Chemosphere 117 (2014) 303–308

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Technical Note

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

Wuxing Liu [a,*], Jinyu Hou [a,b], Qingling Wang [a], Linlin Ding [a], Yongming Luo [a,c]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

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-l-carboxylic acid deaminase activity >1.0 M α-KB mg^{-1} 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.

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 phytoremediation 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 (*Testuca 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

* Corresponding author. Tel.: +86 25 86881842; fax: +86 25 86881126.
E-mail address: liuwuxin@issas.ac.cn (W. Liu).

http://dx.doi.org/10.1016/j.chemosphere.2014.07.026
0045-6535/

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-l-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 $\mu S\ cm^{-1}$; cation exchange capacity 4.94 $cmol\ kg^{-1}$; 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 16 920 $mg\ kg^{-1}$. The petroleum fractions of C12–C16, C16–C21 and >C21 were 441, 2720 and 13 760 $mg\ kg^{-1}$, 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 $(NH_4)_2SO_4$) 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 M α-KB $mg^{-1}\ h^{-1}$, 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 (Ar) was determined by the above method. The quantitative index was the value of A/Ar which was inversely related to siderophore production. General reference standards: A/Ar 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 (OD_{600nm} = 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-