Chiang Mai J. Sci. 2017; 44(4) : 1210-1223
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Diversity and Plant Growth Promoting Activities of Actinomycetes from Mangroves

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Received: 30 December 2016
Accepted: 18 April 2017

ABSTRACT

Four hundred forty eight actinomycetes were isolated from water and sediment samples of mangrove areas at Andaman Coastal Research Station for Development of Kasetsart University Research and Development Institute, Ranong province, Thailand. According to the morphological characteristics and occurrence of diaminopimelic acid (DAP) isomeric form in the cell hydrolysates, 318 isolates of actinomycetes were clustered as members of streptomycete and 130 isolates were clustered as non-streptomycete type. These 448 isolates could be separated into 8 spore-colour groups and 16S rRNA gene sequences of 146 representative isolates, which had different morphologies from each colour group, were analyzed. They were classified into 14 genera: *Actinomadura, Actinoplanes, Gordonia, Isoptericola, Jiangella, Microbispora, Micromonospora, Nocardia, Nocardiopsis, Nonomuraea, Pseudonocardia, Saccharopolyspora, Streptomyces* and *Streptosporangium*. *Streptomyces* was most prevalent genus with 65.1 % occurrence frequency followed by *Micromonospora* (6.8 %), *Nonomuraea* (6.2 %) and *Saccharopolyspora* (6.2 %). Assessment of plant growth promoting activities of the 448 isolates exhibited that 88.4 %, 50.9 % and 38.8 % of them possessed indole-3-acetic acid (IAA), siderophores and phosphate solubilizing activities, respectively. Fifty potent isolates for plant growth promoting activities were further screened for their antagonistic potential against rice pathogenic bacteria (*Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*) and aminocyclopropane carboxylate (ACC) deaminase production. Fourteen isolates inhibited both pathogens and 11 isolates exhibited ACC-deaminase activity. Isolates 1SM5-02, 2SH3-07, 3SH5-05 and 3WH5-01 exhibited high plant growth promoting activities even up to 300 mM of NaCl stress. Their 16S rRNA gene sequences were closely related to *Nocardiopsis yanglingensis* A18^T (98.85 %), *Streptomyces jiujiangensis* JXJ 0074^T (99.35 %), *Streptomyces psammoticus* NBRC 13971^T (99.51 %) and *Pseudonocardia oroxyli* D10^T (99.78 %), respectively. The four isolates significantly enhanced shoot and root weight and root length of rice seedling under non-saline and up to 200 mM NaCl conditions. This is the first report on diversity and plant growth promoting potentials of actinomycetes in mangrove from Ranong province, Thailand.

Keywords: actinomycetes, diversity, mangrove, plant growth promoting

1. INTRODUCTION

Actinomycetes are Gram-stain-positive bacteria in class *Actinobacteria*, generally soil borne, and functionally important for mineral-cycles and organic matter decomposition. Moreover, they are recognized as the producers of bioactive compounds including antibiotics, anti-phytopathogens and plant growth-promoting substances. There are many reports show that actinomycetes have abilities of plant growth promoters, such as plant growth hormone production, phosphate solubilization, siderophores production and N_2-fixation [1-2].

Mangroves are important coastal ecosystems that support wide range of plants, animals and microorganisms. Microbial diversity is the key for ecosystems as they originate food chain that keeps environmental balance. Mangrove environments were reported as the source of novel actinomycetes such as *Micromonospora rifamycinica* sp. nov., isolated from mangrove sediment of the South China sea [3], and *Streptomyces ferrugineus* sp. nov. isolated from mangrove soil in Thailand [4]. Moreover, new species of genus *Jiangella, Nonomuraea* and *Nocardiopsis* were found in mangrove of Ranong province, Thailand [5-7].

Streptomyces genus is well documented for plant growth-promoting substances and anti-phytopathogenic agents. Furthermore, *Streptomyces fradiae, S. avermitilis, S. cinnamonensis, S. canus* and *Leifsonia poae* were reported to promote guava height and plant dry matter content by growth hormones (IAA and GA3) production, phosphate solubilization and siderophore production [1]. In Vietnam, *Streptomyces toxytricini* VN08-A-12 of soil and leaf litter could inhibit 10 major races of *Xanthomonas oryzae* pv. *oryzae* and also increased yield of healthy rice cultivars [8]. Although, use of actinomycetes in agriculture could be promising strategy for promoting plant growth, however there is scarce information on plant growth promoting actinomycetes in mangrove-habitats. Therefore, the objectives of this research were to isolate actinomycetes from mangroves, characterize and assessment of their potential to use as plant growth promoting agents under saline condition.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation of Mangrove Samples

Mangrove sediment and water samples from mangrove areas at Andaman Coastal Research Station for Development of Kasetsart University Research and Development Institute, Ranong province were collected from 3 sampling sites of about ≥ 1 km apart (site A: N09° 21′ 53.6″ E98° 24′ 26.7″; site B: N09° 22′ 50.1″ E98° 24′ 08.1″; site C: N09° 22′ 16.2″ E98° 23′ 59.9″) which covered mangrove from the residential area to the sea (Figure 1). The samples from each site were collected 4 times during rainy season (May to August, 2012) and dry season (October, 2012 to January, 2013). Sediment and water samples were collected from 5 cm sub-surface and kept at 4 °C in plastic bag and bottle for sediment and water samples, respectively. Temperature, pH and water salinity were determined at the collecting sites. Salinity of sediment samples was recorded from dried samples.

2.2 Selective Isolation and Enumeration of Actinomycetes from Sediment and Water Samples

The sediment samples were air dried at room temperature, then ground and sieved, while water samples (200 mL) were separately filtered through cellulose acetate membrane filter (∅ = 0.45 µm) and sediment collected on the membrane was resuspended in 2 mL of sterilized water. The samples were

serially diluted and spread onto humic acid-vitamin agar (HVA) and starch casein agar (SCA), prepared with half-strength synthetic sea water, and 1/10 strength marine agar (1/10MA). All selective media were supplemented with nalidixic acid (25 µg/mL) and nystatin (50 µg/mL) to inhibit Gram-negative bacterial and fungal growth, respectively. Actinomycete population on each medium was recorded after 3 weeks of incubation at 28 ± 1°C. The isolates with different colony morphology were purified and maintained on International *Streptomyces* Project (ISP) 2 agar or stored in 20 % glycerol at -20°C for long-term preservation.

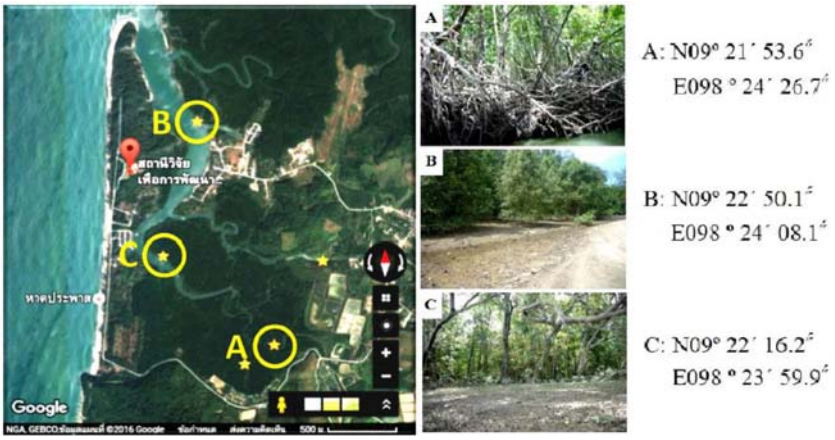

Figure 1. Sampling locations of the area under study. The letters indicate sampling sites including its latitude and longitude of GPS location.

2.3 Characterization of Isolated Actinomycete Strains

The cultural and morphological characteristics of the isolates were observed from their growth on ISP 3 agar after incubation at 28 ± 1°C for 14 d. The colour of the aerial spore mass, substrate mycelium, and diffusible pigment were recorded. The isomeric form of diaminopimelic acid (DAP) in whole-cell hydrolysates of these isolates were also determined by paper chromatography using modified method from Hasegawa *et al.* [9]. The actinomycete isolates were assigned to streptomycetes or non-streptomycetes group based on presence of *LL*-DAP and *meso*-DAP, respectively, besides the morphological characteristics.

2.4 Identification Based on 16S rRNA Gene Sequencing

The genomic DNA of the representative isolates of each spore colour group were extracted using a modified method of Mingma *et al.* [10]. PCR amplification of the 16S rRNA gene was carried out with primers 1F (5′-TCACGGAGAGTTTGA TCCTG-3′) and 1530R (5′-AAGGAGAT CCAGCCGCA-3′). The 16S rRNA gene was sequenced through a commercial service provider (Macrogen Inc., Seoul, Korea). The sequences were compared with other sequences present in the EzTaxon-e database using BLAST program from the Biocloud web site (http://eztaxon-e.ezbiocloud.net). The result of 16S rRNA gene sequences were aligned with related

species and generated a neighbour-joining phylogenetic tree using MEGA version 6.0 software by bootstrap analysis of 1000 replications.

2.5 Screening for Plant Growth Promoting Activity

All 448 actinomycete isolates were screened for their plant growth promoting traits. Siderophore production was determined by agar diffusion method using chrome azurol S agar (CAS) [11]. The ISP 2 agar discs (∅ 0.6 cm) of 7 d old actinomycete cultures were transferred onto CAS agar plate. Isolates which produced orange zone around the disc were considered as siderophore-producing strain. The size of orange halo zone was measured after incubation for 3 d at 28 ± 1°C in the dark.

Phosphate solubility was tested by agar diffusion method using Pikovskaya (PVK) agar [12] supplemented with insoluble tricalcium phosphate (0.5 % w/v). Isolates producing clear zones around the colony after incubation for 14 d at 28 ± 1°C were considered as phosphate-solubilizing isolate. The clear zone and colony diameters were measured and the phosphate solubilization potency was calculated by subtracting the colony diameter from the clear zone diameter.

The ability of the isolate to produce indole-3-acetic acid (IAA) was performed using a colorimetric method [13]. Isolates were grown in glucose-yeast extract broth (10 g/L yeast extract and 10 g/L glucose) supplemented with 0.2 % (w/v) L-tryptophan. After shaking (180 rpm) incubation at 28 ± 1°C in darkness for 7 d, the cultures were centrifuged at 10,000 rpm at room temperature for 10 min. The supernatant (2 mL) was transferred into new tube and mixed with 1 mL of Salkowski's reagent and tubes were incubated in darkness for 30 min at room temperature. Development of pink colour indicated IAA production. Absorbance of reaction mixture was measured with UV-spectrophotometer at 530 nm. The amount of IAA (µg/mL) was calculated using a standard curve of IAA (0.5-100 µg/mL).

2.6 Aminocyclopropane Carboxylate (ACC) Deaminase Production and Antagonistic Activity Against *Xanthomonas oryzae*

The actinomycete isolates, which showed higher plant growth promoting activity (siderophore-producing zone ∅ ≥ 2 cm, phosphate solubilization value ≥ 5 mm, or produced the IAA ≥ 30 µg/mL) in initial screening, were selected to test for ACC-deaminase production and antagonistic activity against rice bacterial pathogens, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) which causes brown spot disease and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) that causes sheath blight disease. ACC-deaminase production was determined by following Palaniyandi *et al.* [14]. Briefly, the selected isolates were inoculated on Dworkin and Foster minimal salts agar without nitrogen source and containing 3 mM of ACC or $(NH_4)_2SO_4$ as sole nitrogen sources. The ACC-deaminase positive strains which utilized ACC as nitrogen source and had comparable growth with $(NH_4)_2SO_4$ after incubation at 28 ± 1°C for 10 d were taken as positive organism.

The antagonistic activity of the actinomycete isolates against the plant pathogens was assayed using agar overlay method. The ISP 2 agar disc (∅ 0.6 cm) of 7 d old actinomycete isolates were transferred separately on a fresh ISP 2 agar plate and incubated at 28 ± 1°C for 7 d. After growth, the plates were gently overlaid with 5 mL modified nutrient glucose soft agar containing 0.5 mL of an overnight culture of *Xoo* or

Xoc (OD_{600} = 0.2), and further incubated at 28 ± 1°C for 24 h. The antagonistic activity was determined by measuring the zone of inhibition around the mycelium disc.

2.7 Screening of Plant Growth Promoting Traits under Saline Condition

The actinomycete strains were tested in triplicate for their plant growth promoting activities in presence of 100, 200, and 300 mM NaCl supplemented in the test medium. Phosphate solubilization and siderophores, IAA and ACC-deaminase production by the organism were assessed according to the methods mentioned in the topics 2.5 and 2.6. Isolates that showed higher plant growth promoting activities were further subjected to growth promotion effects in rice seedlings.

2.8 Effects of Selected Strains Inoculation on Growth Promotion of Rice Seedlings

Yoshida's nutrient solution (YS) [15] soft agar (0.6 % agar, w/v), supplemented with NaCl to a final concentration of 100 and 200 mM and without NaCl, was prepared in glass test tube (2.5 × 20.0 cm). After sterilization, warm YS soft agar was suspended with spore's suspension of each actinomycete strain to get a final concentration of ~10^7 spores/mL, whereas uninoculated tube was used as control. Then, germinated rice seedlings were soaked in spore suspension of each selected isolate (~10^8 spores/mL) for 4 h and individual seed was aseptically planted on prepared YS soft agar. The tubes of rice seedlings were incubated at 28-30°C under ~9000 Lux LED light for 12 h light and dark photoperiod for 14 d. Uninoculated plants served as control. After two weeks growth, the plants were gently uprooted, washed to remove adhering substances. The length, fresh and dry weight of shoot and root were measured for both control and treated plants. The experiment was carried out with ten replications and repeated twice to confirm the results.

2.9 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using SPSS for Windows Version 17.0 (SPSS Inc., Chicago, Illinois). Statistical significance was evaluated using Duncan's multiple range test at $P < 0.05$ confidence limit.

3. RESULTS AND DISCUSSION

3.1 Characteristics of Mangrove Samples and Enumeration of Actinomycetes Population

The temperature at collecting sites was in the range of 24.7 to 34.0 °C. The pH of sediment and water samples was slightly acidic to alkaline (5.7 to 9.0 and 7.1 to 8.7, respectively). Sediment salinity of each site was 0 to 0.5 % and water salinity was between 0 to 3.2 %, depending on the distance of sampling site from the sea. The number of actinomycetes from water samples ranged from 10 to 1.4×10^4 CFU/100 mL, 0 to 1×10^3 CFU/100 mL on humic acid-vitamin agar (HVA) and starch-casein agar (SCA) respectively, but could not be observed on 1/10 strength marine agar (1/10MA) due to the overgrown of other bacteria (Table 1). The sediment samples contained actinomycetes in range of 9×10^3 to 7.55×10^6 CFU/g, 0 to 5.2×10^5 CFU/g and 0 to 4.8×10^5 CFU/g on HVA, SCA and 1/10MA, respectively (Table 1). According to the results, variation of actinomycete populations occurred in both sediment and water samples from each sampling time. This may be due to the effects of weather and tidewater that could influence chemical nature of mangrove soil and water in all four sampling times. Observation on the frequency of occurrence of the actinomycetes suggested that humic acid-vitamin agar was more

effective for the isolation of actinomycetes from mangrove samples than other selective media. Previous studies of Khamna *et al.* [13] have also mentioned that humic acid vitamin agar was the best medium for isolating actinomycetes from plant rhizosphere soils when compared with oatmeal agar and starch-casein agar. Higher recovery rate of actinomycetes on humic acid-vitamin agar might be due to soil humic acid addition which would be more suitable for the saprophytic organisms [16].

Four hundred forty eight actinomycete isolates with different morphological characteristics were recovered from the mangrove samples. Recovery of isolates from the sediment (70.9 %) was higher than water samples (29.1%) as the actinomycetes are known to be generally more abundant in soil than other habitats. The highest number of organisms was obtained from site B and site C (157 and 155 isolates, respectively) which are located near the sea, while lowest number of organisms was recovered from site A (136 isolates) (Table 1). The population density of actinomycetes is not associated with the isolate number of actinomycetes collected from each sampling site. Although the populations in some sampling times were high, few different colony types were isolated (Table 1). Our study revealed that the variation of environmental conditions at sampling sites, such as the distance from the sea, seasons and weather conditions during sampling time, might be the reason of variation in actinomycete community.

Table 1. Actinomycete population on HVA, SCA and 1/10MA, and numbers of recovered isolate from each sampling site at different seasons.

Sample	Season	Site A			Isolate	Site B			Isolate	Site C			Isolate
		HVA	SCA	1/10MA	number	HVA	SCA	1/10MA	number	HVA	SCA	1/10MA	number
water*	rainy 1	340	60	0	21	90	20	0	22	160	30	0	27
	rainy 2	10	0	0	1	220	10	0	21	180	0	0	18
	dry 3	9000	10	0	10	90000	0	0	2	14000	0	0	5
	dry 4	80	10	0	4	20	0	0	3	30	0	0	0
sediment**	rainy 1	295	6	0.30	14	0.90	0	0.05	14	1.50	0.25	0	15
	rainy 2	24	0.05	1.50	43	755	52	48	38	7.50	0.50	1.50	45
	dry 3	2	1.25	0.35	32	2.65	1.20	0.30	39	4.50	1.55	0.90	34
	dry 4	14	1	4	11	1.1	0.05	0.50	18	2.25	0.10	0.15	11

Note: * number of colonies (CFU/100 mL);** number of colonies ($\times 10^4$ CFU/g).

3.2 Characterization and Identification of the Isolates

The isomeric form of DAP in whole-organism hydrolysates together with morphological characteristics of 448 actinomycete isolates indicated that 71.1% contained *LL*-DAP, a chemical marker for members of the streptomycetes group [17]. The remaining isolates (28.9 %) contained *meso*-DAP which were classified into non-streptomycetes group [17]. On the basis of their aerial spore mass colour on International *Streptomyces* Project (ISP) 3 agar, actinomycetes in this study were assigned into 8 spore-colour groups viz. grey (36.1 %), green (17.6 %), yellow (14.1 %), white (11 %), pink (4.7 %), brown (3.1 %), black (0.9 %) and non-spore forming group (12.5 %). This finding of the wide variety of cultural morphologies of mangrove actinomycetes was similar to the study of Malek *et al.* [18] which demonstrated 7 colour series of the actinomycete isolates recovered from Tanjung Lumpur mangrove of Malaysia but members of white series represent the most predominant group. Partial 16S rRNA gene of the representative isolates from each colour group (146 isolates) were analyzed and classified into the following 14 genera: *Actinomadura* (1.4 %), *Actinoplanes* (1.4 %), *Gordonia* (0.7 %), *Isoptericola* (0.7 %), *Jiangella* (0.7 %), *Microbispora* (1.4 %), *Micromonospora* (6.8 %), *Nocardia* (1.4 %), *Nocardiopsis* (5.5 %), *Nonomuraea* (6.2 %), *Pseudonocardia* (2.0 %), *Saccharopolyspora* (6.2 %), *Streptomyces* (65.1 %) and *Streptosporangium* (0.7 %), and belonging to 9 families with 97 to 100 % sequence similarity to their closest type strains. Phylogenetic tree was constructed based on 16S rRNA gene sequences of non-streptomycete isolates to assure their association with 9 familiae viz. *Gordoniaceae, Jiangellaceae, Micromonosporaceae, Nocardiaceae, Nocardiopsaceae, Promicromonosporaceae, Pseudonocardiaceae, Streptosporangiaceae, Thermomonosporaceae* (Figure 2). The results reported by Hong *et al.* [19] showed 237 bioactive actinomycete isolates from mangrove soils and plants in China belonged to 13 genera classified into 7 families. The frequency of actinomycete genera found in all sampling site was not much different (site A, 9 genera; site B, 8 genera; and site C, 8 genera). Genus *Actinomadura* and *Streptosporangium* were found only in site A, genus *Microbispora, Isoptericola* and *Jiangella* were found only in site B and genus *Gordonia* was found only in site C. However, genus *Streptomyces* represented the most predominant in the mangrove of Ranong province. The presence of predominant number of *Streptomyces* isolates in this study was in agreement with results reported by Hong *et al.* [19] and Lee *et al.* [20] which isolated substantial *Streptomyces* isolates from mangrove soils in China and Malaysia, respectively. The sequence similarity level and phylogenetic tree showed that some isolates formed a separate line of descent from most of their closely related strains which were highly potential to be assigned as novel species (Figure 2). Three isolates from three genera (*Jiangella, Nocardiopsis* and *Nonomuraea*) were identified as novel species [5-7]. The report of Lee *et al.* [20] also showed that actinobacteria isolated from mangrove habitats of Malaysia at least 5 isolates from genera *Sinomonas, Microbacterium* and *Streptomyces* were considered as putative novel taxa. These results also supported that mangrove environments provided diverse genera and serves as a good source for novel taxa of actinomycetes.

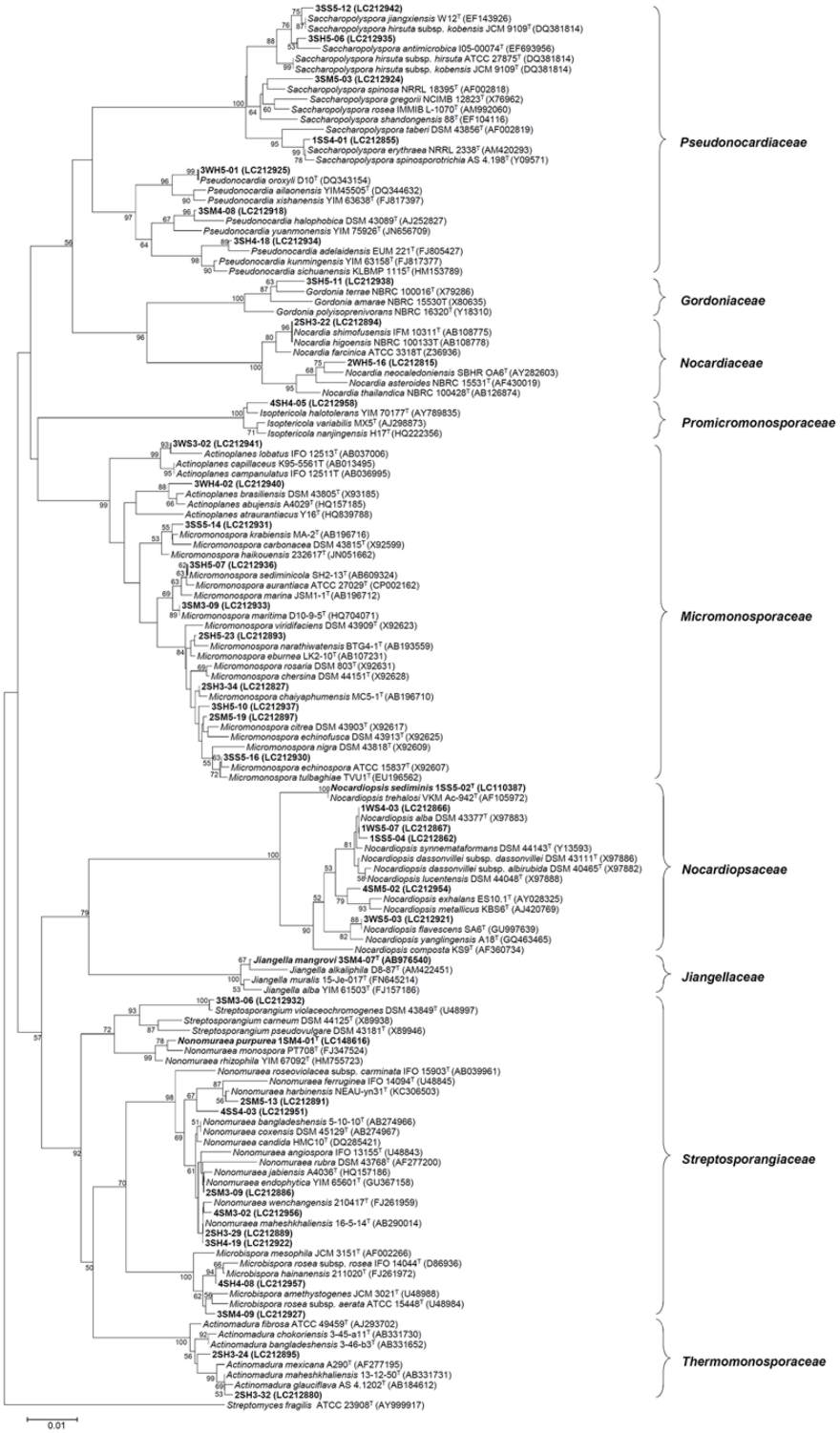

Figure 2. Neighbour-joining tree based on almost complete 16S rRNA gene sequences showing the relationships between non-streptomycete isolates and related type strains. Numbers at the nodes indicate bootstrap values based on 1000 replicates; only values above 50 % are shown.

3.3 Plant Growth Promoting Activities of the Isolates

In this study, 397 actinomycete isolates (88.6 %) produced siderophores on chrome azurol S (CAS) agar indicated by orange halo formation around the colonies, and 174 isolates (38.8 %) showed phosphate solubilizing activity as they formed a clear zone around colony on Pikovskaya's medium (Table 2). Determination of indole-3-acetic acid (IAA) production showed that 228 isolates (50.9 %) produced IAA in the range of 0.21 to 165.74 µg/mL (Table 2). Most of the actinomycete strains showed siderophores production which demonstrated that mangrove provided a rich source of siderophore producing actinomycetes. Marine microbes typically require iron for growth more than the iron concentration in the surface of ocean and more siderophores producing strains are generally found from iron-scarced environments [21]. There are several reports showed that actinomycetes from soil and other terrestrial environments can produce plant growth promoters to enhance plant development such as *Streptomyces* strain C isolated from a wheat field soil had the abilities to produce IAA, siderophores and solubilize phosphate, could increase the growth and development of the wheat plant [22]. Beside this, *Streptomyces* strain mhcr0811, exhibited phosphate solubilization, phytase, chitinase, IAA and siderophore production, significantly improved wheat growth, biomass and mineral (Fe, Mn, P) contents [23]. However, the study of plant growth promoting activities of mangrove actinomycetes has received less attention. Thus, actinomycetes in this study that produced plant growth promoting compounds could be a promising agent for utilization in plant growth improvement. In addition, mangrove ecosystem in Ranong province of Thailand was a suitable ecosystem for the diversity of actinomycetes which could produce plant growth promoting compounds.

Table 2. Number of actinomycete isolates with plant growth promoting activities from each sampling site.

Plant growth promoting activity	Mangrove sampling site			Total isolate
	Site A (136 isolates)	Site B (157 isolates)	Site C (155 isolates)	
Siderophores	118	137	142	397
Phosphate solubilization	52	70	52	174
IAA	78	66	48	228

3.4 Aminocyclopropane Carboxylate (ACC) Deaminase Production and Antagonistic Activity Against *Xanthomonas oryzae*

Fifty representative isolates, which had siderophores-producing zone diameter ≥ 2 cm, phosphate solubilization value ≥ 5 mm, or produced IAA ≥ 30 µg/mL, were subsequently tested for their antagonistic activity against *Xanthomonas oryzae pv. oryzae* (*Xoo*) and *Xanthomonas oryzae pv. oryzicola* (*Xoc*). Twenty three isolates (46 %) inhibited at least one of the bacterial pathogens while 14 isolates (28 %) showed inhibition activity against both test pathogens. Many actinomycete isolates from marine environments were

extensively investigated for new bioactive compounds to control pathogenic bacteria, multiple-drug-resistant bacteria, fungi and tumor cells [19,24]. However, there are no report on the isolation of actinomycetes from mangrove and the potential to control rice-pathogenic bacteria. In this study, most of the potent isolates inhibited both of the test pathogens were member of genus *Streptomyces*. Several investigations indicated that *Streptomyces* are the promising actinomycetes that inhibited the growth diseases causing bacterial of rice [8,25]. The antibacterial activity of these potent actinomycete isolates from mangrove highlighted the important strategy to inhibit the growth of rice bacterial pathogens, *Xanthomonas oryzae*. Moreover, ACC-deaminase exhibit stress alleviation activity in plant by hydrolyzes ACC, the precursor of ethylene in plant to ammonia and α-ketobutyrate [26]. Therefore, ACC-deaminase production of 50 representative isolates was also investigated. Among these, 11 isolates could grow on ACC-supplemented medium as sole nitrogen source. *Streptomyces* isolates 2SH3-07 and 3SH5-05 exhibited this ability together with all the plant growth promoting traits determined in this study. The activities of ACC-deaminase together with IAA are critical to attain maximum growth promotion through the activity of plant growth promoting microbes [27]. Thus, the isolates in this study that possess a wide range of plant-growth promoting activities were selected to study the activities under saline condition.

3.5 Plant Growth Promoting Efficiency of Potent Isolates under Saline Condition

Fourteen potent actinomycete isolates were selected based on their high plant growth promoting abilities from previous screening. These isolates were subsequently determined the *in vitro* plant growth promoting abilities under the presence of 100, 200, 300 mM NaCl and without NaCl. The results revealed that all isolates showed siderophore production and isolate 1SM5-02 showed the highest siderophores activity by forming 24.7±0.6-25.5±0.5 mm of orange halo diameter on CAS agar with 0 to 200 mM NaCl. The zone diameter was slightly decreased to 21.3 mm when the concentration of NaCl reached 300 mM (Table 3). For phosphate solubilization, isolate 2SH3-07 showed the highest ability to solubilize mineral phosphate in Pikovskaya (PVK) agar even though the concentration of NaCl increased to 300 mM (Table 3). Biosynthesis of IAA was detectable in all the test isolates. Isolates 1SM5-02 showed the highest IAA of 94.3±0.8 µg/mL under non NaCl condition and increased to 100.2±1.9 µg/mL after adding NaCl to 300 mM. Moreover, isolate 3WH5-01 also produced high IAA under 0 to 200 mM NaCl condition, similar to isolate 1SM5-02 (Table 3). Among 14 potent isolates, 7 isolates showed ACC-deaminase activity although the concentration of NaCl in the test medium was increased up to 300 mM. Moreover, most of the tested isolates could maintain all the plant growth promoting activities over a wide range of NaCl concentration up to 300 mM. Our data clearly showed the NaCl tolerance of all the test isolates was a preferable property for use in agricultural practices to improve crop productivity in salt affected areas as previously concluded by Rajput *et al.* [28] that salt-tolerant, IAA producing, phosphate solubilizing bacterial strain SAL-15 containing ACC-deaminase activity could enhance growth and yield of wheat (*Triticum aestivum* L. var. TJ-83) under salinity stress. As a result of *in vitro* plant growth promoting abilities under saline

condition, 4 isolates (isolates 1SM5-02, 2SH3-07 and 3WH5-01) were selected to determine their effect on rice seedling growth under saline condition due to their high activities in each plant growth promoting traits under saline conditions. In addition, isolate 3SH5-05 was selected based on its high activities in all tests (Table 3). These 4 selected isolates were identified according to their morphological characteristics and 16S rRNA gene sequences analysis as previously described (Topic 2.4). The result showed that isolates 1SM5-02, 2SH3-07, 3SH5-05 and 3WH5-01 were closely related to *Nocardiopsis yanglingensis* A18^T (98.85 %), *Streptomyces jiujiangensis* JXJ 0074^T (99.35 %), *Streptomyces psammoticus* NBRC 13971^T (99.51 %) and *Pseudonocardia oroxyli* D10^T (99.78 %), respectively. Many of non-streptomycete actinomycetes taxa are therefore rarely reported in literature dealing with routine isolations of biocontrol agents and/or plant growth promoters [29]. Interestingly, non-streptomycete actinomycetes, *Nocardiopsis* isolate 1SM5-02 and *Pseudonocardia* isolate 3WH5-01 showed promising plant growth promoting potentials in this study.

Table 3. Plant growth promoting activities of four potent isolates under saline conditions.

Activity	NaCl conc.	1SM5-02	2SH3-07	3SH5-05	3WH5-01
Siderophores (cm)	0 mM	25.5±0.5	16.5±0.5	23.3±2.4	23.5±0.5
	100 mM	24.7±0.6	15.5±1.3	22.8±0.3	22.3±1.6
	200 mM	25.3±0.6	16.2±0.3	22.7±1.6	22.0±0.5
	300 mM	21.3±0.6	16.3±1.3	17.3±1.1	20.2±0.6
Phosphate solubilization (cm)	0 mM	ND	4.7±0.3	3.7±0.3	2.0±0.0
	100 mM	ND	3.7±0.0	3.7±0.6	0
	200 mM	ND	4.3±1.1	3.2±0.8	0.2±0.2
	300 mM	ND	4.2±0.3	2.7±0.6	0.3±0.3
IAA (µg/mL)	0 mM	94.3±0.8	14.9±2.4	9.2±2.5	94.3±0.6
	100 mM	94.5±0.4	19.4±1.6	26.9±1.9	96.1±0.1
	200 mM	94.4±1.3	29.6±3.2	34.1±1.4	96.2±0.2
	300 mM	100.2±1.9	19.0±1.4	17.5±1.5	95.1±1.0
ACC deaminase	0 mM	ND	ND	+	+
	100 mM	ND	ND	+	+
	200 mM	ND	ND	+	+
	300 mM	ND	ND	+	+

Note: + = detected ND = not detected.

3.6 Effect of the Potent Actinomycetes on Rice Seedling Growth under Saline Condition

Growth promoting effects on rice seedling of isolates 1SM5-02, 2SH3-07, 3SH5-05 and 3WH5-01 were significantly as they enhanced all growth parameters of rice seedling under non-NaCl and 100 mM NaCl condition (Table 4). At 200 mM NaCl, isolate 2SH3-07, 3SH5-05 and 3WH5-01 significantly increased shoot and root weight including root length, while isolate 1SM5-02 increased root length and dry weight (Table 4). Moreover, inoculated seedling not increased only these growth parameters but also enhanced shoot and root appearance that seem to be more succulent than an uninoculated control.

The results of this experiment confirm plant growth promoting ability of all potent actinomycete isolates by increasing all growth parameters even when the seedling were grown in saline condition. Investigation by Tank and Saraf [30] showed that PGPRs which are able to solubilize phosphate, produce phytohormones and siderophores in salt condition promote growth of tomato plants under 2% NaCl stress. So, the increase in growth parameters in this study might result from plant growth promoting activities of actinomycetes which enhanced availability of nutrients and reduced stress effects. Our observations certify the beneficial role of actinomycetes isolated from mangrove for plant growth promoting effects to enhance plant growth and development under salinity stress.

Table 4. Evaluation of the four active actinomycete isolates for growth promoting effect on rice seedling under 0, 100 and 200 mM NaCl stress.

	Isolate	Shoot			Root		
		Length (cm)	Fresh weight (mg)	Dry weight (mg)	Length (cm)	Fresh weight (mg)	Dry weight (mg)
0 mM NaCl	Control	35.5±5.3^{b}	161.2±4.9^{b}	19.3±0.5^{b}	7.7±1.3^{b}	66.7±11.8^{b}	5.2±0.2^{b}
	1SM5-02	40.1±3.0^{a}	173.6±0.5^{a}	21.8±0.5^{a}	10.3±1.5^{a}	110.8±11.1^{a}	8.5±0.6^{a}
	2SH3-07	39.4±2.2^{a}	173.8±0.8^{a}	21.0±0.2^{a}	10.3±0.9^{a}	120.1±4.3^{a}	8.3±0.1^{a}
	3SH5-05	38.7±1.2^{a}	162.5±1.1^{b}	21.6±0.8^{a}	10.7±1.2^{a}	105.0±9.9^{a}	8.2±0.4^{a}
	3WH5-01	39.5±2.3^{a}	171.8±1.7^{a}	21.2±1.0^{a}	11.0±3.6^{a}	111.6±8.5^{a}	7.4±1.6^{a}
100 mM NaCl	Control	10.0±2.9^{c}	36.5±2.5^{b}	5.5±0.4^{b}	7.3±1.9^{b}	29.3±2.7^{b}	2.1±0.1^{d}
	1SM5-02	12.7±1.5^{a,b}	52.7±1.5^{a}	8.1±0.1^{a}	8.9±1.7^{a}	50.5±0.6^{a}	3.3±0.1^{a,b}
	2SH3-07	11.6±1.2^{b,c}	52.7±4.2^{a}	8.2±0.6^{a}	7.9±0.7^{a,b}	68.8±5.5^{a}	3.8±0.3^{c}
	3SH5-05	14.7±3.2^{a}	54.8±4.2^{a}	9.1±1.3^{a}	8.2±1.0^{a,b}	68.9±7.5^{a}	3.6±0.2^{b,c}
	3WH5-01	13.9±2.0^{a,b}	50.5±2.2^{a}	8.0±0.3^{a}	8.2±1.1^{a,b}	69.4±4.9^{a}	4.8±0.2^{a}
200 mM NaCl	Control	2.0±1.7^{a}	8.8±0.7^{c}	1.4±0.3^{b}	3.3±1.7^{b}	8.2±1.6^{c}	0.9±0.2^{c}
	1SM5-02	2.4±2.4^{a}	9.7±0.6^{c}	1.6±0.1^{b}	5.3±2.2^{a,b}	9.9±1.6^{c}	1.6±0.4^{b,c}
	2SH3-07	3.6±2.0^{a}	15.3±0.2^{b}	2.5±0.1^{a}	5.4±1.7^{a}	29.3±2.6^{a}	9.2±0.2^{a}
	3SH5-05	4.1±1.6^{a}	18.7±2.2^{a}	2.8±0.3^{a}	5.1±1.2^{a,b}	25.5±1.3^{a,b}	1.9±0.3^{b}
	3WH5-01	4.3±2.4^{a}	16.3±1.4^{a,b}	2.6±0.3^{a}	4.9±2.4^{a,b}	21.0±1.7^{b}	2.5±0.6^{b}

Note: Data are means of 8 replications.
Data followed by the same letter in the same column are not significantly different ($p < 0.05$) from each other according to Duncan test.

4. CONCLUSION

The results of this study revealed that most of the actinomycetes recovered from sediment and water samples in mangrove forest were members of genus *Streptomyces* (65.07%), *Micromonospora* (6.85%), *Nonomuraea* (6.16%) and *Saccharopolyspora* (6.16%). The cultivable actinomycetes diversity in this study was distributed in 14 genera and could be the good source of novel strains of non-streptomycetes group. Plant growth promoting and anti-*Xanthomonas oryzae* activities of the isolates could be useful for promoting plant growth and control

Xanthomonas oryzae in rice cultivation. Plant growth promoting effects of the four active actinomycete isolates were evident in tube experiments, which showed that all of them caused enhancement in most of the rice seedling growth parameters under non-saline and saline conditions up to 200 mM NaCl. Our results indicate that mangrove actinomycetes are promising candidates to improve the growth and development of plants under salinity stress conditions. The present study is the first report on plant growth promoting potential of actinomycetes from mangrove in Ranong province, Thailand. This appears to be a good strategy for selecting promising strains to apply in stressful environments for sustainable agriculture. Further investigations on these potent actinomycetes in greenhouse condition were necessary for future use as bio-fertilizer.

ACKNOWLEDGEMENTS

This research was supported by the National Research Council of Thailand (NRCT) and the Center of Excellence on Biodiversity (BDC-PG1-160003). We gratefully acknowledge Andaman Coastal Research Station for samples collection and Prof. Dr. Savitree Limtong of Kasetsart University, the director of research program. Paweena Suksaard is grateful to the Science Achievement Scholarship of Thailand (SAST). Wasu Pathom-aree also acknowledges the Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Faculty of Science, Chiang Mai University.

REFERENCES

[1] Mohandas A.T., Poovarasana S., Panneerselvama P., Sarithaa B., Upretia K.K., Kamala R. and Sita T., *Scientia Horticulturae,* 2013; **150**: 371-376. DOI 10.1016/j.scienta.2012.11.019.

[2] Franco-Correa M., Quintana A., Duque C., Suarez C., Rodriguez M.X. and Barea J.M., *Appl. Soil Ecol.*, 2010; **45**: 209-217. DOI 10.1016/j.apsoil.2010.04.007.

[3] Huang H., Lu J., Hu Y., Fang Z., Zhang K. and Bao S., *Int. J. Syst. Evol. Microbiol.*, 2008; **58**: 17-20. DOI 10.1099/ijs.0.64484-0.

[4] Ruan C.Y., Zhang L., Ye W.W., Xie X.C., Srivibool R., Duangmal K., Pathom-aree W., Deng Z.X. and Hong K., *Antonie van Leeuwenhoek*, 2015; **107**: 39-45. DOI 10.1007/s10482-014-0301-6.

[5] Suksaard P., Mingma R., Srisuk N., Matsumoto A., Takahashi Y. and Duangmal K., *Int. J. Syst. Evol. Microbiol.,* 2016; **66**: 4987-4992. DOI 10.1099/ijsem.0.001457.

[6] Muangham S., Suksaard P., Mingma R., Matsumoto A., Takahashi Y. and Duangmal K., *Int. J. Syst. Evol. Microbiol.*, 2016; **66**: 3835-3840. DOI 10.1099/ijsem.0.001273.

[7] Suksaard P., Duangmal K., Srivibool R., Xie Q., Hong K. and Pathom-aree W., *Int. J. Syst. Evol. Microbiol.,* 2015; **65**: 2569-2573. DOI 10.1099/ijs.0.000303.

[8] Van Hop D., Phuong Hoa P.T., Quang N.D., Ton P.H., Ha T.H., Van Hung N., Van N.T., Van Hai T., Kim Quy N.T., Anh Dao N.T. and Thi Thom V., *Biocontrol Sci.*, 2014; **19**: 103-111. DOI 10.4265/bio.19.103.

[9] Hasegawa T., Takizawa M. and Tanida S., *J. Gen. Appl. Microbiol.*, 1983; **29**: 319-322. DOI 10.2323/jgam.29.319.

[10] Mingma R., Pathom-aree W., Trakulnaleamsai S., Thamchaipenet A. and Duangmal K., *World J. Microbiol. Biotechnol.*, 2014; **30**: 271-280. DOI 10.1007/s11274-013-1451-9.

[11] You J.L., Cao L.X., Liu G.F., Zhou S.N., Tan H.M. and Lin Y.C., *World J. Microbiol. Biotechnol.*, 2005; **21**: 679-682. DOI 10.1007/s11274-004-3851-3.

[12] Pikovskaya R.I., *Microbiology USSR*, 1948; **17**: 362-370.

[13] Khamna S., Yokota A. and Lumyong S., *World J. Microbiol. Biotechnol.*, 2009; **25**: 649-655. DOI 10.1007/s11274-008-9933-x.

[14] Palaniyandi S.A., Damodharan K., Yang S.H. and Suh J.W., *J. Appl. Microbiol.,* 2014; **117**: 766-773. DOI 10.1111/jam.12563.

[15] Yoshida S., Forno D.A., Cock J.K. and Gomez K.A., *Laboratory Manual for Physiological Studies of Rice,* International Rice Research Institute, Manila, 1976.

[16] Hayakawa M. and Nonomura H., *J. Ferment. Technol.,* 1987; **66**: 501-509. DOI 10.1016/0385-6380(87)90108-7.

[17] Whitman W.B., Goodfellow M., Kämpfer P., Busse H.J., Trujillo M.E., Ludwig W., Suzuki K.I. and Parte A., *Bergey's Manual of Systematic Bacteriology: Volume 5: The Actinobacteria*, 2nd Edn., Springer, New York, 2012.

[18] Malek N.A., Zainuddina Z., Chowdhury A.J.K. and Abidin Z.A.Z., *J. Teknol.*, 2015; **77**: 37-43. DOI 10.11113/jt.v77.6734.

[19] Hong K., Gao A.H., Xie Q.Y., Gao H., Zhuang L., Lin H.P., Yu H.P., Li J., Yao X.S., Goodfellow M. and Ruan J.S., *Mar. Drugs,* 2009; **7**: 24-44. DOI 10.3390/md7010024.

[20] Lee L.H., Zainal N., Azman A.S., Eng S.K., Goh B.H., Yin W.F., AbMutalib N.S. and Chan K.G., *Sci. World J.*, 2014; **2014**: 1-14. DOI 10.1155/2014/698178.

[21] Luther G.W. and Wu J., *Mar. Chem.*, 1997; **57**: 173-179. DOI 10.1016/S0304-4203(97)00046-7.

[22] Sadeghi A., Karimi E., Dahaji P.A., Javid M.G., Dalvand Y. and Askari H., *World J. Microbiol. Biotechnol.*, 2012; **28**: 1503-1509. DOI 10.1007/s11274-011-0952-7.

[23] Jog R., Pandya M., Nareshkumar G. and Rajkumar S., *Microbiol.*, 2014; **160**: 778-788. DOI 10.1099/mic.0.074146-0.

[24] Peela S., Kurada V.B. and Terli R., *World J. Microbiol. Biotechnol.*, 2005; **21**: 583-585.

[25] Hata E.M., Sijam K., Ahmad Z.A.M., Yusof M.T. and Azman N.A., *Braz. Arch. Biol. Technol.,* 2015; **58**: 821-832. DOI 10.1590/S1516-89132015060263.

[26] Glick B.R., *FEMS Microbiol. Lett.*, 2005; **251**: 1-7. DOI 10.1016/j.femsle.2005.07.030.

[27] El-Tarabily K.A., *Plant Soil,* 2008; **308**: 161-174. DOI 10.1007/s11104-008-9616-2.

[28] Rajput L., Imran A., Mubeen F. and Hafeez F.Y., *Pak. J. Bot.,* 2013; **45**: 1955-1962.

[29] Doumbou C.L., Salove M.K., Crawford D.L. and Beaulieu C., *Phytoprotection.*, 2001; **82**: 85-102.

[30] Tank N. and Saraf M., *J. Plant Interact.*, 2010; **5**: 51-58. DOI 10.1007/s11274-011-0952-7.