Those 5 isolates also were found as non-pathogenic, based on the negative reaction in hypersensitivity test. Based on 16S rRNA sequence analysis, 5 selected Actinomycetes isolates were highly homolog with *Streptomyces* genera in different taxa of species and strains (similarity ≥99%). Our finding reveals a potent application of 5 Actinomycetes isolates as plant growth promoter in soybean agriculture.

Keywords: Actinomycetes, Rhizosphere, Plant Growth Promoting, IAA, 16S rRNA

Introduction

Soybean (*Glycine max* L.) is one of Indonesian's most important legume crops. However, the rate of soybean production in Indonesia is still relatively low, approximately 2.37% per year. Such yield productivity is still not enough to supply soybean needs in Indonesia leading to high import demand of soybean for nearly 70% per year as reported by the MAI (2015). Modern agriculture relies on the use of some chemical fertilizers in increasing soybean productivity. The excessive use of these chemical fertilizers in long-term results in the accumulation of some chemical residues that may cause environmental damage, including groundwater contamination, soil structure alteration and ecological damage. Therefore, it is necessary to increase the soybean productivity by the other approaches mainly an environmentally friendly one.

Plant Growth Promoting Rhizobacteria (PGPR) is considered to be the biological agent for improving plant growth. The bacteria actively colonize the root areas and stimulates the plant growth either through direct or indirect mechanisms. In direct mechanism, they are able to synthesize some phytohormones, including indole-3-acetic acid (IAA), gibberellic acid, cytokine and ethylene (Mohapatra *et al*., 2014) and to supply nutrients through siderophores production (Khamna *et al*., 2009), nitrogen fixation (Ekpo and Nkanang, 2010) and phosphate solubilization (Jog *et al*., 2014). Whereas in the indirect mechanism, PGPR involves in controlling phytopathogens (Chen *et al*., 2018).

Rhizosphere Actinomycetes, a group of Gram-positive bacteria, has highlighted to be the most potential candidates of biofertilizer agents. Some Actinomycetes genera have been widely developed for increasing

agricultural crops productivity including *Actinoplanes, Streptomyces and Micromonospora*. Among them, *Streptomyces* the most explored genera in respect to the plant growth promoting activity. In instance, two *Streptomyces* species isolated from the wheat rhizosphere attributed with high plant growth promoting activities and chitinase-phytase productions could significantly promote wheat growth (Jog *et al*., 2012). Sousa *et al.* (2008) also reported three *Streptomyces* strains (AC-147, AC-95 and AC 29) which capable in producing siderophores, solubilizing phosphate and producing phytohormones IAA, as potential PGPR agents. Based on those potential application of Actinomycetes as PGPR, thus exploration of rhizospheric Actinomycetes is considered as an important work, particularly in developing PGPR agent to promote soybean growth. The objective of this study was to isolate Actinomycetes of soybean rhizosphere and to evaluate their ability in improving soybean growth, *in vitro*.

Materials and Methods

Isolation of Actinomycetes from Soybean Rhizosphere

In this study, the serial dilution method was applied for isolating Actinomycetes from soybean rhizosphere (collected from soybean plantation in Bangbayang Village, Sukabumi, West Java, Indonesia). Two selective media, including Humic Acid Vitamin Agar (HVA; composition: humic acid 1 g, $CaCO_3$ 0.020 g, Na_2HPO_4 0.5 g, KCl 1.7 g, $MgSO_4.7H_2O$ 0.05 g, $FeSO_4.7H_2O$ 0.01 g, Na_2HPO_4 0.5 g, nalidixic acid 0.02 g, cycloheximide 0.05 g, B-vitamins 5 mL, agar 18 g, distilled water 1000 mL, pH 7.2) and Starch Casein Agar (SCA; Composition: Soluble starch: 10 g, casein 0.3 g, K_2HPO_4: 2 g, KNO_3 2 g, $MgSO_4.7H_2O$ 2 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, agar 18 g, nalidixic acid 0.02 g, 0.07 g, distilled water 1000 mL, pH: 7.2) were used. Prior serial dilution, one g soil was preheated at 60°C for 15 min. The preheated soil was then homogenized in 9 mL of 0.85% NaCl solution (10^{-1}) and further used for subsequent, dilution 10^{-6}. About 100 µL of four last dilutions were spread on top of HVA and SCA media and were incubated for 14 days at room temperature (±27°C). The growing Actinomycetes colonies were then purified by using International *Streptomyces* Project medium no.2 (ISP2; composition: Dextrose 4 g, malt extract 10 g, yeast extract 4 g, agar 20 g, distilled water 1000 mL, pH 7.2).

Screening for Indole Acetic Acid (IAA) Production

Colorimetric method was used to measure IAA production by each Actinomycete isolates (Gopalakrishnan *et al*., 2014). Prior to IAA measurement, about two plugs of inoculums (±0.8 diameter) were inoculated on 20 mL of ISP2 liquid medium supplemented with 0.2 mL of 0.2% L-tryptophan and incubated in an agitated incubator at 120 rpm at room temperature (±27°C) for 10 days. About 1 mL supernatant of each culture was mixed with 4 mL of Salkowski reagent. The mixed solutions were then incubated in dark for about 30 min. IAA concentration was calculated based on the standard curve. The NTB 110 (Actinomycete isolated from maize rhizosphere, IAA producer, a collection of Microbiology Laboratory) and *Staphylococcus aureus* (IPBCC collection, Bogor Agricultural University-Indonesia) were used as the positive and negative control, respectively. Each sample was tested in duplo.

Selection of Plant Growth Promoting (PGP) Properties of Actinomycetes

Ragdoll method was applied for evaluating PGP activity of rhizospheric Actinomycetes as described by Sreevidya *et al.* (2016). Prior to use, 3 plugs of Actinomycetes inoculum were cultured on 50 mL of ISP4 medium and then incubated in a shaker (120 rpm) at room temperature for 7 days. Soybean seeds were surface sterilized by using 96% ethanol for 10 s and 3% of H_2O_2: Distilled water solution (1:1 v/v) for 10 min and 10 times washed with sterilized distilled water. The sterilized seeds were then soaked in sterilized distilled water for 60 min. The submerged seeds were selected to be cultivated in sterile wet tissues and incubated for 2 days. The germinated seeds with a radicular length of 0.5-1 cm were selected for testing. The germinated seeds were then soaked in Actinomycetes culture containing individual strain for 60 min. The soaked germinated seeds were cultivated on a wet paper towel, folded and rolled and incubated at ±27°C for 5 days. The seeds treated with sterile distilled water and uninoculated ISP4 medium were served as control. After the incubation period, three parameters including hypocotyl length, radicular length and the number of lateral roots were observed. This assay was conducted in triplicates and each replication consists of nine sprouts. The selected Actinomycetes isolates were classified into 4 experimental groups based on the time of the test conducted. The data were analyzed by ANOVA and tested by Duncan test ($\alpha = 0.05$).

Phosphate Solubilization Test

Phosphate solubilizing activity was tested by using Pikovskaya medium. The medium composition was (g/L): NaCl (0.2), KCl (0.2), $MgSO_4.7H_2O$ (0.1), $MnSO_4.7H_2O$ (0.0025), $FeSO4.7H_2O$ (0.0025), $(NH_4)_2SO_4$ (0.5), glucose (10), yeast extract (0.5), $Ca_3(PO_4)_2$ (5) and bacto agar (15). One plug of Actinomycetes colony was plated onto the surface medium and incubated at ±27°C for 7 days. *Burkholderia cepacia* (IPBCC collection) was used as positive control. Phosphate solubilizing activity was

indicated by the formation of the clear zone around Actinomycetes colonies. Phosphate solubilization index was calculated by using the following formula: (diameter of colony + diameter of clear zone)/ diameter of colony.

Growth Actinomycetes in N-free Medium

The selected Actinomycetes were grown in plate by streaking on N-free medium agar and the plates were incubated for 7 days at room temperature. N-free medium contained (g/L): K_2HPO_4 (1), KH_2PO_4 (3), $MgSO_4$ (0.065), $FeCl_3.6H_2O$ (0.01), $CaCl_2.2H_2O$ (0.07), dextrose (5), $Na_2MoO_4.2H_2O$ (240×10^{-6}), H_3BO_4 (3×10^{-6}), $MnSO_4.H_2O$ (1.83×10^{-6}), $ZnSO_4.7H_2O$ (2.90×10^{-4}), $CuSO_4.5H_2O$ (1.30×10^{-4}), $CoCl_2.6H_2O$ (1.20×10^{-4}), agar (15). *Escherichia coli* strain DH5α was used as a negative control.

Hypersensitivity Reaction Test

Actinomycetes isolates were tested for their pathogenicity on the plant. The pathogenicity on the plant was tested by using Hypersensitivity (HR) test in the tobacco leaf. One mL of seven days-old of each Actinomycetes culture was injected into the interveinal tissue in the abaxial surface of tobacco leaf by using a sterile syringe (without needles). Hypersensitivity reaction was indicated by a necrotic area in injected tissue after 24-48 h. *Xanthomonas oryzae* pv. oryzae (Xoo) and sterile distilled water were used as a positive and negative control, respectively.

Identification of the Potential Actinomycetes

Genomic DNA of the potential Actinomycetes was extracted by using Presto™ Mini gDNA bacteria Kit (Geneaid) according to the manufacturer's procedures. The quality and quantity of the DNA were measured by using Nanodrop™ 1000 Spectrophotometer. 16S rRNA genes were amplified by using universal primer, 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387r (5'-GGG CGG WGT GTA CAA GGC-3') that targeted DNA fragment approximately 1300 bp in size (Fredriksson *et al*., 2013). Fifty µL PCR mix containing: MyTaq™ HS Red Mix 2x (Bioline) (25 µL), 10 pmol 1387r primer (5 µL), 10 pmol 63f primer (5 µL), 100 ng/µL of DNA template (4 µL) and adjusted with nuclease-free water (11 µL) was used as PCR reaction. The PCR conditions were performed in 35 cycles with predenaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 55°C for 45 sec, elongation at 72°C for 1 min 45 sec and post-elongation at 72°C for 10 min. The PCR products were sequenced in First Base, Malaysia. The sequences were aligned by the BlastN program in National Center for Biotechnology Information (NCBI) GenBank database. The sequences were deposited to NCBI GenBank (https://www.ncbi.nlm.nih.gov). The phylogenetic tree was constructed using MEGA 7.0 software by neighbor-joining method with 1000x bootstrap replications.

Results

Soybean Rhizosphere Actinomycetes Producing IAA

A total of 53 isolates were successfully isolated from the soybean rhizosphere in two different media, including HVA and SCA, based on each distinct morphological characters. Of 53 Actinomycetes isolates, 18 isolates were able to produce IAA. IAA concentration produced by these Actinomycetes ranging from 2.08 to 16.70 ppm (Table 1). These Actinomycetes isolates were grouped into high, moderate and low IAA producers. Four isolates were grouped into high IAA producers (>8.9 ppm), 8 isolates were moderate IAA producers (5-8.9 ppm) and 6 isolates were low IAA producers group (1-4.9 ppm). The highest IAA concentration was produced by ARK 48 isolate (16.70 ppm), while the lowest IAA production was shown by ARK 86 (2.08 ppm). The NTB 110 isolate as a positive control was able to synthesize IAA in 13.71 ppm, while there is no IAA detected in *S. aureus*'s supernatants which was used as a negative control.

Plant Growth Promoting Activity of Rhizosphere Actinomycetes (In Planta Assay)

The 18 IAA-producing Actinomycetes were then tested for their plant growth promoting activity, *in planta*. Among isolates tested, 7 isolates were able to highly promote soybean growth as compared to the control treatment (Fig. 1). These 7 Actinomycetes isolates were ARK 116, ARK 86, ARK 13, ARK 63, ARK 94, ARK 17 and ARK 48 which were able to promote all three parameters, i.e., hypocotyl and radicular length as well as the number of lateral roots. In addition, four isolates (ARK 49, ARK 68, ARK 26 and ARK 28) were able to promote two growth parameters, while the other four isolates (ARK 103, ARK 47, ARK 92 and ARK 108) could only promote one parameter. Yet, three isolates (ARK 51, ARK 16 and ARK 87) were unable to promote soybean growth (Table 2).

Phosphate Solubilization Activities of Rhizosphere Actinomycetes

All 18 Actinomycetes isolates were able to solubilize phosphate in the pikovskaya medium containing tricalcium-phosphate as indicated by clear zone formation around the Actinomycetes colonies. Phosphate solubilization index of these isolates was various ranging from 2.05±0.06-2.72±0.08 (Table 3). The highest and the lowest index showed by the ARK 92 and ARK 49, respectively.

The Growth of Rhizosphere Actinomycetes in N-free Medium

Among 18 isolates tested, 15 isolates were able to grow in N-free medium (Table 3). Their ability in nitrogen-fixing was found diverse as indicated by the growth and development of the colony in the medium. The higher nitrogen fixation activity, the more abundance of the colony growth. In this experiment, *E. coli* strain DH5α was unable to grow in the particular N-free medium.

Table 1: The **c**oncentration of IAA produced by the 18 Actinomycetes isolates in a 7-days old culture of 20 mL ISP2 medium containing 0.2 mL of 0.2% L-tryptophan incubated at room temperature

No.	Isolate code	IAA concentration (ppm)*
1.	Positive control (NTB 110)	13.71
2.	Negative control (*Staphylococcus aureus*)	0.00
3.	ARK 94	12.04
4.	ARK 87	9.17
5.	ARK 63	8.83
6.	ARK 108	8.75
7.	ARK 28	8.42
8.	ARK 16	8.29
9.	ARK 13	7.54
10.	ARK 49	7.08
11.	ARK 68	5.37
12.	ARK 116	5.25
13.	ARK 103	4.87
14.	ARK 92	4.46
15.	ARK 26	3.33
16.	ARK 51	3.08
17.	ARK 86	2.08
18.	ARK 48	16.70
19.	ARK 47	16.10
20.	ARK 17	2.30

Note: * The data were calculated from duplo measurement

Table 2: Plant growth promoting activities via *in planta* assay of 18 selected Actinomycetes isolates

Experimental group	Treatment	Hypocotyl length (cm)*	Radicular length (cm)*	The number of lateral roots*
1	Distilled water	8.24^{a}	7.57^{a}	15.81^{b}
	Uninoculated ISP4	8.22^{a}	7.07^{a}	12.52^{ab}
	ARK 116	**10.52^{c}**	**9.33^{b}**	**19.97^{c}**
	ARK 51	7.81^{a}	6.68^{a}	10.89^{a}
	ARK 108	**9.40^{b}**	7.07^{a}	11.56^{a}
	ARK 13	**9.38^{b}**	**9.30^{b}**	**16.78^{c}**
2	Distilled water	4.84^{a}	3.04^{a}	6.26^{a}
	Uninoculated ISP4	5.84^{ab}	5.00^{ab}	7.93^{ab}
	ARK 16	7.27^{bc}	6.40^{bc}	13.59^{bcd}
	ARK 103	7.62^{bc}	6.57^{bc}	**16.11^{cd}**
	ARK 87	5.92^{ab}	5.51^{ab}	12.48^{abc}
	ARK 48	**9.06^{c}**	**8.53^{c}**	**20.48^{d}**
	ARK 47	7.81^{bc}	7.75^{bc}	**17.15^{cd}**
3	Distilled water	6.69^{a}	4.30^{a}	8.07^{a}
	Uninoculated ISP4	6.85^{a}	5.82^{a}	14.26^{ab}
	ARK 17	**9.75^{b}**	**8.98^{bc}**	**21.85^{c}**
	ARK 49	**10.22^{b}**	**9.32^{bc}**	16.81^{bc}
	ARK 68	**9.68^{b}**	**8.76^{b}**	15.89^{bc}
	ARK 26	**9.99^{b}**	**8.33^{b}**	15.63^{bc}
	ARK 28	**10.54^{b}**	**11.43^{c}**	16.04^{bc}
	Distilled water	5.71^{ab}	2.42^{a}	5.74^{a}
4	Uninoculated ISP4	4.57^{a}	2.59^{a}	6.27^{a}
	ARK 92	6.95^{bc}	**5.89^{b}**	**9.41^{b}**
	ARK 86	**8.09^{cd}**	**7.01^{b}**	**15.92^{c}**
	ARK 63	**10.73^{e}**	**8.60^{c}**	**16.07^{c}**
	ARK 94	**11.23^{e}**	**8.89^{c}**	**13.67^{c}**

*The data was calculated as the average of three replications in which each replication consist of 9 soybean sprouts. Data in bold and different letter above the number indicate significant differences with control based on Duncan test (α = 0.05)

Table 3: Plant growth promoting characters of soybean rhizosphere Actinomycetes isolates

No.	Isolate code	Growth parameters promotion			Growth in N-free medium	Phosphate solubilization index ± Standart deviation
		Hypocotyl	Radicula	Lateral roots		
1.	ARK 116	v	v	v	+	2.30±0.01
2.	ARK 86	v	v	v	+	2.28±0.07
3.	ARK 13	v	v	v	+	2.26±0.10
4.	ARK 63	v	v	v	+	2.39±0.02
5.	ARK 94	v	v	v	+	2.17±0.08
6.	ARK 17	v	v	v	-	2.19±0.09
7.	ARK 48	v	v	v	-	2.17±0.07
8.	ARK 108	v	x	x	+	2.12±0.11
9.	ARK 103	x	x	v	+	2.23±0.10
10.	ARK 47	x	x	v	+	2.27±0.24
11.	ARK 49	v	v	x	+	2.05±0.06
12.	ARK 26	v	v	x	+	2.25±0.11
13.	ARK 68	v	v	x	+	2.09±0.01
14.	ARK 28	v	v	x	+	2.33±0.04
15.	ARK 16	x	x	x	+	2.26±0.06
16.	ARK 51	x	x	x	-	2.10±0.01
17.	ARK 87	x	x	x	+	2.29±0.04
18.	ARK 92	-	v	v	+	2.72±0.08

Note: v: Significantly promote growth parameter, x: Not significantly promote; growth parameter +: Grow, - : Not grow

Table 4: Homology of 5 potential Actinomycetes with their closest relative species in GenBank NCBI database

No.	Isolate code (Acc. number)	Related species (Acc. number)	Query cover (%)	E-value	Similarity (%)
1.	ARK 13 (MH612936.1)	*Streptomyces panaciradicis* strain 1 MR-8 (NR_134200.1)	99	0.0	99
2.	ARK 63 (MH612939.1)	*Streptomyces recifensis* strain ST100 (EU216596.1)	100	0.0	99
3.	ARK 86 (MH612940.1)	*Streptomyces polychromogenes* strain GXSS21 (HQ844464.1)	100	0.0	99
4.	ARK 94 (MH612941.1)	*Streptomyces manipurensis* strain USC003 (KX358626.1)	100	0.0	99
5.	ARK 116 (MH612942.1)	*Streptomyces* sp. strain CAH7 (EF025318.1)	100	0.0	99

Note: Actinomycete isolates accession number reported in this study are available in the NCBI GenBank database

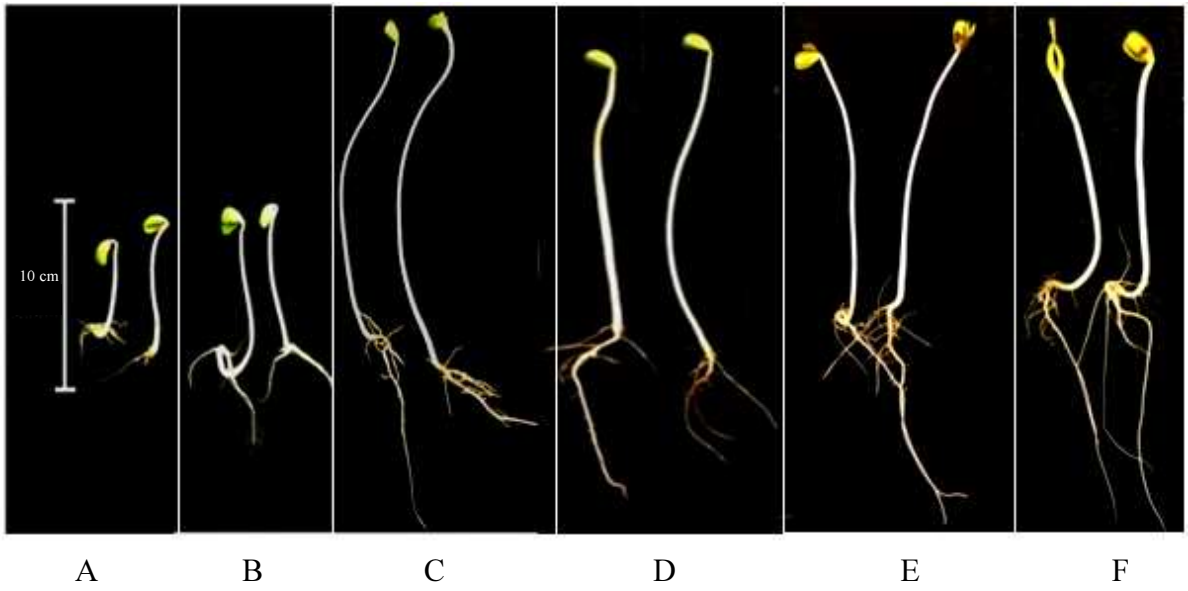

Fig. 1: Soybean growth response after 5 days treated with: (A) distilled water, (B) uninoculated ISP4 medium, (C) ARK 94, (D) ARK 63, (E) ARK 13, (F) ARK 116

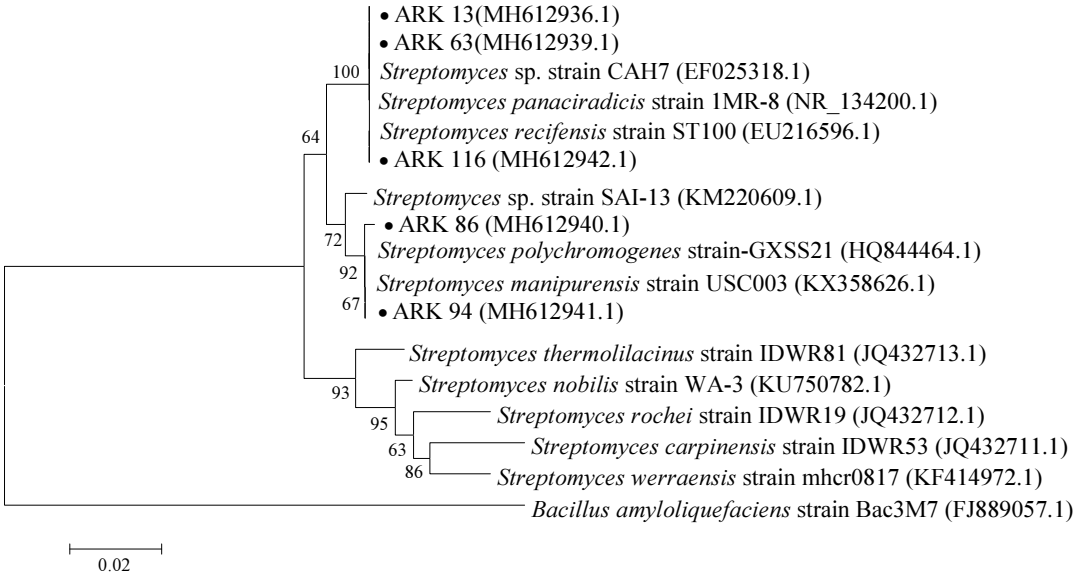

Fig. 2: Phylogenetic tree of five potential Actinomycetes isolates compared with their related species constructed by using the neighbor-joining method with 1000x bootstrap replications

Hypersensitivity Reactions of Actinomycetes Isolates

Based on hypersensitivity test, all of 18 isolates of Actinomycetes producing IAA were unable to induce the hypersensitive reaction in tobacco leaf. Whereas, the positive control as served by *X. oryzae* could develop a necrotic area in the tobacco leaf and there was no necrotic shown by sterile distilled.

The Identity of Actinomycetes Based on 16S rRNA Gene

The five Actinomycetes isolates were selected for identification based on their plant growth promoting properties. In instance, the selected isolates were capable in growing on nitrogen-free medium, promoting growth *in planta*, solubilizing phosphate and negative hypersensitivity reactions. Based on 16S rRNA gene sequences, the 5 isolates were highly homolog to the genus *Streptomyces* (similarity ≥ 99%), yet distinct in the taxa of species and strains (Table 4). Consistently, the phylogenetic tree also confirmed that these five isolates belonged to *Streptomyces* genera (Fig. 2).

Discussion

A total of 53 Actinomycetes isolates have been isolated from soybean rhizosphere by using selective media. Isolation of Actinomycetes in various media may increase the possibility in isolating different genera of species of Actinomycetes. In this case, HVA medium contains humic acid, while SCA medium contains amylum. The distinct nutritional content mainly in carbon sources may induce the growth of different bacteria, thus increasing the diversity of the isolated bacteria. From a total of 53 isolates, 18 isolates (31%) were able to produce IAA. The availability of tryptophan on the medium provides the precursor for IAA synthesis. In this study, ARK 48 and ARK 143 produced the highest and lowest IAA concentration, respectively. Such differences is likely influenced by the genetic and metabolic background of each isolate in converting L-tryptophan to IAA. Actinomycetes isolated from rhizosphere environment are considered to have the capability to synthesize IAA. Jog *et al.* (2012) reported 12 strains (78 % of isolated strains) collected from wheat rhizosphere produced auxins ranging from 2.6-19.22 ppm. Four rhizospheric Actinomycetes also were found to produce IAA ranging from 3.6-14.6 ppm as reported by Sreevidya *et al.* (2015). IAA controls some vital development of the plant including cell division, cell expansion, root development and apical dominance (Majda and Robert, 2018). In this regards, IAA-producing Actinomycetes have a great potential to be further explored as a biofertilizer agent.

In the present investigation, the 18 IAA-producing isolates enhanced plant growth in terms of increasing hypocotyl and radicular length, as well as the number of lateral roots as compared to un-inoculated control plants. Among isolates tested, seven isolates were capable in improving those three plant growth parameters. In instance, ARK 116 isolate exhibited a maximum increase in hypocotyl and radicular length as well as the number of lateral roots. Our study is in line with the discovery of Sreevidya *et al.* (2015), *Streptomyces* sp. strain VAI-7 significantly enhanced

both root and shoot lengths of chickpea. Another study also demonstrated that PGPR inoculation on maize seeds significantly increased stem and total fresh weight (Gholami *et al*., 2009).

In this study, we found that it is unlikely that high IAA production *in vitro* would constantly result in significant growth promoting activity *in planta*. In instance, high and moderate IAA producing isolates, ARK 87 and ARK 16 isolates had lower activity in promoting the growth of soybean, than that treatment without bacterial inoculation. It is likely due to the endogenous IAA as produced by plants is able to influence the process of elongation and plant cell divisions so that Actinomycetes inoculation treatments did not give a significant effect on the plant growth (Pamungkas *et al*., 2009). On the other hand, high IAA concentration may elicit a negative effect on the plant development since it can inhibit the growth of plant embryos (Yoshida *et al*., 2012).

The selected Actinomycetes isolates also investigated for their phosphate solubilization and nitrogen fixation activities. In our study, all of 18 selected actinomycetes isolates exhibited their capability to solubilize phosphate in various activities as indicated by different phosphate solubilization index. These isolates have a potential to mobilize insoluble inorganic phosphate for improving growth of plants under low phosphate availability. Plant growth promoting of Actinomycetes also well known as a solubilizer of inorganic phosphate through soil acidification process (Anwar *et al*., 2016). Among 18 isolates tested, fifteen isolates were able to grow on N-free medium suggesting their activity in fixing-nitrogen. The PGPR candidate also should be a non-pathogenic bacteria in the plant, therefore pathogenicity test of 18 selected isolates was conducted. The hypersensitivity test showed that all isolates were not pathogen on the plant.

We have selected 5 out of 18 potential isolates based on their capability of plant growth promoting characters, including IAA production, plant growth promoting activity *in planta*, phosphate solubilization, nitrogen fixation and non-pathogenic characters. Based on the 16S rRNA gene, 5 selected isolates were all identified as *Streptomyces* genera, yet in various taxa of species and strains. It is well reported that *Streptomyces* spp. have been considered as PGPR agents. This group exhibited an excellent potential in promoting plant growth, including in rice and sorghum (Gopalakrishnan *et al*., 2013), wheat (Jog *et al*., 2014), tomato (El-Tarabily, 2008) and maize (EL-Sayed *et al*., 2015). According to their potential properties, these 5 *Streptomyces* strains need to be further developed as biofertilizer agents for sustainable agriculture, especially on soybean agriculture.

Conclusion

A total of 53 actinomycetes isolates have been isolated from the rhizosphere of the soybean plant. Among them, 18 (34%) isolates could produce IAA in various concentration. Further, we selected 5 isolates which elicit markedly potential plant growth promoting characters based on phosphate solubilizing activity, *in planta* assay and nitrogen-fixing activity as well as non-pathogenic character. ARK 116 shown the most potent plant growth promoting Actinomycetes. Those five Actinomycetes isolates were identified as genera of *Streptomyces* based on their respective 16S rRNA gene.

Acknowledgement

We gratefully thank the Ministry of Research, Technology and Higher Education of Indonesia for all the support. The nucleotide sequences of 16S rRNA genes reported are available in the NCBI GenBank database under Accession Numbers MH612936.1, MH612939.1.1, MH612940.1, MH612941.1 and MH612942.1.

Funding Information

Part of this study was funded by the Ministry of Research, Technology and Higher Education of Indonesia through "Penelitian Dasar Unggulan Perguruan Tinggi (PD-UPT)" to Aris Tri Wahyudi under contract no.1735/IT3.11/PN/2018.

Author's Contributions

Aris Tri Wahyudi: Has led the project, designed the research activities and involved in paper writing.

Jepri Agung Priyanto: Has contributed to the experimental works, data analysis and paper writing.

Resti Afrista: Has involved in the experimental works and data analysis.

Deni Kurniati: Has involved in the experimental works and data analysis.

Rika Indri Astuti: Has contributed to the interpretation of data and paper writing.

Alina Akhdiya: Has contributed to the interpretation and data analysis.

Ethics

This article is originally from the authors works. The corresponding author confirms that all of the other authors have read and recognized the manuscript.

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