Chiang Mai J. Sci. 2017; 44(1) : 83-93
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Rhizobacterial Candidates Isolated from Jerusalem Artichoke (*Helianthus tuberosus* L.) Rhizosphere for Host Plant Growth Promotion

Kamon Sritongon [a,b], Wiyada Mongkolthanaruk [b], Sophon Boonlue [b], Sanun Jogloy [c,d], Darunee Puangbut [c,d] and Nuntavun Riddech*[b]

[a] Graduate school of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
[b] Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
[c] Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.
[d] Peanut and Jerusalem artichoke Improvement for Functional Food Research Group and Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.
*Author for correspondence; e-mail: nunrid@kku.ac.th

Received: 8 April 2015
Accepted: 16 March 2016

Abstract

The objective of this work was to isolate and characterize rhizobacteria from Jerusalem artichoke in order to evaluate their abilities to promote early growth of the plant *in vivo*. Characterization of plant growth promoting activities such as nitrogen fixation, phosphate and potassium solubilization, indole-acetic acid production (IAA), siderophore and phytopathogenic inhibition was conducted. A total of 46 isolates gave positive results for either direct or indirect plant growth promoting activity. Selected stains were identified on the basis of 16s rRNA gene sequences, evaluated individually by being mixed in liquid media which was then used to inoculate pots containing Jerusalem artichoke. Strains identified as *Pseudomonas azotoformans* (N3-903 and C2-114) and *Rhodococcus cercidiphylli* (S1-903) were evaluated in pot experiments under greenhouse conditions. Inoculation of strain C2-114 showed increased shoot dry weight (up to 52.6%), root dry weight (up to 58.5%), and biomass (up to 54.7%). The result of association of IAA activity with plant growth was significant. We provide the first report of plant growth promoting activity by *R. cercidiphylli*. *Pseudomonas* strains has the potential to be use in field-grown Jerusalem artichoke.

Keywords: plant growth promoting rhizobacteria, indole-acetic acid, *Pseudomonas azotoformans*, *Rhodococcus cercidiphylli*

1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is becoming popular as a new crop in Thailand. Its tuber containing inulin can reduce the risk of colon cancer, by suppressing tumour growth [1]. Moreover, the tuber is a source of novel prebiotic foods for human consumption, bioethanol, fructose syrup and animal feed [2]. Given these promising uses, it is important to

develop sustainable strategies for cultivation, including organic production.

It is possible that organic products can find uses as fertilizers, growth regulators and livestock feed additive, substituting partially or completely for synthetic compounds, and thereby improving agricultural sustainability [3]. Biofertilizers and biological control agents have potential as less damaging substitutes [4]. For example, plant growth promoting rhizobacteria (PGPR) can be a part of new, more ecological based agricultural system [5].

Numerous soil bacteria that prosper in the plant rhizosphere, can enhance plant growth with a plethora of mechanisms; these are known collectively as PGPR [6]. They stimulate plant growth via production of the phytohormones indole-acetic acid (IAA), gibberellins and cytokinins as well as mineral solubilization, asymbiotic nitrogen fixation, antibiosis against phytopathogenic microorganisms, and production of hydrogen cyanide, siderophores as well as antagonistic activity [7]. PGPR is the most important population providing available substrate and mineral exchange into vicinity of root system [8].

Previous research revealed endophytic nitrogen fixing bacteria from the root of Jerusalem artichoke in China [9]. However, there are limitations on use of indigenous microorganisms for enhancement of plant productivity, as PGP activity may vary due to environmental factors including climate, weather, soil characteristics or activity of the indigenous microbes of the soil [7]. Thus, the aim of our work was to 1) discover novel plant growth promoting microbial species associated with the rhizosphere of Jerusalem artichoke and 2) to evaluate the potential of representative strains to act as biofertilizers. To the best of our knowledge, this is the first report of effective PGPR isolated from Jerusalem artichoke in Thailand.

2. MATERIALS AND METHODS

2.1 Isolation and Selection

Samples of soil adhering to Jerusalem artichoke roots were collected from various fields in Thailand during August-September, 2012 and soil was removed by shaking. The 18 rhizosphere soil samples from fields located in Nakhon Ratchasima, Saraburi, Udonthani, Phetchabun and Chaiyaphum provinces were analyzed chemical characters (Table 1). Ten grams of the each sample was transferred to 90 mL in sterile distilled water to shake on a gentle shaker for 10 min. Serial dilutions were spread on soil-extract peptone agar [10]. After that, the petri dishes were incubated at 30°C for 48-72 h. Different characters of colony were selected, then these colonies were purified on nutrient agar (NA) by cross-streak technique and maintained on agar slants at 4-7°C.

2.2 Assays for Direct Plant Growth Promotion Activity

Nitrogen fixation was assayed in Ashbey's nitrogen-free mannitol broth [11], which was dissolved in deionized water before sterilization. National Botanical Research Institute Phosphate medium (NBRIP) agar [12] was used for testing capacity of strains to solubilize tricalcium phosphate. Potassium solubilizing bacteria were detected by Aleksandrov agar supplemented with potassium aluminium silicate powder [13]; a clear zone around the colony, indicated to positive solubilization after 7 days of incubation at room temperature (25 to 30°C). Salkowski's reagent [4] was used to screen for IAA production in nutrient medium broth (NB) (with and without 1 g L^{-1} L-tryptophan) and incubation period was 72 h at room temperature. These experiments were performed in triplicate.

2.3 Assays for Indirect Plant Growth Promotion Assay

Siderophore production: Siderophore production was investigated using chrome

Table 1. Description of the rhizosphere soil samples.

Province	Area	Soil texture	% OM	Total elements (g kg^{-1})			Available elements (mg kg^{-1})		pH[a]	Ec[b] (ds m^{-1})
				N	P	K	P	K		
Nakhon Ratchasima	Mueang Pak Chong	S SL	0.53 2.19	0.33 1.32	0.12 0.44	0.47 0.88	41.92 35.33	107.01 108.45	5.72 7.14	0.30 0.33
Saraburi	Ban Mo	SL	2.99	1.45	0.38	0.89	17.29	414.59	7.67	0.40
Udonthani	Mueang	S	0.64	0.39	0.09	0.40	16.47	34.55	4.42	0.15
Phetchabun	Nam Nao	SL	3.52	1.37	0.33	5.54	8.34	119.48	4.10	0.20
Chaiyaphum	Chulabhorn Dam	LS	2.76	1.15	0.88	1.35	343.78	216.89	4.55	0.41

OM: Organic matter, N: Nitrogen, P: Phosphorus, K: Potassium, S: Sand, SL: Sandy loam, LS: Loamy sand

[a] 1:2 (Soil:Distilled water)

[b] 1:5 (Soil:Distilled water)

azurol S (CAS) agar [14]. Briefly, each isolate was transferred by dissecting needle to the surface of a petri dish containing CAS agar, then incubated overnight for 7 days at 28°C to detect the presence of an orange-yellow halo zone denoting siderophore production.

Antipathogenic fungal assay: A phytopathogenic, isolate of the soilborne fungus *Sclerotium rolfsii* obtained from an infected Jerusalem artichoke in a field at Khon Kaen University, was paired with the rhizosphere inhabiting strains of bacteria on potato dextrose agar (PDA) in dual culturing assays. A PDA disc (5 mm diameter) containing the fungus was placed up at the center of the plate and incubated for 24 h. The candidate bacterial strain was then streaked onto the plate 2 cm away from the center. *Sclerotium rolfsii* cultured alone on PDA served as a control. All Petri dishes were incubated at room temperature for 72 h, after which the percentage of radial growth inhibition of *S. rolfsii* by each candidate strain was calculated using the formula of Sarathambal et al. [15].

2.4 16S rDNA Sequencing and Phylogenetic Tree

Genomic DNA was extracted by using a genomic DNA mini kit (Geneaid Biotech Ltd., Taiwan). Amplification of DNA coding 16S ribosomal RNA regions was carried out by PCR with *Taq* polymerase, using two primers, 20F (5'-GAG TTT GAT CCT GGC TCA G-3'), positions 9-27 and 1500R (5'-GTT ACC TTG TTA CGA CTT-3'), position 1509-1492 [16]. Purified PCR products were directly sequenced with an ABI PRISM® BigDye™ Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems). Sequencing of 16s rDNA was obtained by using the primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 800R (5'-TAC CAG GGT ATC TAA TCC-3'), and addition 518F (5'-CCA GCA GCC GCG GTA ATA CG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3'). An ABI Prism® 3730XL DNA Sequence (Applied Biosystems) was used for DNA sequencing. The nucleotide sequences derived from all primers were entered to BioEdit (Cap contig assembly). Alignment

algorithm was used for the calculation of pairwise sequence similarity [17], which was implemented on the EzTaxon-e server [18]. The MEGA 5.10 program was applied for phylogenetic trees.

2.5 Quantitative Analysis *in vitro* of Direct Plant Growth Activity

Selected strains were assessed for bio-compatibility using a modified version of the methods of Yu et al. [19]. Each culture was streaked simultaneously across the central culture line of a 9 cm-diameter petri plate, perpendicular to the central culture, for its bio-compatibility on tryptone soya agar (HiMedia®, India) and incubated at 30°C for 48 h. Occurrence of an antagonistic zone at the intersections of cultures inferred bio-incompatibility of the isolates. Individual strain was grown in 50 mL NB in Erlenmeyer flask, cultured on a shaker (150 rpm) for 18 h. The bacterial cells were suspended in distilled water and diluted to 10^8 CFU mL^{-1}. An equal volume of each single inoculum was mixed and used as a mixture suspension. One milliliter of individual and mixture suspensions was transferred to media for quantitative estimations:

Nitrogen fixation: The inoculum was transferred to Ashbey's nitrogen-free broth, and then incubated on a rotary shaker at 30°C for 72 h. Nitrogen content was processed using micro-kjeldahl method and measured with a Flow Injection Analyzer (FIA5012, Tecator, Sweden).

Phosphate and potassium solubilization: For this assay, 40 mL NBRIP broth was added to 125 mL Erlenmeyer flasks and potassium solubilizing ability was detected in Aleksandrov liquid medium supplemented with potassium aluminium silicate as inorganic potassium source. Both experiments were carried out at 30°C for 7 days with a rotary shaker. After incubation, the supernatant was collected and centrifuged twice at 4,000 rpm for 30 min. For quantitation, soluble phosphate in supernatant was measured by the vanadomolybdate method [20]. Solubilized potassium content in the supernatant was estimated in each of the samples by a flame photometry apparatus at 766.5 nm wavelength.

IAA production: The colorimetric quantification procedure was used following the modified method of Ribeiro and Cardoso [4]. One mL of the culture was inoculated in an Erlenmeyer flask (aperture aluminum foil) containing 40 mL NB amended with L-tryptophan (final concentration of 1 g L^{-1}), and incubated at 30°C for 24 h on a rotary shaker (150 rpm). IAA was separated by centrifugation at 4,000 rpm for 30 min, a 1 mL aliquot was then mixed vigorously with an equivalent volume of Salkowski's reagent, and incubated in darkness for 25 min at room temperature. A pinkish color developed, and was quantified using a spectrophotometer (530 nm) against with an IAA standard.

2.6 Plant Material and Pot Experiment

The Kaentawan 50-4 cultivar (JA102×89) of Jerusalem artichoke was used, supplied by Faculty of Agriculture, Khon Kaen University. Young plants were prepared with tubers following the method of Sennoi et al. [21]. One of two true leaves per plant was transplanted to a 14.5 cm-diameter plastic pot filled with nonsterile sandy soil (Table 2). The experiment was conducted in an open-sided greenhouse at the Faculty of Agriculture (Field Crop Division), Khon Kaen University, in March, 2014. It was designed in a randomized complete block with four replications (three pots per unit). Plant biomass (shoot and root dry weight) and height was determined 30 days after transplantation.

Five treatments included both pure and mixed strains and a non-inoculated control treatment was added. Each inoculum mixture was prepared individually in NB, then incubated overnight at 30°C with shaking at 150 rpm.

Table 2. Description of the characteristics of the soil used for potted experiment.

Soil texture	% OM	Total elements (mg kg^{-1})			Available elements (mg kg^{-1})		CEC (cmol kg^{-1})	pH[a]	Ec[b] (ds m^{-1})
		N	P	K	P	K			
sandy soil	0.36	144.02	67.96	291.45	6.02	47.03	2.57	5.5	0.02

OM: Organic matter, N: Nitrogen, P: Phosphorus, K: Potassium, CEC: Cation exchange capacity

[a] 1:2 (Soil:Distilled water)

[b] 1:5 (Soil:Distilled water)

Viable cells were collected by centrifugation at 4,000 rpm for 20 min, washed with sterile distilled water, thoroughly re-washed again and re-suspended in sterile tap water, and adjusted to a final concentration of 10^8 CFU mL^{-1} (equivalent volume of each strain as mixture treatment). An autopipette was used for transferring 5 mL of bacterial inoculum and inoculated into plant; sterile tap water was applied to pots as control.

2.7 Statistical Analysis

Data analysis was conducted with analysis of variance (ANOVA), using STATISTIX 8 software program (Analytical Software, Tallahassee, Florida, USA). The least significant difference (LSD) test was analyzed with separation of means at $P < 0.05$ and $P < 0.01$ for *in vitro* and potted experiments.

3. Results and Discussion

3.1 Characteristics of Bacterial Isolates Exhibiting Plant Growth Promoting Activity

Forty-six isolates exhibited evidence of direct plant growth promotion (Table 3). Of this total, 21 (45.7 %) were able to grow in liquid nitrogen free medium, and were therefore considered to be diazotrophic bacterial isolates. Solubilization of phosphate and potassium occurred for 26 strains (56.5%), whereas 11 (23.9%) colonies grew on Aleksandrov agar. Only eight strains (17.4%) were positive for IAA. Whole isolates possessing direct plant growth promotion activities were used in indirect activity assays (siderophore and antagonistic plant pathogen); results showed >50% inhibition of *S. rolfsii* mycelia growth for S2-801 and U4-90101 on evaluated agar plates and obtained 23 isolates that exhibited a yellow halo zone on CAS agar. Ribeiro and Cardoso [4] reported that rhizosphere microbes had multiple mechanisms for promoting growth of *Araucaria angustifolia*.

3.2 16S rDNA Sequence and Phylogenetic Tree

Rhizobacteria isolates S1-903, C2-114 and N3-903 had multiple functions for promoting plant growth, so we selected these microbes for characterization by 16s rRNA gene sequences. The full sequences analyzed on homology with available sequences deposited in the database, revealing 99.58% similarity of C2-114 and N3-903 with *Pseudomonas azotoformans* in a clade with type strain (Figure 1.). *Pseudomonas azotoformans* has been found in the potato endosphere, and may support to plant growing [22]. PGP attributes of fluorescent *P. azotoformans* such as nitrogen fixation, solubilization of mineral phosphate and sulphur were reported in work of Levenfors et al. [23], and we report here IAA production, potassium solubilization and siderophore production of *P. azotoformans* N3-

Table 3. Characterization of bacterial isolates for nitrogen fixation, phosphate and potassium solubilization, IAA production, siderophore and phytopathogen inhibition.

Isolate	Nitrogen fixation	Phosphate solubilization	Potassium solubilization	IAA production		Siderophores	Antagonism against *S. rolfsii* (% inhibition)
				with L-tryptophan	without L-tryptophan		
H1-607	-	+	-	-	-	-	-
H1-1003	+	-	-	-	-	-	-
H1-702	+	+	-	-	-	-	-
H1-606	+	-	-	-	-	-	-
H1-801	+	-	-	-	-	-	-
H2-801	-	+	-	-	-	-	-
H2-703	-	-	-	+	-	-	-
H2-606	-	-	-	+	-	-	-
H3-603	-	-	-	+	-	+	-
H3-1002	+	-	-	-	-	-	-
P1-50102	-	+	-	-	-	+	-
P1-702	-	+	-	-	-	-	-
P1-5092	+	-	-	-	-	-	-
P1-504	-	+	+	-	-	+	-
P1-5091	+	-	-	-	-	+	-
P1-5071	+	+	-	-	-	+	-
P2-601	+	-	-	-	-	-	-
P2-502	+	-	-	-	-	+	-
P2-508	+	-	-	-	-	-	-
P3-703	+	-	-	-	-	-	-
S1-1002	+	-	-	-	-	-	-
S1-1003	-	-	-	+	-	-	-
S1-903	**+**	**+**	**+**	**+**	**-**	**+**	**30.24**
S2-1003	-	+	-	-	-	-	-
S2-801	+	-	-	-	-	+	52.37
S2-1002	-	+	-	-	-	+	-
S2-802	-	-	+	-	-	-	-
S2-1001	-	+	+	-	-	+	-
U1-805	-	+	+	-	-	+	-
U1-90101	+	-	-	-	-	+	-
U3-80201	-	+	-	-	-	+	-
U3-9021	-	+	-	-	-	+	-

Table3. (Continued).

Isolate	Nitrogen fixation	Phosphate solubilization	Potassium solubilization	IAA production		Siderophores	Antagonism against *S. rolfsii* (% inhibition)
				with L-tryptophan	without L-tryptophan		
U4-90101	-	+	-	-	-	+	50.67
U4-804	+	-	-	-	-	-	-
U4-100202	+	+	-	-	-	-	-
U4-100201	-	+	-	-	-	+	-
C1-112	-	+	+	+	-	+	-
C1-11201	+	-	-	-	-	-	-
C2-110201	-	+	+	-	-	+	-
C2-114	**+**	**+**	**+**	**+**	**-**	**+**	**-**
N1-90101	-	+	-	-	-	+	-
N2-1042	-	+	+	-	-	+	-
N3-904	-	-	+	-	-	-	-
N3-90102	-	+	+	-	-	-	-
N3-906	-	-	+	-	-	+	-
N3-903	**+**	**+**	**+**	**+**	**-**	**+**	**-**

+ or – indicate positive or negative result respectively

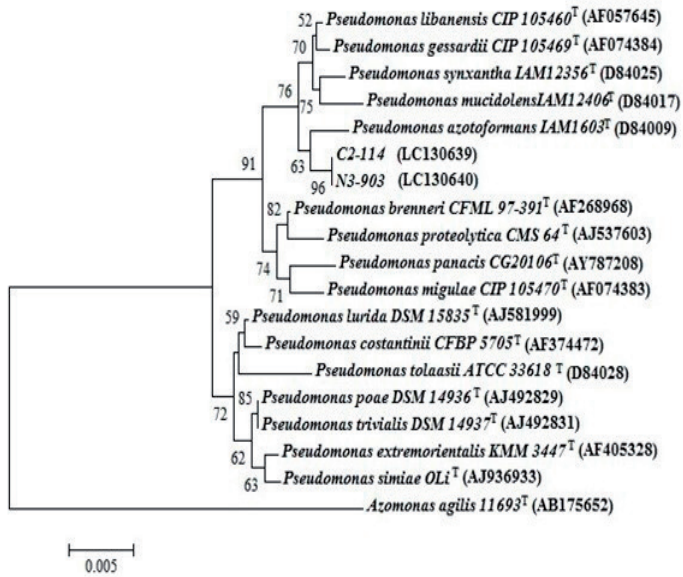

Figure 1. The evolutionary history of C2-114 and N3-903 strains was carried out by using the Neighbor-Joining method with the bootstrap test (1000 replicates) and *Azomonas agilis* 11693^{T} as outgroup.

903 and C2-114. S1-903 had 99.56% similarity *Rhodococcus cercidiphylli* YIM 65003^{T} (Figure 2.). *R. cercidiphylli* first discovered by Li et al. [24] as an endophyte of *Cercidiphyllum japonicum* leaves, was examined as part of research for new bioactive secondary metabolites. This *Rhodococcus* species has not been established in PGPR group. To our knowledge, we present the first evidence that *R. cercidiphylli* displays attributes of PGPR.

Our results showed that *Pseudomonas* sp. was not as antagonistic against *S. rolfsii* under the conditions tested. However, Ahmad et al. [7] found that fluorescent *Pseudomonas* strains had both siderophore synthesis and antagonistic activity against the other fungal phytopathogens *Aspergillus* sp., *Fusarium solani*, *F. ciceri* and *F. oxysporum*. *S. rolfsii*, the causal agent of southern blight, was first reported on Jerusalem artichoke in California, USA [25], and has also caused widespread damage on this crop in Thailand. Future work in our group will focus more explicitly on interaction of PGPR with *S. rolfsii* with the goal of improving southern blight management on Jerusalem artichoke.

3.3 Plant Growth Promoting Activity

There was no evidence of formation of antagonistic zones among strains. Activities of selected bacterial strains in quantitative estimation following screening liquid media showed values (Table 4). We obtained no significant differences among treatments on the amount of available P and K in the crude extract samples. Deceased

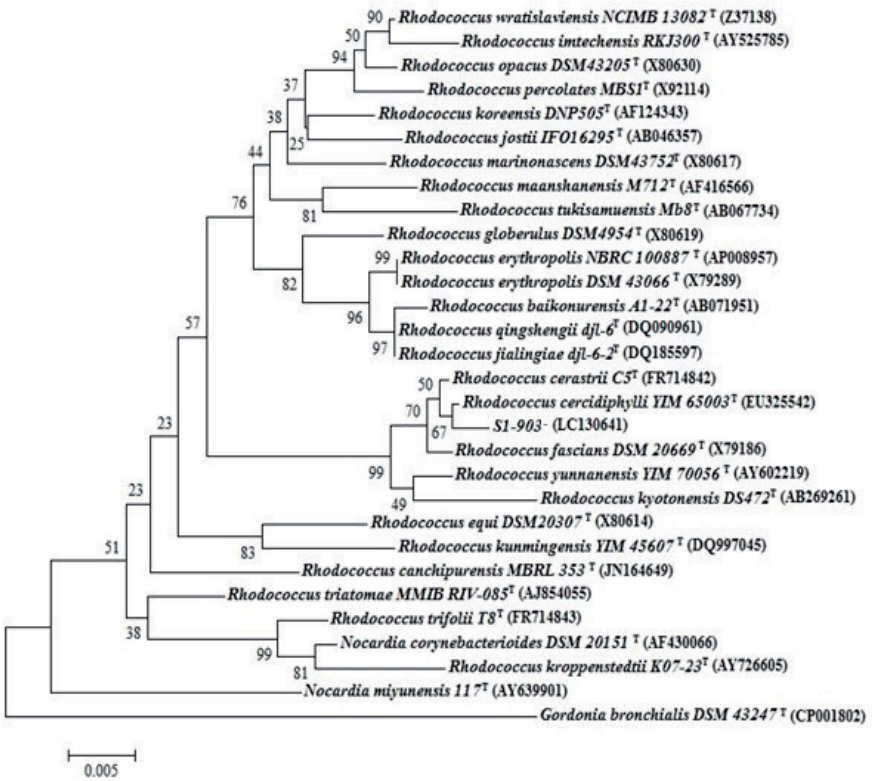

Figure 2. Estimation of the evolutionary history of strain S1-903 was carried out by using the Neighbor-Joining method with the bootstrap test (1000 replicates) and *Gordonia bronchialis* DSM 43247^{T} as outgroup.

Table 4. Plant growth promoting activities of rhizobacterial treatments.

Treatment	Total N ($\mu g\ mL^{-1}$)	Phosphate solubilization		Potassium solubilization		IAA ($\mu g\ mL^{-1}$)
		Available P ($\mu g\ mL^{-1}$)	pH	Available K ($\mu g\ mL^{-1}$)	pH	
P. azotoformans N3-903	11.0 bc	170.68	4.51	4.56	5.02	80.15 a
P. azotoformans C2-114	13.73 ab	169.38	4.60	4.80	5.18	78.75 b
R. cercidiphylli S1-903	17.38 a	166.15	4.26	4.94	5.13	9.81 d
Mixture[a]	6.47 c	174.40	4.58	4.51	5.17	12.38 c
F-test	**	ns		ns		**

[a] Mixture of *P. azotoformans* N3-903*, R. cercidiphylli* S1-903 and *P. azotoformans* C2-114.
ns: non-significant, **: Mean values followed by the different letters in the same column are significant (P<0.01), according to least significant difference (LSD) test .

pH of media was evidence of release of acids that simultaneously increase phosphate and potassium solubilization. Similar results of Hu et al. [13] that described efficiency of mineral solubilizations depending on organic acids and capsule production. Moreover, exopolysaccharide release with the acids (oxalic, gluconic, malic, lactic, citric succinic and fumaric acids) can increase phosphate solubilization activity [26]. Strain S1-903 of *R. cercidiphylli* was shown to excrete more total N in the N free broth than another non-symbiotic nitrogen fixing species (Table 4). Many rhizosphere-associated bacteria act as N fixers that can contribute available nitrogen forms such as ammonium, nitrate and amino acids in N-depleted soils [27]. Both N3-903 and C2-114 *Pseudomonas* strains showed high amount of production of an IAA analog, ranging from 78.74 to 80.15 $\mu g\ mL^{-1}$, which was significantly different (P<0.01) from other treatments in the column (Table 4). It is unclear whether *P. azotoformans* has the ability to produce IAA as no evidence has been reported [23]. The genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Pantoea*, *Pseudomonas*, and *Serratia* have been reported as IAA biosynthesizing PGPR by using amino tryptophan [28]. Some microbes (*Azotobacter* spp. and *Pseudomonas* spp.) had IAA production activity without tryptophan [29], but our data on IAA production in the absence on tryptophan showed a negative result.

3.4 Pot Experiment

Inoculation of pots with the *P. azotoformans* strains resulted in significantly (P<0.01) greater root dry weight, shoot dry weight and biomass than the control, whereas *R. cercidiphylli* had no significant impact on growth (Figure 3.). The mixture of the three strains resulted in significantly greater shoot growth but not greater root growth than the control. This association of PGPR-induced IAA levels and biomass accumulation was also observed by Khalid et al. [30]. It was clear that the presence of some PGPR strains enhanced biomass accumulation by Jerusalem artichoke. We found significant positive correlations in IAA production and total nitrogen production of these strains *in vitro* (Table 4) for plant parameters (Table 5); IAA production and root dry weight (r=0.73, P<0.01), shoot dry weight (r=0.48, P<0.05), biomass (r=0.64, P<0.01); total nitrogen related with plant height (r=0.47, P<0.05). In the

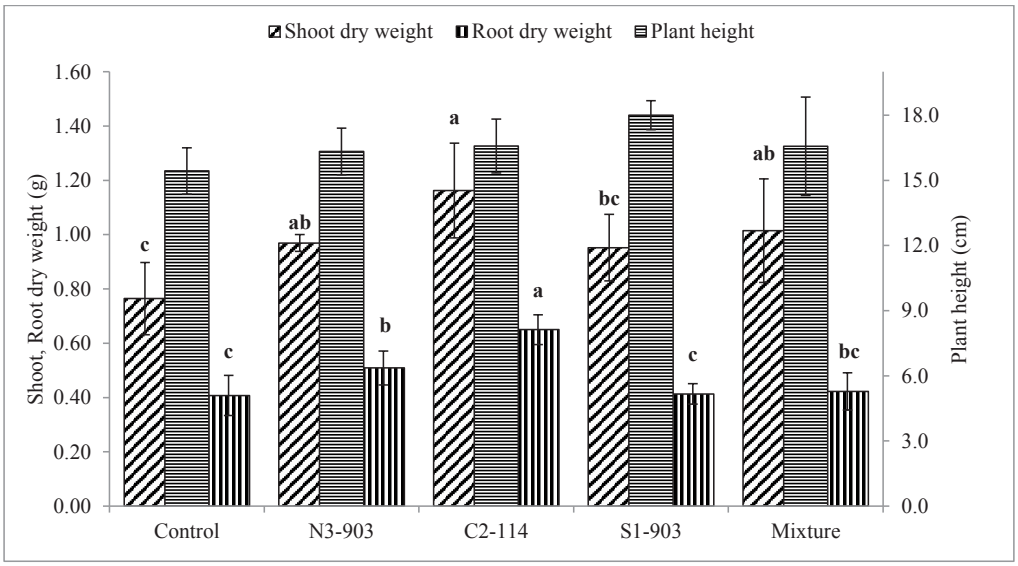

Figure 3. Effect of bacterial treatments (Single inoculum: *P. azotoformans* N3-903; *P. azotoformans* C2-114; *R. cercidiphylli* S1-903; Mixture inoculum: *P. azotoformans* N3-903, *R. cercidiphylli* S1-903 and *P. azotoformans* C2-114) on plant growth parameters of Jerusalem artichoke in pot condition. Bar represents mean ± SD of four replications.

present study, there was a statistically strong association between IAA levels and greater root and shoot development of growth. Similar to Khalid et al. [30] who reported that *in vitro* IAA production correlated with root and shoot growth of wheat. The production of IAA by PGPR enhances plant growth, particularly root development. PGPR may perform via multiple plant growth promoting mechanisms, such as nitrogen fixation and phosphate solubilization, simultaneously with IAA biosynthesis, for plant growth enhancement [7, 15].

4. Conclusion

To our knowledge, we present the first evidence that *R. cercidiphylli S1-903* act as PGPR. *P. azotoformans* C2-114 and N3-903 promoted shoot and root dry weight of Jerusalem artichoke. Thus, these strains could use for development of bio-fertilizer.

Acknowledgments

This work was financially supported by Khon Kaen University. The author would like to gratefully acknowledge Dr. Mark L. Gleason, Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, USA for revision of the manuscript. The authors are grateful for the Thailand Research Fund (TRF) for providing financial support for manuscript preparation through the Senior Research Scholar Project of Professor Dr. Sanun Jogloy (Project no. RTA 5880003).

References

[1] Pool-Zobel B.L., *Br. J. Nutr.*, 2005; **93**: S73-S90. DOI 10.1079/BJN20041349.

[2] Pimsaen W., Jogloy S., Suriharn B., Kesmala T., Pensuk V. and Patanothai A., *Asian J. Plant Sci.*, 2010; **9(1)**: 11-19. DOI 10.3923/ajps.2010.11.19.

[3] Esitken A., Yildiz H.E., Ercisli S., Donmez M.F., Turan M. and Gunes A., *Scientia Horticulturae*, 2010; **124**: 62-66. DOI 10.1016/j.scienta.2009.12.012.

[4] Ribeiro C.M. and Cardoso E.J.B.N., *Microbiol. Res.*, 2012; **167**: 69-78. DOI 10.1016/j.micres.2011.03.003.

[5] Bhattacharyya P.N. and Jha D.K., *World J. Microbiol. Biotechnol.*, 2012; **28**: 1327-1350. DOI 10.1007/s11274-011-0979-9.

[6] Vessey J.K.., *Plant Soil*, 2003; **255**: 571-586. DOI 10.1023/A:1026037216893.

[7] Ahmad F., Ahmad I. and Khan M.S., *Microbiol. Res.*, 2008; **163**: 173-181. DOI 10.1016/j.micres.2006.04.001.

[8] Hartmann A., Schmid M., van Tuinen D. and Berg G., *Plant Soil*, 2009; **321**: 235-257. DOI 10.1007/s11104-008-9814-y**.**

[9] Meng X., Long X., Kang J., Wang X. and Liu Z., *Acta Prataculturae Sinica*, 2011; **20**: 157-163.

[10] Bunt J.S. and Rovira A.D., *J. Soil Sci.*, 1955; **6**: 199-288. DOI 10.1111/j.1365-2389.1955.tb00836.x.

[11] Atlas R.M., *Handbook of Microbiological Media*, 3rd Edn., CRC Press, Boca Raton, 2004.

[12] Nautiyal C.S., *FEMS Microbiol. Lett.*, 1999; **170**: 265-270. DOI 10.1111/j.1574-6968.1999.tb13383.x.

[13] Hu X., Chen J. and Guo J., *World J. Microbiol. Biotechnol.*, 2006; **22**: 983-990. DOI 10.1007/s11274-006-9144-2.

[14] Schwyn B. and Neilands J.B., *Anal. Biochem.*, 1987; **160**: 46-56. DOI 10.1016/0003-2697(87)90612-9.

[15] Sarathambal C., Ilamurugu K., Balachandar D., Chinnadurai C. and Gharde Y., *Appl. Soil Ecol.*, 2015; **87**: 1-10. DOI 10.1016/j.apsoil.2014.11.004.

[16] Kawasaki H., Hoshino Y., Hirata A. and Yamasato K., *Arch. Microbiol.*, 1993; **160**: 358-362. DOI 10.1007/BF00252221.

[17] Myers E.W. and Miller W., *Comput. Appl. Biosci.*, 1988; **4**: 11-17. DOI 10.1093/bioinformatics/4.1.11.

[18] Kim O.S., Cho Y.J., Lee K., Yoon S.H., Kim M., Na H., Park S.C., Jeon Y.S., Lee J.H., Yi H., Won S. and Chun J., *Int. J. Syst. Evol. Microbiol.*, 2012; **62**: 716-721. DOI 10.1099/ijs.0.038075-0.

[19] Yu X., Liu X., Zhu T.H., Liu G.H. and Cui M., *Eur. J. Soil Biol.*, 2012; **50**: 112-117. DOI 10.1016/j.ejsobi.2012.01.004.

[20] Barton C.J., *Anal. Chem.*, 1948; **20**: 1068-1073. DOI 10.1021/ac60023a024.

[21] Sennoi R., Jogloy S., Saksirirat W., Kesmala T. and Patanothai A., *Euphytica*, 2012; **190**: 415-424. DOI 10.1007/s10681-012-0813-y.

[22] Andreote F.D., de Araújo W.L., de Azevedo J.L., van Elsas J.D., da Rocha U.N. and van Overbeek L.S., *Appl. Environ. Microbiol.*, 2009; **75**: 3396-3406. DOI 10.1128/AEM.00491-09.

[23] Levenfors J., Welch C.F., Fatehi J., Wikstrom M., Rasmussen S. and Hökeberg M., *United States Pat. No.* US 20130005572 A1 (2013).

[24] Li J., Zhao G.Z., Chen H.H., Qin S., Xu L.H., Jiang C.L. and Li W.J., *Syst. Appl. Microbiol.*, 2008; **31**: 108-113. DOI 10.1016/j.syapm.2008.03.004.

[25] Koike S.T., *Plant Dis.*, 2004; **88**: 769. DOI 10.1094/PDIS.2004.88.7.769B.

[26] Yi Y., Huang W. and Ge Y., *World J. Microbiol. Biotechnol.*, 2008; **24**: 1059-1065. DOI 10.1007/s11274-007-9575-4.

[27] Richardson A.E., Barea J.M., McNeill A.M. and Prigent-Combaret C., *Plant Soil*, 2009; **321**: 305-339. DOI 10.1007/s11104-009-9895-2.

[28] Dastager S.G., Deepa C.K. and Pandey A., *Plant Physiol. Biochem.*, 2010; **48**: 987-992. DOI 10.1016/j.plaphy.2010.09.006.

[29] Ahmad F., Ahmad I. and Khan M.S., *Turk. J. Biol.*, 2005; **29**: 29-34.

[30] Khalid A., Arshad M. and Zahir Z.A., *J. Appl. Microbiol.*, 2004; **96:** 473-480. DOI 10.1046/j.1365-2672.2003.02161.x.