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Effect of Indole-3-Acetic Acid-Producing Bacteria on Phytoremediation of Soil Contaminated with Phenanthrene and Anthracene by Mungbean

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Abstract

The use of indole-3-acetic acid (IAA)-producing bacteria isolated from non-contaminated weed rhizosphere to enhance plant growth and PAH phytoremediation capacity was investigated. IAA-producing bacterial isolates, designated NSRU1, NSRU2, and NSRU3, were isolated from the rhizosphere of *Eleusine indica* (Poaceae) and *Chromolaena odorata* (Asteraceae). The isolates were able to produce IAA in nutrient broth. However, when grown in the presence of 100 mg/l of either phenanthrene or anthracene, the amount of IAA produced by each isolate was reduced significantly. Mungbean seedlings were planted in 100 mg/kg phenanthrene - or anthracene-contaminated soil without or with inoculation of ≈ 10^6 CFU/g dry soil with one of the bacterial isolates. Inoculation with either NSRU1 or NSRU2 was effective at enhancing shoot length of mungbean in phenanthrene-contaminated soil on day 16. Also, inoculation with isolate NSRU1 led to increased root dry weight of mungbean in phenanthrene-contaminated soil on day 30. Phenanthrene and anthracene degradation on day 16 and 30 in planted and inoculated soil ranged between 92 - 93.8% and 92.2 - 94.1%, respectively, which were not significantly different from planted and uninoculated soil (93.9 and 94.9%). These data showed that IAA-producing bacteria could enhance plant growth, but was unable to increase PAH biodegradation under the conditions tested.

Keywords : IAA-producing bacteria; phytoremediation; polycyclic aromatic hydrocarbons

1. Introduction

Indole-3-acetic acid (IAA) is a natural plant auxin which exerts important effects on plant growth and development, such as elongation, differentiation, apical dominance and flowering. IAA biosynthesis in plant occurs via either Trp-dependent or Trp-independent pathway (Mano and Nemoto, 2012). IAA-producing bacteria are a group of plant growth promoting bacteria (PGPB) generally detected in the rhizosphere and in plant tissues. Strains from several bacterial genera have been reported to produce IAA, such as *Bacillus, Klebsiella, Pseudomonas, Rhizobium, Sphingomonas* and *Xanthomonas* (Tsavkelova *et al.,* 2006). IAA-producing bacteria have been used to enhance crop growth. For example, immersion of seeds of Tanzania grass in Yeast Manitol (YM) broth containing 10^8 CFU/ml IAA-producing *Mesorhizibium* sp. or *Bradyrhizobium* sp. and then planted in soil led to increased root dry weight of this plant on day 60 after germination. These bacterial strains could produce about 0.2 - 4.0 µg/ml of IAA in YM broth supplemented with 50 mg/l Trp for 48 h (Machado *et al.,* 2013). In another study, immersion of cowpea seeds in broth containing 10^8 CFU/ml of IAA-producing *Enterobacter* strain for 45 min increased shoot and root lengths of cowpea 3 weeks after seed germination. This strain could produce 23.8 - 104.8 µg/ml of IAA in broth supplemented with 1 g/l Trp for 48 h (Deepa *et al.,* 2010).

IAA-producing bacteria have been used in phytoremediation to enhance plant growth in polluted soil as well as accumulate heavy metals in plant tissues. In one study, suspensions of the IAA-producing and copper-tolerant *Burkhoderia* sp. strain LD-11 (10^9 CFU/ml) were inoculated in surface soil near the plant root system of 7 day-old flowering Chinese cabbage (*Brassica campestris* ssp. *chinensis*) and sweet mustard (*B. juncea*) seedling. There were 2 plants of the same species/pot and each pot contained soil contaiminated with 800 mg/kg copper and 1,000 mg/kg lead. After 42 days, bacterial inoculation was found to increase copper accumulation in the shoots of sweet mustard and roots of flowering Chinese cabbage without any apparent enhancement of plant growth. Bacterial inoculation also increased lead accumulation in the shoots of flowering Chinese cabbage and roots of sweet mustard (Huang *et al.,* 2013). In another study, Golubev *et al.* (2011) examined the effect of IAA-producing bacteria on PAH phytotoxicity. These authors studied the effect of phenanthrene on the

IAA-producing and PAH-degrading *Sinorrhizobium meliloti* and growth of sorghum in phenanthrene-contaminated sand. The bacterial cells suspended in Ruakura nutrient solution were applied to planted sand to final concentration of 10^6 cell/g sterile sand. In the presence of 100 mg/kg phenanthrene, the amount of IAA (measured by HPLC) produced was 1.56×10^{-10} M in the uninoculated rhizosphere; the amount increased to 2.18×10^{-10} M when *S. meliloti* was inoculated. Furthermore, inoculation with *S. meliloti* led to increased root biomass of sorghum grown in 100 mg/kg phenanthrene-contaminated sand but the effect of bacterial inoculation on phenanthrene removal was not reported (Golubev *et al.,* 2011).

The toxicity of PAH on IAA production can adversely affect the success of IAA-producing bacteria used in PAH phytoremediation. This in turn can affect the positive effect of these bacteria on plant growth and the plant's capacity to enhance PAH biodegradation. In this study, several bacteria isolated from non-contaminated rhizosphere of Thai weeds were shown to produce IAA when exposed to PAH.The ability of these bacteria to enhance phenanthren and anthracene phytoremediation in soil was assessed The plant selected for phytoremediation was mungbean which is a legume previously found to not tolerate PAH- and engine oil-contaminated soil well (Chouychai *et al.,* 2007). We were interested in testing if the IAA-producing bacterial isolates could enhance the growth of mungbean in PAH-contaminated soil and if so whether improved growth may translate into improved PAH degradation.

2. Materials and Methods

2.1 Isolation of IAA-producing bacteria

Bacteria used in this experiment were isolated from the rhizospheric soil of *Chromolaena odorata* (Asteraceae) and *Eleusine indica* (Poaceae), two common weeds in Thailand. The weeds were collected from grassland behind building 7 in Nakhonsawan Rajabhat University (Nakhonsawan, Thailand). The 1-g soil sample from the rhizosphere of each plant was placed in 9.5 ml of sterile distilled water and shaken at 180 rpm for 2 h. The suspension was serially diluted in sterile distilled water and spread on Nutrient agar (NA) plates. After 48 h, different colonies were picked and streaked onto fresh NA plates to obtain single colonies. In total, 36 isolates were obtained from the rhizospheric soil of both plants. All isolated bacteria were tested for IAA production as described by Ahmad *et al.* (2008). Samples of each colony were grown individually in Nutrient Broth (NB) supplemented with 0.05 g/l Trp for 72 h. The culture was centrifuged at 1400 xg and two ml of supernantant were transferred to a new tube and mixed with 2 ml of Salkowski's reagent and 2 drops of orthophosphoric acid. A pink color indicates the presence of IAA. Based on this test, ten isolates were selected and subcultured individually in fresh 50 ml NB media in a 125-ml flask for 3 days. Then 10^6 cfu/ml (enumerated by plate count in NA) of each culture were transferred to 50 ml NB supplemented with 0.05 g/l Trp in a 125-ml flask. The IAA concentration was determined spectrophotometrically at 530 nm. IAA concentrations in the samples were estimated from a standard curve constructed with 1 - 100 µg/ml IAA (Fluka, purity 90%) dissolved in distilled water.

2.2 Effect of PAHs on IAA production

Each bacterial isolate was grown in 50 ml NB supplemented with 0.05 g/l Trp in a 125-ml flask which was shaken at 150 rpm at room temperature (25 ^{0}C) for 72 h. Then 10^6 cfu/ml of each culture were transferred to NB supplemented with 0.05 g/l Trp with or without PAHs. Phenanthrene (Sigma-Aldrich, purity 98%) or anthracene (Fluka, purity 98%) dissolved in dimethyformamide was added to separate flasks to final concentrations 100 mg/l in NB. The experiment was done in triplicate. After 3 and 10 days, IAA production in the culture supernatant was measured according to Ahmad *et al.* (2008) as described above.

2.3 Soil preparation

Alkaline agricultural soil with no previous history of hydrocarbon contamination was collected at the Khaorad Agricultural Station, Faculty of Agricultural Technology and Industrial Technology, Nakhonsawan Rajabhat University, Nakhonsawan, Thailand as described in Chouychai and Lee (2012). The soil was air-dried at room temperature (25^{0}C) for at least 24 h to constant weight before use. The soil used in this experiment was alkaline (pH 8.9) with low total phosphorus content (below 0.29 g/100 g soil). The soil contained (per 100 g dry soil): 0.21 g total nitrogen (N), 0.13 g total potassium (K), and 1.78 g organic matter. Soil was mixed with either phenanthrene or anthracene individually dissolved in acetone to final concentration of 100 mg/kg.

2.4 Experimental design.

The following five experimental treatments were used for each of the PAH-contaminated soil: (a) soil inoculated with isolate NSRU1 and planted with mungbean, (b) soil inoculated with isolate NSRU2 and planted with mungbean, (c) soil inoculated with isolate NSRU3 and planted with mungbean, (d) soil planted with mungbean, (e) soil which received the NB only. The following four experimental treatments were done in non-contaminated soil: (f) soil inoculated with isolate NSRU1 and planted with mungbean, (g) soil inoculated with isolate NSRU2 and planted with mungbean, (h) soil inoculated with isolate NSRU3 and planted with mungbean, (i) soil planted with mungbean. Each uninoculated treatment received the same amount of NB as that used in bacterial inoculation. Cells of each isolate were inoculated into soil to final concentrations of about 10^6 CFU/g dry soils (enumerated by plate count in NA) and mixed thoroughly with a digger. Each treatment was done in triplicate.

2.5 Pot experiment

Ten-day old mungbean seedlings were planted in soil containing 100 mg of either phenanthrene or anthracene per kg dry soil at one plant per pot. This phenanthrene concentration has been reported that there were toxic to mungbean root (Chouychai *et al.,* 2007). The diameter of the pot was 7 inches, with each pot holding 1 kg dry weight of soil. The pots were kept in a nursery in which the temperature was around 24°C during the day and 21°C at night and exposed to natural sunlight. Each pot was watered every day with about 20 ml water for 30 days. The experiment was conducted in triplicate. On days 16 and 30, the mungbean plants were removed and the pots terminated. The length, fresh weight, and dried weight of shoot and root of mungbeans were measured.

One g of soil sample was collected from each treatment on days 16 and 30 for analysis of remaining PAHs. The amount of phenanthrene or anthracene remaining in soil was determined by gas chromatography using a Shimadzu model AOC-5000 GC equipped with a mass spectroscopic detector (Shimadzu MS-QP2010) according to Somtrakoon *et al.* (2014). One g of soil was mixed with an equal amount of anhydrous Na_2SO_4, and subjected to Soxhlet extraction prior to GC analysis. Phenanthrene and anthracene were separated on a Rtx®-5MS capillary column (30 m x 25 mm, I.D. = 25 μm) as described in Somtrakoon *et al.* (2014). The detection limit of this analysis was 0.4 mg/kg.

3. Results

3.1 Isolation of IAA-producing bacteria

Three bacterial isolates which showed the highest IAA concentrations were selected for use in subsequent experiment. All were Gram-positive bacteria, and they were designated NSRU1, NSRU2, and NSRU3. NSRU1 was isolated from the rhizosphere of *E. Indica* and its colonies were white, circular, transparent, raised and smooth. NSRU2 and NSRU3 were isolated from the rhizosphere of *C. odorata*. Colonies of NSRU2 were yellow, circular, undulated, opaque and umbonate while colonies of NSRU3 were yellow, circular, and umbonate.

3.2 Effect of PAHs on IAA production

Different amounts of IAA were produced by each of the 3 bacterial isolates when cultured in NB supplemented with 0.05 g/l Trp for 3 days. Isolate NSRU1 produced the highest amount of IAA (37.9 ± 9.1 μg/ml), while isolates NSRU2 and NSRU3 produced lower amounts of 17.4 and 15.9 μg/ml, respectively. When 100 mg/l phenanthrene were added to the culture, the amounts of IAA produced by isolates NSRU1, NSRU2 and NSRU3 were reduced to 10.6, 4.8, and 7.7 μg/ml, respectively (Table 1). Addition of anthracene reduced the amount of IAA produced by each strain to 12.5 - 6.3 μg/ml. The amounts of IAA remaining in the medium on day 10 were generally lower than those on day 3. In particular, the amounts of IAA remaining were below the detection limit in the supernatant of isolate NSRU3 grown in the presence of the PAHs (Table 1).

3.3 Effect of IAA-producing bacteria on mungbean growth

In non-contaminated soil, growth of mungbean appeared normal and was not affected by inoculation with any of the 3 IAA-producing bacterial isolates (Tables 2-4). The presence of phenanthrene in soil led to decreased root length of mungbean on day 16 to 3.9 ± 3.0 cm from that in non-contaminated soil (8.4 ± 3.0 cm). Inoculation with isolates NSRU1 or NSRU2 led to increased shoot length of mungbean in phenanthrene-contaminated soil on day 16 to 24.1 and 26.0 cm, respectively, from that in non-inoculated treatment (21.0 cm). On day 30, only the treatment which was inoculated with isolate NSRU1 showed increased root dry weight of mungbean to 15.2 ± 3.7 mg in phenanthrene-contaminated soil; this value was significantly higher than the root dry weight of mungbean in other treatments (Table 4). In anthracene-contaminated soil, innoculation of any of the isolates did not result in altered growth parameters of mungbean (Tables 2-4).

Table 1. IAA production of selected bacterial isolates cultured in NB containing 0.05 g/l tryptophan and 100 mg/l PAH for 3-10 days. Starting cell concentration of each bacterial isolate was 10^6 CFU/ml.

Bacterial isolate	IAA concentration (μg/ml)		
	Without PAHs	100 mg/l phenanthrene	100 mg/l anthracene
Day 3			
NSRU1	37.9 ± 9.1[a]	10.6 ± 0.8[b]	12.5 ± 0.9[b]
NSRU2	17.4 ± 2.2[a]	4.8 ± 0.3[b]	6.3 ± 0.6[b]
NSRU3	15.9 ± 4.0[a]	7.7 ± 0.7[b]	8.1 ± 0.3[b]
Day 10			
NSRU1	21.9 ± 3.4[a]	12.1 ± 1.8[b]	4.5 ± 0.2[b]
NSRU2	14.3 ± 1.4[a]	4.6 ± 1.5[b]	B.D.
NSRU3	5.0 ± 2.6	B.D.	B.D.

Different lower case letters showed significant difference (*P<0.05*) between treatments in same strain.
B.D. = below detection limit

Table 2. Shoot and root length of mungbean grown in non-contaminated or PAH-contaminated soil with different bacterial strain inoculation

Treatment	Shoot length (cm)			Root length (cm)		
	NCS	PHE	ANT	NCS	PHE	ANT
Day 16						
Non-inoculated	22.3 ± 2.1[a]	21.0 ± 2.1[b]	21.6 ± 1.4[b]	8.4 ± 3.0[a]	3.9 ± 0.5[a*]	7.3 ± 1.1[a]
NSRU1	24.2 ± 2.3[a]	24.1 ± 1.1[a]	21.3 ± 1.3[b]	8.7 ± 2.5[a]	5.8 ± 1.5[a]	9.4 ± 2.2[a]
NSRU2	22.4 ± 1.9[a]	26.0 ± 3.2[a]	25.1 ± 0.2[a]	7.0 ± 1.4[a]	4.3 ± 1.4[a]	9.6 ± 2.4[a]
NSRU3	24.4 ± 1.5[a]	20.0 ± 2.4[b]	23.6 ± 1.2[a, b]	8.0 ± 1.8[a]	6.1 ± 2.2[a]	9.0 ± 3.2[a]
Day 30						
Non-inoculated	23.4 ± 2.7[a]	22.2 ± 2.0[a]	25.0 ± 1.3[a]	7.1 ± 1.9[a]	6.0 ± 1.0[a]	6.8 ± 2.4[a]
NSRU1	24.2 ± 2.8[a]	21.8 ± 0.4[a]	26.3 ± 1.3[a]	8.3 ± 1.9[a]	5.8 ± 0.6[a]	9.3 ± 4.2[a]
NSRU2	23.5 ± 1.6[a]	23.6 ± 1.7[a]	22.6 ± 1.8[a]	8.0 ± 1.2[a]	6.5 ± 0.7[a]	4.5 ± 1.6[a]
NSRU3	25.1 ± 1.8[a]	19.5 ± 3.8[a*]	25.6 ± 2.9[a]	8.1 ± 1.8[a]	7.8 ± 2.8[a]	6.2 ± 0.8[a]

Different lower case letters showed significant difference ($P<0.05$) between the same soil on the same day; [*]showed significant difference ($P<0.05$) from non-contaminated soil
Symbol: NCS = non-contaminated soil, PHE = phenanthrene-contaminated soil and ANT = anthracene-contaminated soil.

Table 3. Shoot and root fresh weight of mungbean grown in non-contaminated or PAH-contaminated soil with different bacterial strain inoculation

Treatment	Shoot fresh weight (mg)			Root fresh weight (mg)		
	NCS	PHE	ANT	NCS	PHE	ANT
Day 16						
Non-inoculated	473.8 ± 64.9[a]	382.3 ± 156.7[a]	372.5 ± 28.6[a]	113.9 ± 20.4[a]	66.7 ± 15.3[b]	118.0 ± 52.9[a]
NSRU1	504.9 ± 78.3[a]	443.3 ± 51.3[a]	548.3 ± 227.6[a]	132.6 ± 31.1[a]	133.3 ± 28.9[a]	159.5 ± 75.2a
NSRU2	428.3 ± 52.5[a]	480.0 ± 99.0[a]	381.6 ± 7.1[a]	103.4 ± 31.2[a]	120.0 ± 14.1[ab]	123.2 ± 25.1[a]
NSRU3	481.8 ± 59.7[a]	373.3 ± 85.0[a]	522.9 ± 9.4[a]	119.0 ± 23.0[a]	116.7 ± 5.8[ab]	148.1 ± 15.3[a]
Day 30						
Non-inoculated	492.3 ± 79.5[a]	550.2 ± 244.6[a]	461.3 ± 85.7[a]	113.4 ± 10.5[a]	110.5 ± 9.1[a]	116.7 ± 28.5[a]
NSRU1	652.0 ± 224.2[a]	495.1 ± 125.5[a]	614.2 ± 82.2[a]	166.3 ± 79.8[a]	159.4 ± 47.0[a]	167.7 ± 105.7[a]
NSRU2	557.1 ± 72.2[a]	627.8 ± 115.5[a]	519.1 ± 16.8[a]	180.0 ± 51.9[a]	167.2 ± 73.0[a]	89.0 ± 20.7[a]
NSRU3	616.1 ± 107.6[a]	451.3 ± 203.8[a]	597.3 ± 112.6[a]	208.5 ± 49.7[a]	136.6 ± 67.5[a]	175.4 ± 28.0[a]

Different lower case letters showed significant difference ($P<0.05$) between the same soil on the same day
Symbol: NCS = non-contaminated soil, PHE = phenanthrene-contaminated soil and ANT = anthracene-contaminated soil

Table 4. Shoot and root fresh weight of mungbean grown in non-contaminated or 100 mg/kg each PAH-contaminated soil with different bacterial strain inoculation

Treatment	Shoot dried weight (mg)			Root dried weight (mg)		
	NCS	PHE	ANT	NCS	PHE	ANT
Day 16						
Non-inoculated	45.9 ± 6.7[a]	39.5 ± 12.1[a]	37.9 ± 7.0[a]	5.5 ± 0.6[ab]	4.5 ± 1.2[b]	7.0 ± 3.5[a]
NSRU1	51.0 ± 7.1[a]	46.8 ± 8.6[a]	57.2 ± 25.0[a]	7.1 ± 1.4[a]	8.9 ± 3.5[a]	7.0 ± 1.3[a]
NSRU2	50.5 ± 8.1[a]	42.6 ± 11.8[a]	51.0 ± 12.9[a]	4.6 ± 0.6[b]	5.6 ± 2.3[b]	7.5 ± 0.4[a]
NSRU3	47.9 ± 11.1[a]	40.1 ± 7.9[a]	54.5 ± 3.4[a]	5.5 ± 1.0[ab]	7.5 ± 1.9[a]	7.9 ± 1.6[a]
Day 30						
Non-inoculated	62.2 ± 14.2[a]	70.5 ± 23.0[a]	79.9 ± 28.8[a]	10.9 ± 2.7[a]	7.1 ± 0.9[b]	14.1 ± 2.1[a]
NSRU1	85.9 ± 34.7[a]	66.0 ± 19.3[a]	102.4 ± 29.0[a]	19.5 ± 9.2[a]	15.2 ± 3.7[a]	16.2 ± 2.7[a]
NSRU2	74.5 ± 19.0[a]	85.0 ± 24.5[a]	64.8 ± 23.9[a]	17.7 ± 5.2[a]	8.3 ± 2.1[b]	12.8 ± 1.1[a]
NSRU3	92.5 ± 27.7[a]	58.6 ± 26.3[a]	101.3 ± 27.8[a]	17.5 ± 4.0[a]	5.9 ± 0.9[b]	19.4 ± 1.6[a]

Different lower case letter showed significant difference ($P<0.05$) between same soil in same day.
Symbol: NCS = non-contaminated soil, PHE = phenanthrene -contaminated soil and ANT = anthracene-contaminated soil

3.4 Effect of IAA-producing bacteria on PAH removal

In non-planted soil, phenanthrene and anthracene were degraded rapidly with the extent of degradation reaching 81 and 42%, respectively on day 16. Planting with mungbean increased the removal of phenanthrene and anthracene from soil to 93.9 and 94.9%, respectively, on day 16 (Table 5). Further inoculation of planted soil with bacterial strains did not result in faster rate or extent of phenanthrene and anthracene degradation relative to that seen in planted soil. This trend was seen also on day 30 (Table 5). Likely, the PAHs were degraded so rapidly that any differences were masked and the removal of both PAHs seems to depend on mungbean and soil indigenous bacteria more than the inoculated IAA-producing bacteria.

4. Discussion

In this study, 100 mg/l phenanthrene and anthracene were found to adversely affect IAA production by the 3 bacterial isolates. IAA production by NSRU 1 was inhibited by 72.0 and 67.0%, while IAA production by NSRU2 and NSRU3 was inhibited by 72.4% and 63.8%, and 51.6% and 49.0% when phenanthrene and anthacene, respectively, were added to the cultures. There were previous reports that IAA production by IAA-producing bacteria was sensitive to pesticides. For example, addition of 0.04 - 0.12 mg/l hexaconazole reduced IAA production by *Mesorhizobium* sp. MRC4 culture by 36.4 - 52.3% from that produced by the culture in a pesticide-free medium (44.0 µg/ml) (Ahemad and Khan, 2012). In another study, IAA production by *Gluconacetobactor diazotrophicus* strain PAL5 was reduced by 77.6 - 93.2% to 0.63 - 0.19 µg/ml in the presence of either 6.0 mg/l Monocrotophos or 2.8 mg/l Dichorvos from that produced in a pesticide-free medium (2.81 µg/ml). Interestingly, addition of 2.5 mg/l lindane completely inhibited IAA production by *G. diazotrophicus* PAL5 (Madhaiyan *et al.,* 2006).

Although pesticides could reduce IAA production, some authors reported the use of IAA-producing bacteria to improve plant growth in pesticide-contaminated soil. For example, inoculation of *Mesorhizobium* sp. MRC4 significantly increased the dry weight of chickpea grown in 0.6 mg/kg Fibronil or 3.9 mg/kg pyrifroxyfen-contaminated soil to 2.67 and 2.61 g/plant from those of chickpea in un-inoculated soil of 1.42 and 1.08 g/plant, respectively. In our study, bacterial inoculation led to increased shoot length and dry root weight of mungbean grown in phenanthrene-contaminated soil on day 16. NSRU1 which produced the highest amount of IAA seemed to be the most beneficial to mungbean in phenanthrene-contaminated soil.

The removal of PAHs in planted soil was reported in many literatures. The main mechanism was the stimulating microbial growth by plant root. Exudation of organic acid, nutrient and detergent from root could enhance biodegradation rate by microorganism in soil (Siciliano and Germida, 1998). The inoculation of IAA-producing bacteria did not seem to enhance phenanthrene or anthracene biodegradation when compared with planted and uninoculated soil. This may be because both PAHs tested in this experiment were degraded rapidly in mungbean planted soil. The ability of synthetic IAA to increase PAH removal from soil has been reported. Application of 2.4 mg/kg IAA to ryegrass planted in 200 mg/kg fluoranthrene increased fluoranthene removal from soil. The amount of fluoranthene remaining in soil planted with ryegrass and treated with IAA was 100 mg/kg while the amount remaining in soil planted with ryegrass without IAA treatment was 130 mg/kg soil. IAA could increase fluoranthene removal by enhance fluoranthene uptake to shoot and increase number of soil microorganism (Li *et al.,* 2015). However, application with

Table 5. Percentages of phenanthrene or anthracene remaining in soil grown with mungbean and non-inoculated or inoculated with IAA producing bacteria in phenanthrene or anthracene -spiked soil for 30 days

Plant	% remaining	
	Day 16	Day 30
Phenanthrene		
Non-planted soil	19.0 ± 1.0[a]	15.2 ± 3.8[a]
Planted soil	6.1 ± 2.1[b]	2.8 ± 1.8[b]
Planted soil + NSRU1	6.2 ± 1.6[b]	3.6 ± 2.7[b]
Planted soil + NSRU2	7.3 ± 4.9[b]	2.0 ± 0.8[b]
Planted soil + NSRU3	8.0 ± 1.8[b]	1.4 ± 1.1[b*]
Anthracene		
Non-planted soil	58.4 ± 6.0[a]	8.4 ± 6.0[a*]
Planted soil	5.1 ± 0.6[b]	6.6 ± 2.4[a]
Planted soil + NSRU1	5.9 ± 4.0[b]	3.0 ± 1.1[a]
Planted soil + NSRU2	7.8 ± 7.6[b]	5.0 ± 3.6[a]
Planted soil + NSRU3	8.8 ± 3.3[b]	5.2 ± 0.8[a]

Different lower case letter showed significant difference ($P<0.05$) between same PAH in same day and * showed significant difference ($P<0.05$) between PAH-remaining on day 16 and 30.

indolebutyric acid, which is auxin also, did not enhance corn growth but could increase hexachlorocyclohexane removal in soil within 30 days (Chouychai *et al.,* 2015). The role of IAA on phytoremediation process in PAH-contaminated soil should be studied in more detail.

5. Conclusions

In summary, IAA-producing bacteria isolated from non-contaminated weed rhizosphere exhibited some tolerance to PAHs and produced IAA in PAH-containing media. In soil experiment, inoculation with these IAA-producing bacterial strains led to enhanced root growth of mungbean in phenanthrene-contaminated soil but not PAH biodegradation. Given the potential benefit of IAA-producing bacteria to phytoremediation, further studies are warranted. These may include inoculum size and selecting conditions in which PAHs were poorly degraded to further assess the potential beneficial effect of IAA-producing bacteria.

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