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Vol. 9(49), pp. 2337-2344, 14 December, 2015
DOI: 10.5897/AJMR2015.7796
Article Number: 1C15E8456541
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Enhanced plant growth and/or nitrogen fixation by leguminous and non-leguminous crops after single or dual inoculation of *Streptomyces griseoflavus* P4 with *Bradyhizobium* strains

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Received 5 October, 2015; Accepted 19 November, 2015

Plant growth-promoting endophytic bacteria have been widely used during crop production for either single inoculation or co-inoculation with nitrogen-fixing bacteria. The effect of *Streptomyces griseoflavus* P4 on growth of various crops was studied and the effects of co-inoculation on plant growth, nodulation and nitrogen fixation by soybean was investigated. Pot experiments were conducted in an environmentally controlled room. Nitrogen fixation was evaluated based on acetylene reduction activity (ARA). Significant increases in soybean and maize growth performance were observed after inoculation with *S. griseoflavus* P4, although some growth parameters were not significant in some crops. *S. griseoflavus* P4 was co-inoculated with high nitrogen-fixing strains. Co-inoculation of *Bradyrhizobium elkanii* AHY3-1 with P4 into the Yezin-6 (non-*Rj*) soybean cultivar resulted in significantly increased root dry weight. Nitrogenase activity also increased due to co-inoculation of *B. elkanii* AHY3-1 with P4 when compared with single inoculation of this strain. Co-inoculation of *Bradyrhizobium japonicum* SAY3-7 and *B. elkanii* AHY3-1 with *S. griseoflavus* P4 into the Yezin-11 (Rj_4) soybean cultivar increased nitrogen fixation significantly by 45 and 31%, respectively, when compared with single inoculation of these strains. Moreover, single or dual inoculation of bradyrhziobial strains resulted in significantly higher shoot biomass than that in the control and P4 alone. These results show that plant growth was promoted by *S. griseoflavus* P4 and that plant growth and nitrogen fixation were enhanced in soybean after co-inoculation of *S. griseoflavus* P4 with the *Bradyrhizobium* strains, *B. japonicum* SAY3-7 and *B. elkanii* AHY3-1.

Key words: Co-inoculation, growth, nitrogenase activity, single inoculation, soybean, *Streptomyces griseoflavus* P4.

INTRODUCTION

Soils have been the predominant reservoir for the isolation of actinomycetes, particularly the genus Streptomyces and soil microbes have been regarded as important sources of biologically active compounds (Balachandran et al., 2015). Endophytic bacteria live in the internal tissues of plants, as symbionts without causing visible harmful effects (Hallmann et al., 1997). Plant growth-promoting rhizobacteria have been used widely in agriculture. They promote plant growth by producing plant growth hormones, such as auxins (Tien et al., 1979), gibberellins (Bottini et al., 1989), cytokinins (Strzelczyk et al., 1994; Tien et al., 1979), ethylene (Strzelczyk et al., 1994) and indole-3-acetic acid (IAA) to improve root growth (Leong, 1996; Merckx et al., 1987; Nimnoi et al., 2010), induce plant resistance to disease and stressors (Wang et al., 2009) and enhance biological nitrogen fixation (Ashraf et al., 2011).

The symbiosis between the nodulating bacteria and legumes results in the fixation of atmospheric nitrogen in the nodules. Bio-fertilizer promotes nodulation efficiency and increases yield by 16-60% (DAR, 2004). Moreover, symbiotic nitrogen fixation by soybean provides 65 to greater than 160 kg fixed N ha^{-1} (Klubeck et al., 1988), accounting for 40–70% of the total nitrogen requirement. Therefore, maintaining this significant nitrogen input is important for economical and sustainable soybean yields, particularly in soils with low available soil nitrogen (Zablotowicz and Reddy, 2004). Therefore, the symbiosis between leguminuous plants and rhizobia is of considerable importance for the environment and agriculture (Ogutcu et al., 2009).

Co-inoculation of rhizobia with plant growth-promoting bacteria rather than a single inoculation of rhizobia has become popular because co-inoculation improves soybean yield and contributes to sustainable agriculture (Hungria et al., 2015). Co-inoculation of *Bradyrhizobium japonicum* with an *Azospirillum brasilense* strain improved nitrogen fixation in soybean (14.1%) as compared to that of the control (Hungria et al., 2013). Hungria et al. (2015) demonstrated that co-inoculation of *Bradyrhizobium* spp. with an *A. brasilense* strain increases soybean yield without additional nitrogen application to soils with an indigenous rhizobial population.

The endophytic actinomycete strain, P4, was isolated from sweet pea root at Kurima, Tsu-City, Japan by Thapanapongworakul (2003). Tang-um and Niamsup (2012b) reported that the P4 16S rRNA sequence has 99.7% sequence similarity with that of *Streptomyces griseoflavus* (Accession number: EU741217). The production of plant growth-promoting hydrolytic enzymes, such as chitinase, amylase (Tang-um and Niamsup, 2012a) and IAA (Soe, 2013) from *S. griseoflavus* P4 has been reported. N uptake by adzuki beans and Thai sweet pea occurred due to co-inoculation of *Streptomyces* spp. P4 with nitrogen-fixing rhizobia (Thapanapongworakul, 2003). A P4 symbiotic interaction similar to that with Myanmar rhizobia has been reported by Soe et al. (2012) and Soe and Yamakawa (2013a, 2013b) who stated that the synergistic effect of P4 with soybean-nodulating rhizobia increases nodulation and nitrogen fixation in Myanmar soybean cultivars. Moreover, Soe (2013) reported that *S. griseoflavus* P4 increases growth performance in leguminous and non-leguminous cereal and vegetable crops.

Using this endophytic strain as a single inoculant in non-leguminous crops or as a co-inoculant with indigenous rhizobial strains to increase plant growth and nitrogen fixation rates of leguminous crops would be of interest. Therefore, this study was conducted to test the effect of a single inoculation of *S. griseoflavus* P4 on various crops and to evaluate the co-inoculation effects of *S. griseoflavus* P4 with various *Bradyrhizobium* strains on plant growth, nodulation and nitrogen fixation in soybean cultivars.

MATERIALS AND METHODS

Inoculum preparation

S. griseoflavus P4 was cultured in Inhibitory Mold Agar (IMA)-2 liquid medium on a rotary shaker (100 rpm) at 30°C for 5 days. IMA-2 medium was prepared by mixing the following reagents (glucose; 5.0 g, soluble starch; 5.0 g, beef extract; 1.0 g, yeast extract; 1.0 g, NZ-case; 2.0 g, NaCl; 2.0 g, $CaCO_3$;1.0 g) with 1000 mL of sterilized distilled water as described in Shimizu et al. (2000). The P4 bacterial suspension was prepared to obtain 10^5 cells mL^{-1}. The bradyrhizobial strains *B. japonicum* SAY3-7, *B. elkanii* AHY3-1 and *B. liaoningense* SMY3-1 were cultured in A1E liquid media (Kuykendall, 1979) and incubated on rotary shaker at 30°C for 7 days. One millimeter of liquid culture medium from each isolate was diluted with 99 mL half-strength Modified Hoagland Nutrient (MHN) solution (Nakano et al., 1997) to prepare bacterial suspensions of approximately 10^7 cells mL^{-1}. These three strains were isolated from Myanmar (Htwe et al., 2015a). Htwe et al. (2015c) previously reported that these three strains showed higher nitrogen fixing efficiencies on non-*Rj* and Rj_4 soybean cultivars.

S. griseoflavus P4 symbiosis with various crops

Two cereal crops, rice (*Oryza sativa* L.) cv. Manawthuka and maize (*Zea mays* L.) cv. Kakuteru (Nihon nousan shubyo Co., Ltd.); two horticultural crops, spinach (*Spinacia oleracea* L.) cv. O-rai hourennsou (Nihon nousan shubyo Co., Ltd.) and Japanease radish (*Raphanus sativus*) cv. Tokinashi-daikon (Nihon nousan shubyo Co., Ltd.); and two leguminous plants, common bean (*Phaseolus vulgaris* (L.)) cv. Suzinashi-saitou (Nihon nousan shubyo Co., Ltd.) and soybean (*Glycine max* (L.) Merr.) cv. Yezin-6 were used to evaluate the effect of *S. griseoflavus* P4 on growth under nitrogen-free conditions. The seeds were surface sterilized by soaking in 2.5% sodium hypochlorite solution for 5 min, rinsed five times in 10 mL 99.5% ethanol, and washed five times in sterilized MHN solution to remove any trace of sodium hypochlorite and ethanol. Ten seeds per pot were grown in prepared culture pots (1 L volume) filled with 1 L vermiculite and 0.6 L MHN solution and autoclaved at 120°C for 20 min. Vermiculite was sieved

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through a 4-mm sieve to obtain fine growth medium for the crops. *S. griseoflavus* P4 was inoculated at 2 mL per seed. The plants were cultivated in a Phytotron (25°C and 75% relative humidity) for 2 weeks. Autoclaved deionized water was used for watering. This experiment was conducted during July of 2015. Five replicate plants were used from pots of the various crops to measure the growth parameters.

Symbiosis of the *Bradyrhizobium* strains and *S. griseoflavus* P4

Growth conditions occurred under nitrogen-free conditions. The treatments were single inoculations of P4, *B. japonicum* SAY3-7, *B. elkanii* AHY3-1 and *B. liaoningense* SMY3-1 and co-inoculation of P4 with each of the *Bradyrhizobium* strains. Six seeds per pot were grown in prepared culture pots (1 L volume) filled with 1 L vermiculite and 0.6 L MHN solution and autoclaved at 120°C for 20 min. A control un-inoculated treatment was also provided. *S. griseoflavus* P4 was inoculated at 2 mL per seed. *Bradyrhizobium* strain was inoculated at 5 mL per seed. The effectiveness of the single and co-inoculations was determined using the *Rj* cultivars Yezin-6 (non-*Rj*) and Yezin-11 (Rj_4). *Rj* genes of these two varieties and their symbiotic effectiveness with *B. japonicum* USDA110 were described in Htwe et al. (2015b). This experiment was conducted from July to August of 2015. Three replicate plants of the two soybean varieties were used to measure acetylene reduction activity (ARA).

ARA was measured by flame ionization gas chromatography (GC-14A; Shimadzu, Kyoto, Japan) after 4 weeks of cultivation as described by Soe and Yamakawa (2013a). Nodules were removed from the roots after the assay, and the number of nodules was recorded. Shoots, roots and nodules were collected separately and oven dried at 70°C for 24 h to obtain dry weights.

Data analysis

The data were analyzed statistically using STATISTIX 8 analytical software (Tallahassee, FL, USA) and means were compared using Tukey's HSD test at $P < 0.05$.

RESULTS

Symbiosis of *S. griseoflavus* P4 with various crops under nitrogen-free condition

The growth performance of the leguminous plants is presented in Tables 1 and 2. Soybean growth parameters, such as shoot and root length and shoot and root dry weight, differed significantly between the plants inoculated with *S. griseoflavus* P4 and the control plants (Table 1). Shoot length in common bean was affected significantly by inoculation with *S. griseoflavus* P4 (Table 2) although none of the other growth parameters differed from the control.

The growth performance of the cereal crops is presented in Tables 3 and 4. Maize shoot length and shoot and root biomass were significantly higher than those of the un-inoculated control (Table 3). In rice, no differences were observed in any of the growth parameters between the inoculated treatment and the control (Table 4).

Table 1. The effect of P4 on soybean growth at two weeks after sowing.

Treatment	SL (cm)	RL (cm)	SDW (mg plant^{-1})	RDW (mg plant^{-1})
Control	17.84^{b}	20.08^{b}	152.00^{b}	64.00^{b}
P4	19.66^{a}	24.08^{a}	182.00^{a}	88.00^{a}
P value	0.0111	0.0059	0.0127	0.0209
CV%	4.67	7.70	8.88	17.41

Means in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). SL, RL, SDW, and RDW are shoot length, root length, shoot dry weight, and root dry weight, respectively.

Table 2. The effect of P4 on common bean growth at two weeks after sowing.

Treatment	SL (cm)	RL (cm)	SDW (mg plant^{-1})	RDW (mg plant^{-1})
Control	13.82^{b}	24.78^{a}	154.00^{a}	76.00^{a}
P4	17.16^{a}	27.70^{a}	202.00^{a}	104.00^{a}
P value	0.0032	0.3363	0.0641	0.0528
CV%	8.20	17.20	19.86	21.66

Means in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). SL, RL, SDW, and RDW are shoot length, root length, shoot dry weight, and root dry weight, respectively.

Table 3. The effect of P4 on rice growth at two weeks after sowing.

Treatment	SL (cm)	RL (cm)	SDW (mg plant^{-1})	RDW (mg plant^{-1})
Control	11.78^{a}	16.16^{a}	7.38^{a}	12.50^{a}
P4	12.16^{a}	17.86^{a}	7.88^{a}	14.00^{a}
P value	0.4064	0.1156	0.2831	0.1974
CV%	5.73	8.96	9.00	12.23

Means in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). SL, RL, SDW and RDW are shoot length, root length, shoot dry weight, and root dry weight, respectively.

The growth performance of the vegetable crops is presented in Tables 5 and 6. Spinach biomass did not increase after inoculation with *S. griseoflavus* P4 (Table 5); however, root and shoot lengths differed significantly from the control. Radish root biomass was affected significantly by inoculation with *S. griseoflavus* P4 (Table 6), although none of the other growth parameters differed from the control.

Taken together, the bacterial inoculation treatments increased shoot length, root length, shoot biomass and root biomass when compared with the control.

Table 4. The effect of P4 on maize growth at two weeks after sowing.

Treatment	SL (cm)	RL (cm)	SDW (mg plant^{-1})	RDW (mg plant^{-1})
Control	17.64^{b}	31.34^{a}	45.90^{b}	79.60^{b}
P4	22.30^{a}	33.68^{a}	84.70^{a}	116.30^{a}
P value	0.0032	0.4619	0.002	0.0422
CV%	8.2	14.73	20.81	24.5

Means in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). SL, RL, SDW, and RDW are shoot length, root length, shoot dry weight, and root dry weight, respectively.

Table 5. The effect of P4 on spinach growth at two weeks after sowing.

Treatment	SL (cm)	RL (cm)	SDW (mg plant^{-1})	RDW (mg plant^{-1})
Control	1.96^{b}	8.46^{b}	5.70^{a}	3.88^{a}
P4	3.42^{a}	13.90^{a}	7.64^{a}	4.40^{a}
P value	0.01	0.0016	0.3524	0.3499
CV%	25.55	16.53	46.58	20.01

Means in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). SL, RL, SDW, and RDW are shoot length, root length, shoot dry weight, and root dry weight, respectively.

Table 6. The effect of P4 on radish growth at two weeks after sowing.

Treatment	SL (cm)	RL (cm)	SDW (mg plant^{-1})	RDW (mg plant^{-1})
Control	4.94^{a}	12.84^{a}	23.50^{a}	3.56^{b}
P4	5.22^{a}	16.12^{a}	22.90^{a}	7.44^{a}
P value	0.697	0.1248	0.08748	0.0017
CV%	21.59	20.89	24.33	24.12

Means in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). SL, RL, SDW, and RDW are shoot length, root length, shoot dry weight, and root dry weight, respectively.

Symbiosis of three selected *Bradyrhizobium* strains and *S. griseoflavus* P4

The performance of the *Bradyrhizobium* strains and *S. griseoflavus* P4 on two different soybean varieties is shown in Tables 7 and 8.

The numbers of nodules and the nodule dry weights in the Yezin-6 (non-*Rj*) cultivar differed significantly among treatments but were not significantly different between single and dual inoculations of each strain. Shoot dry weight did not increase among the treatments; however, root dry weight differed significantly among treatments. A single inoculation of *B. japonicum* SAY3-7 resulted in the highest root dry weight but the difference was not significant from those of a single inoculation of P4, single and dual inoculation of *B. liaoningense* SMY3-1, or a dual inoculation of *B. elkanii* AHY3-1. Co-inoculation of *B. elkanii* AHY3-1 with P4 resulted in significantly higher root dry weight when compared with a single inoculation of this strain. Nitrogen fixation as indicated by the ARA value differed significantly between the P4 co-inoculation and single inoculation treatments, and the control. Co-inoculation of P4 with either *B. japonicm* SAY3-7 or *B. liaoningense* SMY3-1 did not increase the nitrogen fixation rate when compared with a single inoculation of these strains. Nitrogenase activity increased approximately 24% over the control after co-inoculation of P4 with *B. elkanii* AHY3-1, although no difference was found between single and dual inoculation. These results show that co-inoculation of *B. elkanii* AHY3-1 with P4 promotes nitrogenase activity and root growth in the Yezin-6 (non-*Rj*) cultivar.

Table 7. Effect of single and coinoculation of bradyrhizobial strains on plant growth, nodulation and acetylene reduction activity of Yezin-6 soybean cultivar at 28 DAS.

Treatment	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1})
Control	0.00^{c}	0.00d	0.39^{a}	0.25^{b}	0.00^{b}
P4	0.00^{c}	0.00d	0.47^{a}	0.32^{ab}	0.00^{b}
SAY3-7	10.33^{ab}	51.40^{a}	0.48^{a}	0.36^{a}	1.24^{a}
AHY3-1	16.00^{a}	40.00^{abc}	0.47^{a}	0.25^{b}	0.88^{ab}
SMY3-1	10.67^{ab}	22.20^{bc}d	0.46^{a}	0.27^{ab}	1.60^{a}
SAY3-7 + P4	6.33^{b}	17.20^{c}d	0.42^{ab}	0.25^{b}	1.15^{ab}
AHY3-1 + P4	16.33^{a}	42.70^{ab}	0.49^{a}	0.28^{ab}	1.09^{ab}
SMY3-1 + P4	11.67^{ab}	26.00^{bc}	0.43^{a}	0.27^{ab}	1.61^{a}
P value	0.0000	0.0000	0.4471	0.0135	0.0010
CV%	24.97	35.05	12.87	12.38	48.83

Mean in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, NDW, SDW, and RDW indicate nodule number, nodule dry weight, shoot dry weight, and root dry weight, respectively. DAS means days after sowing.

Table 8. Effect of single and coinoculation of bradyrhizobial strains on plant growth, nodulation and acetylene reduction activity of Yezin-11 soybean cultivar at 28 DAS.

Treatment	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1}
Control	0.00^{c}	0.00^{b}	0.36^{c}	0.25^{a}	0.00^{c}
P4	0.00^{c}	0.00^{b}	0.45^{bc}	0.33^{a}	0.00^{c}
SAY3-7	8.00^{bc}	32.60^{a}	0.46^{abc}	0.27^{a}	0.77^{b}
AHY3-1	24.67^{a}	33.20^{a}	0.53^{ab}	0.28^{a}	0.75^{b}
SMY3-1	18.00^{ab}	27.70^{a}	0.48^{abc}	0.31^{a}	0.95^{ab}
SAY3-7 + P4	8.00^{bc}	38.20^{a}	0.46^{abc}	0.29^{a}	1.39^{a}
AHY3-1 + P4	18.67^{ab}	36.20^{a}	0.56^{ab}	0.29^{a}	1.08^{ab}
SMY3-1 + P4	13.33^{abc}	31.40^{a}	0.57^{a}	0.27^{a}	0.97^{ab}
P value	0.0001	0.0000	0.0002	0.4269	0.0000
CV%	43.67	22.85	8.43	13.37	24.30

Mean in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, NDW, SDW, and RDW indicate nodule number, nodule dry weight, shoot dry weight, and root dry weight, respectively. DAS means days after sowing.

The nodule dry weight of the Yezin-11 (*Rj*$_4$) cultivar did not differ between the co-inoculation and single inoculation treatments with the *Bradyrhizobium* strains. The number of nodules differed significantly among treatments but it was not significantly different between single and dual inoculations of each strain. Root dry weight did not change among treatments. However, shoot dry weight was significantly different among the treatments. Both single and dual inoculation of the tested strains resulted in a significant increase in shoot growth when compared with the control and P4 alone. A significant difference in the nitrogenase activity of the Yezin-11 (*Rj*$_4$) cultivar was observed between the single and dual inoculation treatments (Table 8). The highest ARA per plant was found after dual inoculation of *B. japonicum* SAY3-7, followed by dual inoculation of *B. elkanii* AHY3-1, but these ARA values were not significantly different from those of single or dual inoculation of *B. liaoningense* SMY3-1. These results show that co-inoculation of *B. japonicum* SAY3-7 and *B. elkanii* AHY3-1 with P4 increased nitrogen fixation significantly by approximately 45 and 31%, respectively, when compared with single inoculation of these strains.

DISCUSSION

In this study, the growth and plant biomass of soybean was enhanced after inoculation with *S. griseoflavus* P4, which agrees with the results of Soe (2013) who reported that root and shoot biomass was significantly higher in plants inoculated with *S. griseoflavus* P4 than in un-inoculated controls. Soe et al. (2010) reported improved shoot growth of the SJ5 Thailand soybean cultivar after inoculation with P4. Moreover, maize shoot and root biomass amounts were significantly higher than those of un-inoculated controls. These findings agree with those of Soe (2013) who reported that maize shoot and root dry weights increased significantly after inoculation with *S. griseoflavus* P4 when compared with the control. Radish root biomass increased significantly after inoculation with *S. griseoflavus* P4, although other growth parameters did change relative to the un-inoculated control. These results support the findings of Soe (2009, 2013), who found that the root biomass of sweet pea inoculated with P4 was significantly higher than that of the un-inoculated control, although the shoot biomass did not differ significantly (Soe, 2013). Moreover, inoculation of *S. griseoflavus* P4 increased the root dry weight of Myanmar soybean, Hinthada by approximately 63% over the control (Soe, 2009).

Soybean, common bean, maize and spinach shoots and roots elongated significantly after inoculation with the P4 strain. The plant growth-promoting effects of *S. griseoflavus* P4 on leguminous and non-leguminous crops may be due to the production of plant growth-promoting compounds, such as IAA. Soe (2013) reported that *S. griseoflavus* P4 can produce IAA. Meguro et al. (2006) reported that an endophytic strain of *Streptomyces* spp. MBR-52 caused pronounced enhancement of emergence and elongation of plant adventitious roots. IAA-producing microorganisms are known to stimulate root elongation and enhance plant growth (Patten and Glick, 2002).

In this study, the symbiotic interaction between P4 and *B. elkanii* AHY3-1 improved root growth significantly in the Yezin-6 (non-*Rj*) cultivar as compared to that of a single inoculation of a *Bradyrhizobium* strain and *S. griseoflavus* P4. Co-inoculating P4 with the selected indigenous bradyrhizobial strains *B. japonicum* SAY3-7 and *B. elkanii* AHY3-1 in the Yezin-11 (Rj_4) cultivar significantly increased shoot dry weight as compared to a single inoculation of a *Bradyrhizobium* strain. Bai et al. (2002) reported that the endophytic *Bacillus subtilis* strains NEB4 and NEB5 and *Bacillus thuringiensis* strain NEB17 have excellent potential as plant growth promoters in soybean. They stated that the weight of soybean increased after co-inoculation of *B. japonicum* with another strain under nitrogen-free conditions when compared with plants inoculated with *B. japonicum* alone. The symbiotic interaction between P4 and *B. elkanii* AHY3-1 improved nitrogen fixation significantly as compared to a single inoculation of a *Bradyrhizobium* strain and *S. griseoflavus* P4 in the Yezin-6 (non-*Rj*) cultivar. The symbiotic interaction between P4 and the selected indigenous bradyrhizobial strains *B. japonicum* SAY3-7 and *B. elkanii* AHY3-1 increased nitrogen fixation significantly in the Yezin-11 (Rj_4) cultivar as compared to a single inoculation of a *Bradyrhizobium* strain. This result was supported by findings of others that dual inoculation of a bradyrhizobial strain and an endophytic actinomycete (*Streptomyces* spp. P4) increases nodulation and the nitrogen fixation rate in various soybean varieties (Soe et al., 2012; Soe and Yamakaw, 2013a, b). Thapanapongworakul (2003) reported that dual inoculation of P4 *S. griseoflavus* and the *B. japonicum* USDA 110 bradyrhizobial strain increases N uptake by SJ5 (Thailand soybean cultivar) with approximately 44.3% as compared to single inoculation of the same strain.

The *Rj* genes play roles in adaptation and preference for specific rhizobial strains (Ishizuka et al., 1991; Saeki et al., 2000). In addition, non-*Rj* is compatible with all bradyrhizobial strains, but Rj_4 has unique features that restrict nodulation with specific *Bradyrhizobium* strains (Vest and Caldwell, 1972). Soe and Yamakawa (2013a) reported that symbioses were observed after dual inoculation of P4 and MAS34 and dual inoculation of P4 and MAS23 in Yezin-3 (Rj_4) and Yezin-6 (*non-Rj*), respectively. The MAS34 and MAS23 strains have been isolated from Rj_4- and non-*Rj*-genotype cultivars, respectively (Soe et al., 2013b). In this study, dual inoculation of P4 with *B. japonicum* SAY3-7 and *B. elkanii* AHY3-1 isolated from Rj_4 cultivars increased the symbiotic nitrogen fixation rates in Yezin-11 (Rj_4) cultivars. No difference was found for the Yezin-6 (*non-Rj*) cultivar.

Streptomyces strains are frequently reported to be plant growth-promoting microbes (Nassar et al., 2003; El-Tarabily, 2008). Plant growth-promoting traits such as IAA production and ACC deaminase, cellulase and nitrogenase activities by *Bacillus megaterium* LNL6 and *Methylobatcterium oryzae* CBMB20, contribute to the improvement of the overall symbiosis of nitrogen fixation (Subramanian et al., 2015). Plant growth-promoting traits, including the activity of hydrolytic enzymes such as chitinase and amylase (Tang-um and Niamsup, 2012a and 2012b) and IAA production (Soe, 2013), have been documented for *S. griseoflavus* P4. Therefore, the enhancement of nitrogen fixation observed in this study may be related to the induction of plant growth hormones by *S. griseoflavus* P4. Sturz et al. (2003) and Sarr et al. (2010) reported that beneficial, detrimental and neutral effects of endophytic bacteria depend on the co-inoculated strains of nitrogen-fixing bacteria. In this study, co-inoculation of P4 with either *B. japonicum* SAY3-7 or *B. elkanii* AHY3-1 had a synergistic effect on plant growth and nitrogen fixation in the Yezin-11 cultivar; however, co-inoculation of P4 with *B. liaoningense* SMY3-1 was not beneficial or detrimental to growth or nitrogen fixation rates in either tested cultivar. Therefore, co-inoculation of this endophytic bacterium with *B. japonicum* SAY3-7 or *B. elkanii* AHY3-1 may be useful for producing bio-fertilizer and reduce the need for nitrogen fertilizer by enhancing growth and nitrogen fixation rates in soybean.

Conclusion

A beneficial effect of *S. griseoflavus* P4 on the growth of leguminous and non-leguminous crops was observed in this study. It was shown that *S. griseoflavus* P4 was effective in both leguminous and non-leguminous crops. The root dry weight of the Yezin-6 (non-*Rj*) cultivar increased significantly after co-inoculation of *B. elkanii* AHY3-1 with P4 when compared with a single inoculation of this strain. Moreover, nitrogenase activity increased (24%) after inoculating *B. elkanii* AHY3-1 with P4 when compared with a single inoculation of this strain, although no difference among treatments was observed. Co-inoculation of *B. japonicum* SAY3-7 and *B. elkanii* AHY3-1 with P4 increased nitrogen fixation in the Yezin-11 (Rj_4) cultivar by approximately 45 and 31%, respectively, when compared with single inoculation of these strains. The results demonstrate that *S. griseoflavus* P4 promotes growth and nitrogen fixation in soybean after co-inoculation with the *Bradyrhizobium* strains *B. japonicum* SAY3-7 or *B. elkanii* AHY3-1. This study was conducted in pots under controlled conditions. Further studies should be conducted under open field conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work was supported by Ministry of Education, Culture, Sports, Sciences and Technology (MEXT) of Japan.

REFERENCES

Ashraf MA, Rasool M, Mirza MS (2011). Nitrogen fixation and indole acetic acid production potential of bacteria isolated from rhizosphere of sugarcane (*Saccharum officinarum* L.). Adv. Biol. Res. 5:348-355.

Bai Y, D'Aoust F, Smith DL, Driscoll BT (2002). Isolation of plant-growth-promoting Bacillus strains from soybean root nodules. Can. J. Microbiol. 48:230-238.

Balachandran C, Duraipandiyan V, Emi N, Ignacimuthu S (2015). Antimicrobial and cytotoxic properties of Streptomyces sp. (ERINLG-51) isolated from Southern Western Ghats. South Indian J. Biol. Sci. 1:7-14.

Bottini R, Fulchieri M, Pearce D, Pharis R (1989). Identification of gibberelins A1, A3, and iso-A3 in cultures of *A. lipoferum*. Plant Physiol. 90:45-47.

DAR (2004). Rhizobium biofertilizer: The result of research of agricultural research, Golden Jubilee, Department of Agricultural research. pp. 114-118.

El-Tarabily KA (2008). Promotion of tomato (*Lycopersicon esculentun* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing *Streptomycete* actinomycetes. Plant Soil 308:161-174.

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997). Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43:895-914.

Htwe AZ, Saeki Y, Moe K, Yamakawa T (2015b). Determining nodulation regulatory (*Rj*) genes Myanmar soybean cultivars and their symbiotic effectiveness with *Bradyrhizobium japonicum* USDA110. Am. J. Plant Sci. 6:2799-2810.

Htwe AZ, Yamakawa T, Moe K, Dien DC (2015c). Evaluation of the symbiotic effectiveness of different indigenous *Bradyrhizobium* strains on different *Rj*-genes harboring Myanmar soybean cultivars. Afr. J. Microbiol. Res. (submitted).

Htwe AZ, Yamakawa T, Sarr PS, Sakata T (2015a). Diversity and distribution of soybean-nodulation bradyrhizobia isolated from major soybean-growing regions in Myanmar. Afr. J. Microbiol. Res. 9(43):2183-2196.

Hungria M, Nogueira MA, Araujo RS (2013). Co-inoculation of soybeans and common beans with rhizobia and azospirilla: Strategies to improve sustainability. Biol. Fertil. Soils 49:791-801.

Hungria M, Nogueira MA, Araujo RS (2015). Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. Am. J. Plant Sci. 6:811-817.

Ishizuka J, Suemasu Y, Mizogami K (1991). Preference of *Rj*-soybean cultivars for *Bradyrhizobium japonicum* for nodulation. Soil Sci. Plant Nutr. 37:15-21.

Klubeck BP, Hendrickson LL, Zablotowicz RM, Skwara JE, Varsa EC, Smith S, Isleib TG, Maya J, Valdes M, Dazzo FB, Todd RL, Walgenback DD (1988). Competitiveness of selected *Bradyrhizobium japonicum* strains in Midwestern USA soils. Soil Sci. Soc. Am. J. 52:662-666.

Kuykendall LD (1979). Transfer of R factors to and between genetically marked sublines of *Rhizobium japonicum*. Appl. Environ. Microbiol. 37:862-866.

Leong J (1996). Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. Ann. Rev. Phytopathol. 24:187-209.

Meguro A, Ohmura Y, Hasegawa S, Shinizu M, Nishimura T, Kunoh H (2006). An endophytic actinomycete, *Streptomyces* sp. MBR-52, that accelerates emergence and elongation of plant adventitious roots. Actinomycetologica 20:1-9.

Merckx R, Dijkra A, Hartog AD, Veen JAV (1987). Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biol. Fertil. Soils 5:126-132.

Nakano Y, Yamakawa T, Ikeda M, Ishizuka J (1997). Nodulation of *Rj*-soybean varieties with *Rhizobium fredii* USDA193 under limited supply of nutrients. Soil Sci. Plant Nutr. 43:929-932.

Nassar AH, ElTarabily KA, Sivasithamparam K (2003). Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine producing isolate of Streptomyces griseoluteus. Plant Growth Regul. 40:97-106.

Nimnoi P, Pongsilp N, Lumyong S (2010). Endophytic actinomycetes isolated from *Aquilaria crassna* Pierre ex Lec and screening of plant growth promoters production. World J. Microbiol. Biotechnol. 26:193-203.

Ogutcu H, Adiguzel A, Gulluce M, Karadayi M, Sahin F (2009). Molecular characterization of Rhizobium strains isolated from wild chickpeas collected from high altitudes in Erzurum-Turkey. Rom. Biotechnol. Lett. 14(2):4294-4300.

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68:3795-3801.

Saeki Y, Akagi I, Takaki H, Nagatomo Y (2000) Diversity of indigenous *Bradyrhizobium* strains isolated from three different *Rj*-soybean cultivars in terms of randomly amplified polymorphic DNA and intrinsic antibiotic resistance. Soil Sci. Plant Nutr. 46:917-926.

Sarr PS, Yamakawa T, Asatsuma S, FujimotoS, Sakai M (2010). Investigation of endophytic and symbiotic features of *Ralstonia* sp. TSC1 isolated from cowpea nodules. Afr. J. Microbiol. Res. 4(19):1959-1963.

Shimizu M, Nakagawa Y, Sato Y, Furumai T, Igarashi Y, Onaka H, Yoshida R, Kunoh H (2000). Studies on endophytic actinomycetes (I) *Streptomyces sp*. isolate from rhododendron and its antifungal activity. J. Gen. Plant Pathol. 66:360-366.

Soe KM (2009). Effect of endophytic actinomycetes and bradyrhizobia on nodulation and nitrogen fixation of different soybean varieties. Master Thesis, Chiang Mai University, Thailand.

Soe KM (2013). Effect of co-inoculation of Myanmar bradyrhizobia with *Streptomyces griseoflavus* P4 on nodulation, nitrogen fixation and seed yield of Myanmar soybean cultivars. PhD Thesis. Laboratory of Plant Nutrition, Kyushu University, Japan.

Soe KM, Bhromsiri A, Karladee D (2010). Effect of selected endophytic actinomycetes (*Streptomyces* sp.) and bradyrhizobia from Myanmar on growth, nodulation, nitrogen fixation and yield of different soybean

varieties. CMU. J. Nat. Sci. 9(1):95-109.
Soe KM, Bhromsiri A, Karladee D, Yamakawa T (2012). Effects of endophytic actinomycetes and *Bradyrhizobium japonicum* strains on growth, nodulation, nitrogen fixation and seed weight of different soybean varieties. Soil Sci. Plant Nutr. 58:319-325.
Soe KM, Yamakawa T (2013a). Evaluation of effective Myanmar *Bradyrhizobium* strains isolated from Myanmar soybean and effects of coinoculation with *Streptomyces griseoflavus* P4 for nitrogen fixation. Soil Sci. Plant Nutr. 59:361-370.
Soe KM, Yamakawa T (2013b). Low-density co-inoculation of Myanmar *Bradyrhizobium yuyanmingense* MAS34 and *Streptomyces griseoflavus* P4 to enhance symbiosis and seed yield in soybean varieties. Am. J. Plant Sci. 4:1879-1892.
Strzelczyk E, Kamper M, Li C (1994). Cytocinin-like-substances and ethylene production by Azospirillum in media with different carbon sources. Microbiol. Res. 149:55-60.
Sturz AV, Christie BR, Nowak J (2000). Bacterial endophytes: Potential role in developing sustainable systems of crop production. Crit. Rev. Plant Sci. 19:1-30.
Subramanian P, Kim K, Krishnamoorthy R, Sundaram S, Sa T (2015) Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. Plant Growth Regul. 76:327-332.
Tang-um J, Niamsup H (2012a). Extracellular amylase activity from endophytic *Streptomyces griseoflavus* P4. Chiang Mai J. Sci. 39(2):346-350.
Tang-um J, Niamsup H (2012b). Chitinase production and antifungal potential of endophytic *Streptomyces* strain P4. Maejo Int. J. Sci. Technol. 6(1):95-104.
Thapanapongworakul P (2003). Characterization of endophytic actinomycetes capable of controlling sweet pea root rot diseases and effects on root nodule bacteria. Master Thesis, Chiang Mai University, Thailand.
Tien TM, Gaskins MH, Hubbell DH (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. 37:1016-1024.
Vest G, Caldwell BE (1972). Rj_4-A gene conditioning ineffective nodulation in soybean. Crop Sci. 12:692-693.
Wang S, Huijun W, Junqing Q, Lingli M, Jun L, Yanfei X, Xuewen G (2009). Molecular mechanism of plant growth promotion and induced systemic resistance to tobacco mosaic virus by *Bacillus* spp. J. Microbiol. Biotechnol. 19:1250-1258.
Zablotowicz RM, Reddy KN (2004). Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate resistant transgenic soybean: A mini review. J. Environ. Qual. 33:825-831.