



Technical Note

Isolation and characterization of plant growth-promoting rhizobacteria and their effects on phytoremediation of petroleum-contaminated saline-alkali soil

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HIGHLIGHTS

- 115 PGPR strains were isolated from petroleum-contaminated saline-alkaline soils.
- *Klebsiella* sp. D5A displayed the highest plant-growth-promoting activity.
- D5A grew well on the LB medium containing 9% NaCl and at pH 4–10.
- Inoculation of D5A promoted phytoremediation of petroleum-contaminated soils.

ARTICLE INFO

Article history:

Received 7 April 2014

Received in revised form 3 July 2014

Accepted 4 July 2014

Handling Editor: O. Hao

Keywords:

PGPR

Klebsiella sp.

Soil contamination

Salinity

Alkalinity

ABSTRACT

This study aimed to isolate promising halotolerant and alkalotolerant plant growth-promoting rhizobacteria and to study their effects on the growth of tall fescue and phytodegradation efficiency in a petroleum-contaminated saline-alkaline soil. A total of 115 PGPR strains were isolated from the rhizosphere of tall fescue grown in petroleum-contaminated saline-alkaline soils. Of these, 5 strains indicating 1-aminocyclopropane-1-carboxylic acid deaminase activity $>1.0 \text{ M } \alpha\text{-KB mg}^{-1} \text{ h}^{-1}$ were selected for further studies. The isolate D5A presented the highest plant-growth-promoting activity and was identified as *Klebsiella* sp. It grew well on the Luria–Bertani medium containing 9% NaCl and at a pH range of 4–10. A pot experiment was then conducted to study the effect of isolates on phytoremediation. The results showed that inoculation of D5A promoted tall fescue growth and enhanced remediation efficiency in petroleum-contaminated saline-alkaline soil.

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1. Introduction

Contamination of soil environment by petroleum hydrocarbons is becoming prevalent across the globe. A number of methods have been used to clean up the petroleum contaminated soils, but most of them are costly and difficult to get optimum results (Liu et al., 2010a,b). Bioremediation is an appealing and cost-effective approach to cleaning up this type of contaminants. There are many approaches of bioremediation including phytoremediation, land farming, slurry bioreactor treatment and composting. Among bioremediation methods, phytoremediation is a green technology that uses plants to remediate contaminated soils. Different from phyto-

remediation of heavy metals through plant uptake, phytoremediation of petroleum-contaminated soils relies on plant root exudation to create a biologically active soil region (i.e. the rhizosphere) that enhances contaminant bioavailability and encourages microbial degradation (Mohsen et al., 2010; Glick and Stearns, 2011).

Previous studies have shown that tall fescue (*Festuca arundinacea* L.), a perennial species with a highly branched fine fibrous root system, could significantly increase the efficiency of hydrocarbon degradation in the soil (Huang et al., 2005; Gerhardt et al., 2009; Gurska et al., 2009; Liu et al., 2010a,b). It has also been reported hydrocarbon-degraders are able to aggressively colonize the root surface following root exudation. So the number of hydrocarbon-degraders in the rhizosphere is generally higher than that in the bulk soil (Sumia et al., 2013). Clearly, extensive root growth is a

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prerequisite of maximizing the effectiveness of phytoremediation processes. However, most areas of oilfields such as Dagang, Shengli and Daqing Oilfields in China are located in saline–alkaline regions. The biomass accumulation and root growth of tall fescue can be severely affected by soil saline–alkaline stresses which consequently decrease the efficiency of phytoremediation.

Plant growth-promoting rhizobacteria (PGPR) are nonpathogenic beneficial soil rhizobacteria which play a key role in plant health and nutrition by a number of mechanisms. These include the synthesis of siderophores that can solubilize iron in the soil and make it available to the plant, the production of phytohormones, especially indole-3-acetic acid (IAA), and the presence of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase that hydrolyzes ACC, the immediate precursor of the phytohormone ethylene (Glick et al., 1997). Earlier studies indicated that bacteria having plant-growth-promoting (PGP) activity could reduce the level of ethylene and result in better growth of plants under various stress conditions such as salinity, heavy metal toxicity and pathogen attack (Bal et al., 2013). Therefore, the application of PGPR is a promising approach to alleviating saline–alkaline stress on plants and improves the efficiency of phytoremediation in petroleum-contaminated soils.

Because soils of some oilfields located at saline–alkaline sites are natural habitats of haloalkaliphilic bacteria, isolation and utilization of PGPR from such natural habitats could prove to be beneficial for mitigating the saline–alkaline stress to the plants growing in such an environment. Though earlier work has involved isolation of salt-tolerant rhizobacteria from halophytic environments, little is known about their tolerance to alkaline environments where the contaminated soils have high pH (Qadir and Schubert, 2002).

The objectives of the present study were: (1) to isolate and characterize efficient ACC deaminase producing PGPR from the rhizosphere of tall fescue grown in saline-alkali soils, (2) to evaluate other PGP activities of the most promising ACC deaminase producing isolates under various saline–alkaline stresses, and (3) to study the effect of the selected isolates on tall fescue growth and phytoremediation of a petroleum-contaminated saline–alkaline soil.

2. Materials and methods

2.1. Media and soil

The compositions of *Pseudomonas* Agar F (PAF), Dworkin-Foster (DF) and tryptic soy broth media used to isolate and grow PGPR were based on Penrose and Glick (2003). The medium I comprised (g L^{-1}) 10 g tryptone, 5 g yeast extract and 3 g NaCl was used to study the effect of alkalinity stress on the growth of strain D5A.

Rhizospheric soil used for isolating PGPR were randomly collected from the roots of tall fescue in the top 15 cm depth at oil contaminated soil from Dagang and Shengli oilfields of China. The petroleum-contaminated soil used for phytoremediation was collected from Shengli oilfield, Shandong, China. The soil had the following basic properties: pH 9.7 (1: 2.5 water), electrical conductivity (1: 5) $404 \mu\text{S cm}^{-1}$; cation exchange capacity $4.94 \text{ cmol kg}^{-1}$; organic matter 4.26 g kg^{-1} ; hydrolyzable nitrogen 23.7 mg kg^{-1} ; NaHCO_3 -extractable P 8.6 mg kg^{-1} and NH_4OAc -extractable K 176 mg kg^{-1} . TPH (total petroleum hydrocarbon) concentration of soil is 16920 mg kg^{-1} . The petroleum fractions of C12–C16, C16–C21 and >C21 were 441, 2720 and 13760 mg kg^{-1} , respectively, but fraction of <C12 was not detected. Soil properties were measured according to Lu (1999) and TPH and petroleum fractions based on the method of Yang et al. (2014).

2.2. PGPR isolation

PGPR with ACC deaminase activity were isolated according to the method of Penrose and Glick (2003). Briefly, an aliquot of 1 g soil was added to 50 mL sterile PAF medium in a 250-mL flask and incubated aerobically at 21°C on a reciprocal shaker at 200 rpm for 24 h. Then, one mL aliquot was removed from the growing culture, transferred to 50 mL of sterile PAF medium and incubated in the same manner for 24 h. Following these two incubations, the population of bacteria with ACC deaminase activity was enriched and the number of fungi in the culture was reduced. One mL aliquot was removed from the second culture and transferred to 50 mL sterile DF salts minimal medium. After incubation at 21°C on a reciprocal shaker at 200 rpm for 24 h, one mL aliquot was removed from this culture and transferred to 50 mL sterile DF salts minimal medium containing 3.0 mM ACC (instead of $(\text{NH}_4)_2\text{SO}_4$) as the source of nitrogen, namely ADF medium, the culture was placed in a shaking water bath at 200 rpm and grown at 21°C for 24 h. Dilutions of this final culture were plated onto solid ADF salts minimal medium (2% agar) and incubated for 48 h at 28°C . Colonies of different morphologies were picked up and purified.

2.3. ACC deaminase, phosphate solubilization, IAA and siderophore assay

The ACC deaminase activity of cell-free extracts was measured based on the determination of α -ketobutyrate (α -KB) resulting from ACC cleavage by ACC deaminase and enzyme activity was expressed as $\text{M } \alpha\text{-KB mg}^{-1} \text{ h}^{-1}$, as described in Liu et al. (2013). Siderophore secretion by the strains was detected by the improved method of Payne (1994). The strains were cultured in the modified sugar-aspartic acid medium and shaken at 150 rpm at 30°C for 48 h. After centrifugation, 1.5 mL of cell-free culture supernatant was mixed with 1.5 mL of chrome azurol sulfonate assay solution (Wolicka et al., 2009). After 1 h, the absorbance (A) of the mixture is measured at 630 nm. The non-inoculated supernatant used as a reference of which absorbance (A_r) was determined by the above method. The quantitative index was the value of A/A_r which was inversely related to siderophore production. General reference standards: A/A_r 0–0.2, + ++ ++; 0.2–0.4, + ++ +; 0.4–0.6, + ++; 0.6–0.8, + +; 0.8–1.0, +.

Production of IAA was measured according to the Salkowski colorimetric assay (Glickmann and Dessaux, 1995). The phosphate solubilization activity of the isolates was analyzed according to Sundara-Rao and Sinha (1963).

2.4. PGP ability using tall fescue under salt stress

Uniform seeds of tall fescue were sterilized in 70% ethanol for 2 min and in 1% sodium hypochlorite for 10 min and then rinsed twice with sterile water. The sterilized seeds were immersed for 6 h in the isolate suspension ($\text{OD}_{600\text{nm}} = 0.5$) or in 0.03 M MgSO_4 solution as the control. Twenty pretreated seeds were grown in a Petri dish containing two layers of Whatman No. 1 filter paper, moistened with different concentrations of NaCl solution (0–9%). The Petri dishes were placed in biochemistry incubators at 28°C . Seed germination rate, shoot height and root length of seedlings were recorded after 2 weeks. All the operations were done under sterilized conditions and care was taken to avoid contamination during growth and handling of the plants.

2.5. Effects of pH and salinity on the growth and IAA production of strain D5A

The LB medium containing 0.5 mg L^{-1} of tryptophan was used to study effects of pH and salinity on the growth and IAA produc-

tion of strain *Klebsiella* sp. D5A. The medium (100 mL) with different concentrations of NaCl (0–21%) was prepared to test salt tolerance of the bacterial isolate. To test pH response, the pH of LB medium was adjusted to 9 different levels (pH 3–11) using 1 M HCl or NaOH. Flasks with salt and pH treatments were inoculated with suspension of *Klebsiella* sp. D5A cultured overnight in a shaker at 28 °C, 200 rpm for 24 h. In all cases, the concentration of IAA and OD value reflecting the biomass in the medium were determined directly.

2.6. Effect of different levels of Na_2CO_3 and NaHCO_3 stress on the growth of strain D5A

The medium I (5 mL) with different concentrations of (0–300 mM) Na_2CO_3 and NaHCO_3 was used to study the alkalinity stress on the growth of strain D5A, pH was also tested at the same time. Inoculated 50 μL suspension of *Klebsiella* sp. D5A into the culture, 28 °C, 160 rpm. The $\text{OD}_{600\text{nm}}$ was determined after shaking 48 h.

2.7. Physiological characterization and 16S rRNA gene sequencing of strain D5A

Morphological properties of *Klebsiella* sp. D5A were examined by light and transmission electron microscopy. 16S rDNA sequences were amplified from chromosomal DNA by PCR using universal oligonucleotide primers and sequenced as described by Lee et al. (2010). The sequence was then compared to the 16S rDNA sequence in the GenBank database by BLAST. Multiple sequence alignment was done using CLUSTAL X software and a phylogenetic tree was constructed by the neighbor-joining method using MEGA (Version 4.1) software. The confidence level of each branch (1000 repeats) was tested by bootstrap analysis. The 16S rDNA sequence of isolate D5A was deposited in the GenBank database with accession numbers JQ227465.

2.8. PGPR assisted phytoremediation of petroleum contaminated soil

To evaluate the effect of the isolate *Klebsiella* sp. D5A on the growth of tall fescue and petroleum-removal efficiency in the saline-alkaline soil, a pot experiment was conducted in growth chambers. The experiment consisted of 3 treatments in 4 replicates. The treatments were (1) no-plant control, (2) tall fescue and (3) tall fescue inoculated with *Klebsiella* sp. D5A (tall fescue + D5A). Plastic pots were filled with 2.0 kg air-dry soil, and water content of the soil was maintained at about 80% field capacity during the experimental period. Inorganic nutrients $(\text{NH}_4)_2\text{SO}_4$ and K_2HPO_4 was added to all treatments to give a final rates of 250 mg N kg^{-1} and 100 mg P kg^{-1} . Other nutrients in the soil were at the adequate range and thus were not added.

Uniform seeds were surface sterilized by immersing in 0.3% sodium hypochlorite for 5 min and rinsing with sterilized distilled water. An aliquot of 50 seeds were then sown in each pot of the planted treatments. For the tall fescue + D5A treatment, the strain D5A at the exponential growth phase in the LB medium were collected by centrifugation at 9000 rpm for 15 min at 4 °C, washed with sterile distilled water and centrifuged. The inoculum was prepared by re-suspending pelleted cells in sterile distilled water and bacterial suspension (10 mL pot^{-1}) was mixed with the soil before sowing seeds. Plants were harvested at 120 d.

2.9. Plant analysis

Plants were harvested on day 120. Plant biomass was determined after oven dried at 70 °C for 48 h. RA was determined using the triphenyl tetrazolium chloride method (Clemensson-Lindell

1994). The root activity was expressed as μg triphenyl tetrazolium formazane $\text{g}^{-1} \text{h}^{-1}$ on a fresh-weight basis.

Chlorophyll concentration was measured according to Moran and Porath (1980). Two hundred milligrams of plant leaves were cut into 0.5 cm segments and incubated in 80% acetone for 24 h, at 4 °C, in the dark. Absorbance of the solutions was measured with a spectrophotometer at 645 and 663 nm. Chlorophyll concentrations (mg mL^{-1}) were calculated using the following equations (Moran and Porath, 1980):

$$\text{Chlorophyll} = [8.02 \times A_{633}] - [20.2 \times A_{645}] \quad (1)$$

2.10. Statistical analysis

All experiments were conducted in four replicates or otherwise specified. The data collected were analyzed statistically using SPSS 16.0 software. Duncan's multiple range tests were used to compare the means of the treatments, variability in the data was expressed as the standard deviation, and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. PGP features of the isolates

A total of 115 rhizobacteria which could use ACC as a sole nitrogen source on the ADF plate were isolated from five rhizosphere soil samples and tested qualitatively for ACC deaminase activity. Of these, 5 strains shown ACC deaminase activity $>1.0 \text{ M } \alpha\text{-KB mg}^{-1} \text{h}^{-1}$ were selected for further study. The potential PGP characters of the selected strains were evaluated *in vitro* based on phosphate solubilization activity, and production of siderophore and phytohormone (IAA) in chemically defined media. IAA production was observed in 5 isolates in the range 8–112 mg L^{-1} , phosphate solubilization activity in the range 31–131 mg L^{-1} and all 5 isolates produced siderophore. Apart from siderophore production, isolate D5A exhibited the highest level in ACC deaminase activity, phosphate solubilization and IAA production (Table 1).

3.2. PGP ability of isolates on tall fescue seedlings under salt stress

The effects of the isolates on seed germination rate, root length and shoot height of tall fescue under different salt stresses were summarized in Table 2. Without inoculation, increasing salinity level decreased the germination rate, shoot height and root length with root growth being most sensitive. The inoculation of PGPR isolates increased root length and shoot height when NaCl was added. Among the isolates, D5A showed the best plant growth-promoting ability. The inoculation of D5A increased germination rate, root length and shoot height by 6%, 12% and 3% under no NaCl, 14%, 17% and 11% under 3 g kg^{-1} NaCl, 13%, 43% and 29% under 6 g kg^{-1} NaCl, and 109%, 29% and 54% under 9 g kg^{-1} NaCl, respectively (Table 2).

3.3. Morphological characteristics and phylogenetic position of isolate D5A

The colonies of strain D5A are translucent, smooth and convex on solid ADF plates. Cells are Gram-negative, rod-shaped (0.7–0.9 μm thick and 1.8–3.0 μm long) (figure not shown). Physiological and biochemical tests indicated D5A to be aerobic, positive for starch utilization, glucose fermenters, indole production, Voges-Proskauer test, but negative for gelatin hydrolysis and citrate positive. 16S rRNA gene sequences (GenBank accession No. JQ227465) of D5A were also used to identify the strain. In the phylogenetic

Table 1

Traits of bacteria containing ACC deaminases isolated from the petroleum-contaminant soil.

Strains	ACC deaminase activity (M α -KB mg ⁻¹ h ⁻¹)	IAA (mg L ⁻¹)	Phosphate solubilization (mg L ⁻¹)	Siderophore production (A/Ar)
D1A	2.40 ± 0.35b	110 ± 6a	31 ± 2c	+
D5A	5.04 ± 0.12a	112 ± 9a	131 ± 5a	++
S1C	2.28 ± 0.17b	8 ± 1d	83 ± 6b	++++
W2	2.22 ± 0.09b	90 ± 4b	34 ± 2c	+
W50	1.20 ± 0.09c	62 ± 4c	36 ± 3c	+

A/Ar 0–0.2, +, +, +, +; 0.2–0.4, +, +, +, +; 0.4–0.6, +, +, +; 0.6–0.8, +, +; 0.8–1.0, +. The data represent the mean ± standard deviation of three replicates, and data followed by different letters in the same row indicate a significant difference at $p < 0.05$ according to Duncan's multiple range tests.

Table 2Effects of PGPR on the growth of tall fescue seedlings grown for 10 d in the Petri dish containing 0–9 g kg⁻¹ NaCl.

	Germination rate (%)				Shoot height (cm)				Root length (cm)			
	0	3	6	9	0	3	6	9	0	3	6	9
Control	92 ± 6a	83 ± 3bc	78 ± 29bc	35 ± 0b	6.7 ± 0.2c	6.1 ± 0.1c	3.7 ± 0.4c	2.8 ± 0.2b	3.6 ± 0.2a	2.9 ± 0.1bc	1.1 ± 0.1bc	0.9 ± 0.1c
D1A	95 ± 5a	92 ± 6ab	92 ± 10a	43 ± 6c	7.2 ± 0.1ab	6.8 ± 0.1b	5.6 ± 0.3a	3.1 ± 0.1 ab	3.6 ± 0.1a	3.1 ± 0.1ab	1.4 ± 0.5b	1.1 ± 0.1b
D5A	97 ± 3a	95 ± 0a	88 ± 13b	73 ± 3ba	7.5 ± 0.1a	7.1 ± 0.1a	5.2 ± 1.2ab	3.6 ± 0.2a	3.7 ± 0.1a	3.2 ± 0.1a	1.8 ± 0.2a	1.3 ± 0.2a
W50	95 ± 5a	83 ± 3bc	77 ± 6bc	40 ± 9bc	7.0 ± 0.4abc	6.8 ± 0.1b	4.3 ± 0.4bc	2.9 ± 0.7b	3.2 ± 0.2a	2.7 ± 0.2bc	1.3 ± 0.2bc	1.1 ± 0.1b
S1C	93 ± 8a	87 ± 10abc	47 ± 8d	42 ± 3b	7.0 ± 0.3bc	6.8 ± 0.2b	3.1 ± 0.4c	2.8 ± 0.2b	3.6 ± 0.1a	2.8 ± 0.2abc	1.0 ± 0.1c	1.1 ± 0b
W2	95 ± 5a	80 ± 5a	73 ± 3c	32 ± 3c	6.9 ± 0.3bc	5.9 ± 0.2c	4.2 ± 0.8bc	2.0 ± 0.2c	3.6 ± 0.1a	2.4 ± 0.1c	1.3 ± 0.1bc	0.8 ± 0.1c

The data represent the mean ± standard deviation of three replicates, and data followed by different letters in the same row indicate a significant difference at $p < 0.05$ according to Duncan's multiple range tests.

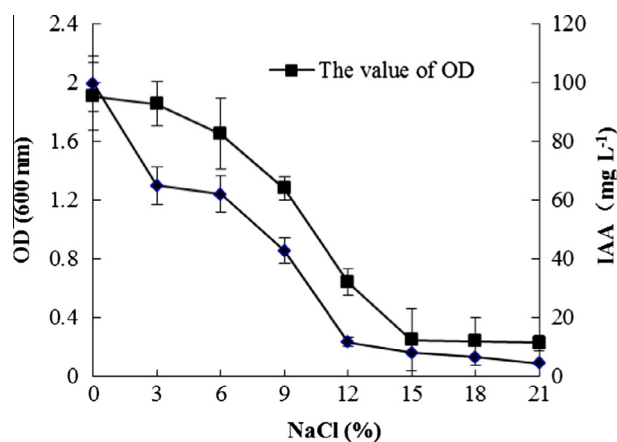


Fig. 1. The biomass and IAA production of D5A strains cultured in the LB medium with various salt concentrations. Error bars are \pm s.e. ($n = 3$).

tree (data not shown), the strain D5A in the *Klebsiella* branch exhibited highest (99.5%) sequence similarity to *Klebsiella variicola* (HQ259961). This strain has been deposited in Chinese Culture Collection Management Committee General Microbiology Center (CGMCC) and the number of the strain is CGMCC No. 7248.

3.4. Response of D5A to salinity and pH

The growth of strain D5A, based on OD_{600nm} values obtained at 24 h, was not affected by NaCl up to 6% and only decreased by 33% at 9% NaCl (Fig. 1), indicating that D5A was highly tolerant to salinity. IAA production was linearly suppressed with increasing salt content from 0.5% to 15%. In general, both the growth and IAA production were not affected by pH between 4 and 10 (Fig. 2), suggesting that the strain could adapt to a wide range of pH.

3.5. Resistance of D5A to alkalinity stress

The OD_{600nm} value of strain D5A was hardly affected by NaHCO₃ up to 100 mM and then decreased when NaHCO₃ concentration exceeded 100 mM. Nevertheless, the OD_{600nm} value was still as

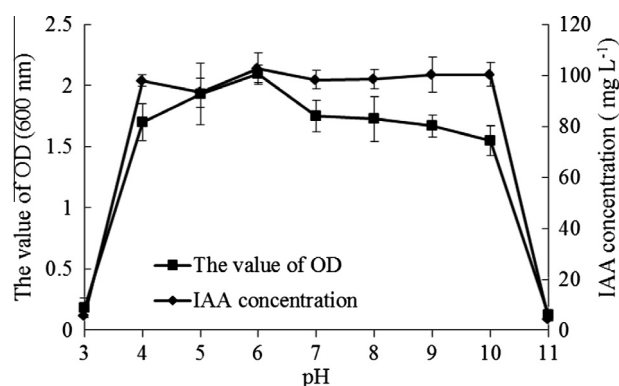


Fig. 2. The biomass and IAA production of D5A strains in the LB medium with different pH. Error bars are \pm s.e. ($n = 3$).

high as 1.03 even at 200 mM (pH 7.68). In comparison, the OD_{600nm} value of strain D5A continuously declined with increasing Na₂CO₃ in the range of 0–50 mM. There was no obvious growth after exposure to 250 mM NaHCO₃ (pH 7.72) or 50 mM Na₂CO₃ (pH 9.93). As for the 1:1 mixture of NaHCO₃ and Na₂CO₃, the strain was virtually undetectable even at the concentration of 75 mM.

3.6. Influence of D5A on plant growth and TPH removal

The inoculation of D5A significantly improved seed germination, biomass production and chlorophyll contents in leaves of tall fescue grown in the petroleum-contaminated saline-alkaline soil (pH 9.7 and EC 404 μ S cm⁻¹). Shoot dry weight was almost 3 times greater in the inoculated than non-inoculated treatment by the end of the experiment (Table 3). The inoculation also increased root dry weight, RA and chlorophyll contents by 73%, 101% and 170%, respectively.

The TPH contents in the soil after 120 d decreased by 42%, 50% and 66% in the non-plant control, tall fescue and tall fescue + D5A treatments, respectively. The content of TPH was significantly lower ($P < 0.05$) in two treatments with plants than the control, and the inoculation of D5A further decreased TPH contents. As is

Table 3

Effect of D5A inoculation on TPH concentration in soil, shoot and root dry weights, root activity and chlorophyll in leaves of tall fescue grown for 120 d in the petroleum-contaminated soil.

	No-plant control	Tall Fescue	Tall Fescue+D5A
TPH concentration (mg kg ⁻¹)	9793 ± 286a	8415 ± 464b	5700 ± 433c
<C12 concentration (mg kg ⁻¹)	ND	ND	ND
C12–C16 concentration (mg kg ⁻¹)	277 ± 32a	195 ± 10b	105 ± 15c
C16–C21 concentration (mg kg ⁻¹)	1307 ± 53a	1145 ± 128b	795 ± 72c
>C21 concentration (mg kg ⁻¹)	8209 ± 210a	7075 ± 330b	4800 ± 346c
Shoot dry weight (g pot ⁻¹)	–	0.91 ± 0.11b	3.67 ± 0.97a
Root dry weight (g pot ⁻¹)	–	1.51 ± 0.35b	2.61 ± 0.25a
Chlorophyll contents (mg g ⁻¹ f. wt.)	–	1.47 ± 0.15b	3.97 ± 0.28a
Root activity (μg h ⁻¹ g ⁻¹ f. wt.)	–	721 ± 73b	1446 ± 47a

The data represent the mean ± standard deviation of four replicates, and data followed by different letters in the same row indicate a significant difference at $p < 0.05$ according to Duncan's multiple range tests. ND, not detectable; –, not applicable.

expected, the fraction below C12 was not detectable and over 80% of TPH were >C21 fractions in all treatments given the soil used in this experiment was contaminated for many years and the lighter fraction are easily removed by evaporation and degradation (Table 3).

4. Discussion

ACC deaminase, IAA and siderophore production, and phosphate solubilization are important contributors to plant growth (Glick 1995). Thus, these characters were used to isolate PGPR. Over one hundred rhizobacteria which could use ACC as a sole nitrogen source on the ADF plate were obtained and five most promising strains with higher activity of ACC deaminase were further evaluated for their ability to produce IAA and siderophores (Table 1). Among these, the isolated D5A showed high PGP activities and the best growth-promoting ability (Table 2).

On the basis of morphological, physiological, biochemical characteristics, phylogenetic position and genes sequences of 16S rRNA, the isolated strain D5A was identified as *Klebsiella* sp. This study demonstrated that D5A was highly tolerant to extreme pH, high salinity and high alkalinity, and thus has potential to develop as a PGPR inoculum for improving plant growth and TPH removal in highly saline-alkaline soils. The results showed that the isolate D5A was able to grow and produce a high amount of IAA even in the presence 9% NaCl (Fig. 1). The isolate D5A was capable of producing a large amount of mucous and exopolysaccharides (data not shown) which bind cations including Na⁺ and decrease the content of Na⁺ available for plant uptake, thus helping alleviate salt stress in plants (Ashraf et al., 2004). Extreme pH values may be one of the major limiting factors for the presence of microorganisms in soils. D5A kept optimal growth and producing IAA at the pH range from 4 to 10 (Fig. 2). In contrast, common PGPR have a narrow range of optimal pH (Shrivastava, 2013). This indicates that the isolate could also be a strong candidate for the improvement of plant growth in highly alkaline or acidic soils. Compared with Cl⁻, HCO₃⁻ and CO₃²⁻ at same pH are more toxic for organisms. Unfortunately, the chemical properties of saline-alkaline soils are related tightly to the presence of Na₂CO₃ and NaHCO₃ in the soil (Vorob'eva and Pankova, 2008). Although many studies have examined the Na₂CO₃ tolerance of plants, little is known about the resistance of bacterial strains to Na₂CO₃. In this study, Strain D5A could grow well in 25 mM Na₂CO₃ (OD_{600nm} = 1.375) (Fig. 3) while plant growth could be seriously affected when the Na₂CO₃ concentration in soil solution reached 5 mM (Lu et al., 2010). So D5A might be used to alleviate the saline-alkaline stress on plants and to improve the efficiency of phytoremediation in saline-alkali soils.

The degradation of most hydrocarbons is believed to enhance through a rhizosphere effect. Our present study showed that, as

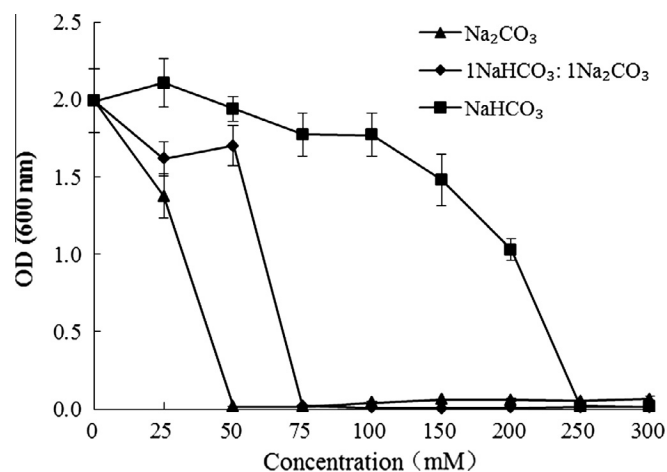


Fig. 3. Different concentration of Na₂CO₃ and NaHCO₃. Error bars are \pm se ($n = 3$).

expected, salt stress decreased shoot growth and root length of tall fescue. However, the inoculation of PGPR strains, especially D5A, improved all plant growth parameters in the salt treatments in a Petri dish. The pot experiment also showed that the inoculation of D5A increased root biomass by up to 73% and RA by up to 100% and TPH removal efficiencies by 16% in the petroleum-contaminated saline-alkaline soil.

5. Conclusion

In this study, a total of 115 PGPR were isolated from the rhizosphere of tall fescue grown in the petroleum-contaminated saline-alkali soils. Among them, the isolate D5A showed the highest PGP activities and growth-promoting ability. It also had a good adaptability to extreme pH and high salinity and alkalinity levels. The D5A was identified as *Klebsiella* sp. The inoculation of this isolate enhanced the phytoremediation efficiency by further 16% and also promoted the growth of host plants in the petroleum-contaminated saline-alkaline soil. Thus, the D5A can be used as a potential inoculum to improve phytoremediation of the organic contaminants in petroleum-contaminated saline-alkaline soils.

Acknowledgements

We thank Jiangsu Provincial Natural Science Foundation of China (BK2012891), National High Technology Research and Development Program of China (863 Program) (2012AA06A204) and the National Natural Science Foundation of China (41001182) for financial support.

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