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REGULAR ARTICLE

Collection and analysis of root exudates of *Festuca arundinacea* L. and their role in facilitating the phytoremediation of petroleum-contaminated soil

Wuxing Liu · Jinyu Hou · Qingling Wang · Huijuan Yang · Yongming Luo · Peter Christie

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Abstract
Background and aims The objectives of this study were to elucidate the mechanisms of interaction between root exudates of tall fescue and functional bacteria associated with petroleum degradation and whether components of the exudates can enhance petroleum removal from soil.
Methods Root exudates of tall fescue were collected through a continuous root exudate trapping system and identified by GC-MS. Chemotaxis, swarming, and in vitro assay were conducted to assess the effects of the organic acids of root exudates on *Klebsiella* sp. D5A (plant growth promoting rhizobacterium), *Pseudomonas* sp. SB (biosurfactant producing bacterium), and *Streptomyces* sp. KT (petroleum-degrading bacterium). A pot experiment with organic acid amendment was conducted to study the effects of these components of root exudates on petroleum remediation. Microbial physiological metabolisms affected by organic acids were tested using Biolog Eco plates.
Results Palmitic acid was found to be most effective in promoting D5A colonization on tall fescue. ρ-Hydroxybenzoic and palmitic acids significantly stimulated the growth of strains D5A, SB, and KT. Furthermore, palmitic acid amendment significantly enhanced petroleum removal in pot experiment.
Conclusions Palmitic acid was the critical organic acid to facilitate petroleum removal during phytoremediation. These findings provide insight into the mechanisms by which tall fescue enhances the degradation of petroleum.

Keywords *Festuca arundinacea* L. · Root exudates · Functional bacteria · Petroleum phytoremediation

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Introduction

Petroleum hydrocarbons are some of the most universally detected organic pollutants in the environment because of the common industrial use of petroleum products worldwide. The large scale and economic importance of this contamination has resulted in a determined effort in developing remediation technologies for environmental clean-up. Phytoremediation is considered to be a promising and cost-effective method to enhance the bioremediation of polluted soils. Over the last few years, decreases in soil contaminants in the

presence of plants have been reported (Cunningham et al. 1995; Schnoor et al. 1995; Usarla et al. 2002; Agamuthu et al. 2010; Liu et al. 2013). Phytoremediation can use several different mechanisms for remediating contaminants including phytoaccumulation/phytoextraction, phytostabilization, and rhizosphere degradation (Batty and Dolan 2013). Unlike phytoremediation of heavy metals by extracting contaminants and accumulating them above-ground, the primary mechanism of phytoremediation of petroleum-contaminated soils is the provision of a biologically active soil region (i.e., the rhizosphere) by plant root systems which can encourage microbial degradation by stimulating the growth of related functional bacteria (Abhilash and Singh 2010).

The rhizosphere effect influences phytoremediation in several ways. Growing roots improve physicochemical and biological soil conditions by assisting aeration and water retention. In addition, root growth increases the bioavailability of organic pollutants through the desorption of hydrocarbons from soil micropores (Khan et al. 2013). Plant roots also modify microbial habitats to enhance bacterial populations, diversity, and activity (Meng and Zhu 2010), and consequently, the removal of hydrocarbons. There is a body of evidence to suggest that plant roots influence the rhizosphere microenvironment by the release of root exudates (Segura and Ramos 2013) which play an important role in selectively fostering a specific microbial community from the pool of soil microbes in the rhizosphere (Dennis et al. 2010; Hartmann et al. 2009; Zhang et al. 2014). A previous study found that application of selected plant secondary metabolites (naringin, caffeic acid, and limonene) resulted in large differences in bacterial community structure between the amendment with natural compounds and a control with no amendment (Uhlik et al. 2013).

Organic acids are important components of root exudates and have received considerable attention due to their role in providing substrates for microbial metabolism and serving as intermediates for biogeochemical reactions in soils (Shukla et al. 2013). In addition, several studies have been conducted on their effects on phytoremediation under different environmental stresses (Sun et al. 2010; Phillips et al. 2012). For example, it was found that linoleic acid, a common component present in relatively high proportions in plants used in phytoremediation, can stimulate the degradation of polycyclic aromatic hydrocarbons (PAHs; Yi and Crowley 2007). Similar studies have also addressed this issue by adding root-derived substances as simulated rhizodeposits to enhance PAHs degradation (Tejeda-Agredano et al. 2013). Recent research has focused mainly on the influence of whole original root exudates on amendment shifts in microbial community structure, degradative microbial populations, and removal rates of pollutants. However, the interactions between plant root exudates and microbial degradation processes remain poorly understood.

Our previous studies found that the perennial grass tall fescue (*Festuca arundinacea* L.) has a high capability to remove petroleum contaminants and to resist saline–alkaline-contaminated soils, especially when inoculated with plant growth promoting bacteria (PGPR; Liu et al. 2014). This species has been commonly used for phytoremediation and has been shown to be effective in enhancing the dissipation of organic pollutants (Ho et al. 2007). Other properties of the root exudates of tall fescue remain to be explored and this species was, therefore, chosen as the test species in the present study.

The aims of the present work were (1) to collect and determine the organic acids in root exudates of tall fescue in undisturbed conditions, (2) to study the interactions between organic acids from tall fescue roots and functional bacteria associated with petroleum degradation, and (3) to determine the impact of organic acid amendment on petroleum removal and the soil microbial community.

Materials and methods

Chemicals and bacterial strains

XAD-4 resin, benzoic acid, ρ -hydroxybenzoic acid, palmitic acid, and stearic acid were purchased from Sigma. All other reagents were ACS grade or better.

Klebsiella sp. D5A, *Pseudomonas* sp. SB, and *Streptomyces* sp. KT were obtained from the Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. They had been deposited in the Chinese Culture Collection Management Committee General Microbiology Center (CGMCC) and the accession numbers are CGMCC Nos.7246, 7248, and 9610, respectively.

Collection of root exudates

The apparatus used to collect tall fescue root exudates was adapted from other studies (Tang and Young 1982) and is shown in Fig. 1. Quartz sand was used with Hoagland nutrient solution. Prior to charging the glassware with quartz and the joint with glass wool, white quartz sand of 0.5–1 mm granule diameter and glass wool were soaked in 2 M HCl for 24 h, flushed with deionized water, dry heat sterilized at 170 °C for 2 h, and rinsed in sterile saturated $CaSO_4$ solution before use. XAD-4 neutral exchange resin used to fill the resin column was rinsed with deionized water several times, placed in a Soxhlet extractor, extracted for 24 h with acetone, flushed with distilled methanol for 24 h to remove any remaining acetone, and preserved in methanol under low light and low temperature before transfer into glass columns with sufficient glass wool inserted above and below.

Seeds of tall fescue were sterilized (75 % ethanol, 1 min, and 0.1 % mercury (I) chloride, 5 min), germinated in sterile Petri dishes for 7 days and then 50 seedlings transferred to the apparatus in a phytotron (daytime temperature 25 °C, night temperature 16 °C) unconnected to the resin column. Fifteen days later, the resin column was connected to the glassware to collect the exudates and the collection system was placed in a plant growth chamber (30±2 °C, 16 h light/8 h dark). Culture medium was passed through the apparatus at a rate of 5 mL min^{-1}. Water loss was compensated for every day and exudates were collected continuously for 2 days. The compounds were eluted with spectral-grade methanol and evaporated with a vacuum rotary evaporator at 45 °C until dry. Dried compounds were dissolved in 2 mL CH_2Cl_2 and 1 mL dissolved compounds lyophilized. Two hundred fifty microliters salinization solution (BSTFA to pyridine ratio, 5:1) was added to each dried specimen and sealed with a lid. The solution was heated in a water bath between 75 and 80 °C for 1 h, filtered through a 0.45-μm membrane filter and analyzed by GC-MS (Wang et al. 2011).

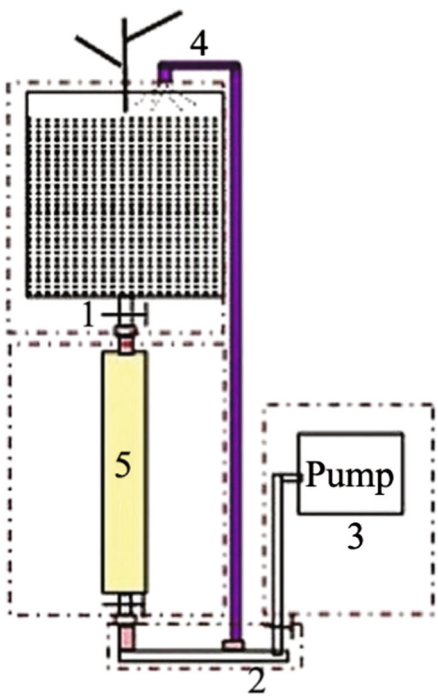

Fig. 1 Cycling device for collection of root exudates. *1* Glassware; *2* Connection tube; *3* Air pump; *4* Water sprinkler; *5* Resin

Examination of root exudates

GC-MS analysis of the silylated extracts was performed on a fused silica column (DB-5) in a GC ultra gas chromatograph. An Aglient 7890N GC system and Agilent 5795B series MSD equipped with G6500-CTC autosampler was used. The gas chromatographic conditions were as follows: helium gas was used as a carrier gas at a flow rate of 1.0 mL min^{-1}; oven initial temperature 50 °C, 30 °C min^{-1} up to 200 °C, 6 °C min^{-1} up to 250 °C, and 30 °C min^{-1} up to 270 °C. Injections of 1 μL were made in a splitless mode. The mass spectrometric detector with ion trap analyzer Thermo Electron Polaris Q was set as follows: electron impact mode with ion source temperature set at 230 °C, transmission line temperature at 280 °C, analyzed mass interval *m/z* 60–640, and mass spectra were generated at 70 eV. Spectrum acquisition was realized 10 min after injection in order to avoid saturation of the detector. Compounds are tentatively identified ($P \geq 95$ % match) on the basis of the NIST98 Mass Spectral Library.

Chemotaxis assay

A capillary assay was performed according to the method of Ling et al. (2011). *Klebsiella* sp. D5A was grown in LB medium until the log phase ($OD_{600\ nm}$ of 0.6). The assay was comprised a 200-μL pipette tip as a space for holding 100 μL bacterial suspensions in LB medium. A 4-cm 25-gauge needle (Becton-Dickinson) used as the chemotaxis capillary was attached to a 1-mL syringe containing 200 μL of the compound (organic acids at

different concentrations were introduced to the syringe separately in LB liquid medium) and LB liquid medium (control). After incubation for 2 h at room temperature, the needle syringe was removed from the bacterial suspension. Then the contents were diluted and plated on LB medium plate. Accumulation of bacteria in the capillaries was calculated as the average from the colony-forming units (CFUs) obtained in triplicate plates and the results were expressed as the mean of three separate capillary assays for each treatment. The relative chemotaxis index (RCI) was calculated as the ratio of the accumulation value for the bacteria that entered the test capillary to the corresponding value for the control capillary. An RCI of 2 or greater is considered as positive chemotactic response (Mazumder et al. 1999). The experiment was conducted in four replicates.

Swarming assay

Klebsiella sp. D5A and *Pseudomonas* sp. SB strains were incubated in LB liquid culture on a shaker at 28 °C and 150 rpm. Bacterial cells reaching the logarithmic growth phase ($OD_{600\ nm}$ 0.4) were centrifuged for 3 min at 6,000 rpm and 4 °C and resuspended in 2 mL 50 mM sterile phosphate buffer. *Streptomyces* sp. KT grew in actinobacteria culture medium (Kong et al. 2008) and the spores were washed with sterile phosphate buffer (pH 6.8) to a final concentration of 10^8 CFUs mL^{-1}.

Migratory agar plates were prepared with 30 μM organic acid standard sample on a 1.0 % agar plate. Plates were dried on a cleaning bench for 30 min. Five microliters bacterial suspension loaded onto sterile paper was placed in the center of each plate. The bacteria were incubated at 28 °C for 3 days. Colony diameters were measured in three directions on each plate with four replicates. The results are expressed as the average of four separate assays for each determination.

In vitro assay

An in vitro assay was performed in glass tubes (6 cm in diameter and 20 cm in height) containing 80 mL of solid nutrient medium containing 0.8 % agar. The solid nutrient medium was prepared according to Ling et al. (2011). Four germinated seeds of tall fescue were sown in the nutrient medium in the center of each glass tube for 4 days in a growth chamber at 28 °C with a 16-h light regime. Then 10 μL of bacterial suspension ($OD_{600\ nm}$ 0.4) was injected into the nutrient medium at a point located 1.5 mm from the plant roots and 20 μL aliquots of different organic acid solutions (30 mM) were dropped onto the plant roots. Sterile water (20 μL) was dropped onto the plant roots as control. After an additional 2 weeks of incubation under the same conditions, the roots were sampled to count the number of bacterial cells on the root surfaces. All the materials used in the in vitro assay were sterilized. The germinated seeds were sterilized as previously described and the organic acid solutions were sterilized by filtration (0.22 μm). To count the number of bacterial cells, root tissues (50 mg of fresh weight basis) of tall fescue were rinsed with sterile water and homogenized in 1 mL of sterile water with a tissue grinder. The suspension was then serially diluted in sterile water and plated on LB solid medium to conduct bacterial cell counts. Each experiment was conducted in triplicate.

Pot experiment

Aged-contaminated soil (available nitrogen, 34.0 mg kg^{-1}; available P, 28.0 mg kg^{-1}; pH 8.7; salt content, 0.17 %; and total petroleum hydrocarbon concentration, 3,075 mg kg^{-1}) obtained near an individual oil production well at Shengli oilfield, Shandong province, north China was air dried, passed through a 2-mm sieve. Inorganic nutrients $(NH_4)_2SO_4$ and K_2HPO_4 were added to all treatments to give final rates of 250 mg N kg^{-1} and 100 mg P kg^{-1}. Five treatments with four replicates were set up using glass jars containing 200 g petroleum-contaminated soil amended with different organic acids (benzoic acid, ρ-hydroxybenzoic acid, palmitic acid, and stearic acid) and diethyl ether as control. Organic acid dissolved in 5 mL diethyl ether was added to the soil to a final concentration of 500 mg kg^{-1} and the same volume of diethyl ether applied to the control soils every week. Soil moisture content was maintained regularly at 50 % field capacity with deionized water during the study period by weighing every day. Soil samples were incubated at 30 °C and 95 % relative humidity for 4 weeks. Total petroleum hydrocarbon was extracted ultrasonically and determined by FT-IR according to the method of Liu et al. (2013). Biolog Eco™ plates (Biolog, Inc., Hayward, CA, USA) containing 31 different C sources were used to determine the bacterial community metabolic activity based on carbon source utilization. The soil samples (5×*g* d.w.) were shaken with 45 mL of

autoclaved-sterilized 0.85 % NaCl solution for 30 min and then brought to a final dilution of 10^{-3}. An aliquot of 150 μL soil suspensions was inoculated into each plate well. The plates were read every 12 h ($OD_{590\ nm}$) over 240 h using a Biolog automated plate reader. Average well color development (AWCD) was calculated as described by San Miguel et al. (2007). Thirty-one carbon sources were organized into five groups referring to Weber and Legge (2009): carbohydrates, carboxylic and acetic acids, amino acids, amines/amides, and polymers to measure species metabolic diversity. AWCD changes in each group referred to functional shifts in the bacterial community. A single time point absorbance at 144 h, when the optical density did not increase for the highest number of wells over all plates, was used for well comparisons. Total petroleum hydrocarbon (TPH) degraders of soil were enumerated by the most-probable-number (MPN) technique (Wrenn and Venosa 1996).

Statistical analysis

One-way analysis of variance for independent samples was performed using the SAS 9.1 software package. Treatments means were compared by the least significant difference at P=0.05.

Results

Identification of organic acids in tall fescue root exudates

Analysis of organic acids from tall fescue by GC-MS full scan was performed. In all root exudate samples, benzoic acid, ρ-hydroxybenzoic acid, palmitic acid, and stearic acid formed the dominant composition of the organic acids in tall fescue root exudates (Fig. 2). Some other peaks appeared in the mass spectrum of tall fescue root exudates, but in most cases, they showed low similarities with the standards. Also the molecular weight provided by MS could not confirm the compounds in the corresponding unknown peaks. More standards may aid in future analysis.

Effects of different organic acids at different concentrations on chemotaxis of *Klebsiella* sp. D5A

Capillary-like chemotaxis experiments were adopted to detect chemotaxis behaviors of *Klebsiella* sp. D5A and chemotactic responses to different organic acids of *Klebsiella* sp. D5A represented by RCI are shown in Table 1. Significant chemotactic responses were observed in all the organic acid treatments at concentrations greater than 20 uM and reached a maximum at 30 μM compared with LB liquid medium (control). In addition, the chemotactic response of *Klebsiella* sp. D5A towards palmitic and benzoic acids was higher than the response to the other acids.

Swarming motility toward different organic acids of *Klebsiella* sp. D5A, *Pseudomonas* sp. SB, and *Streptomyces* sp. KT

This experiment examined swarming of petroleum degradation associated functional bacteria under the induction of different organic acids. In the case of *Klebsiella* sp. D5A, ρ-hydroxybenzoic acid,

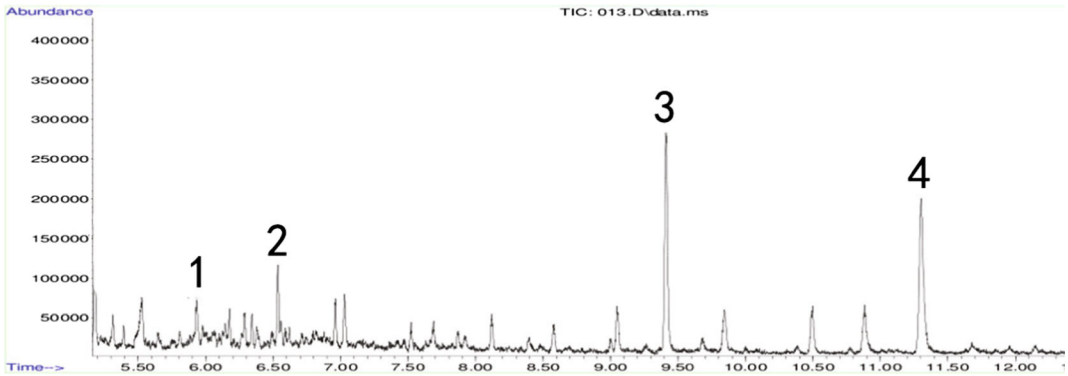

Fig. 2 GC mass spectrum of *Festuca arundinacea* root exudates

Table 1 Chemotactic response of *Klebsiella* sp. D5A towards various organic acids with different concentration evaluated by capillary assay

	Organic acid concentration (μM)				
	0	10	20	30	40
ρ-Hydroxybenzoic acid	1.00±0.11 c	2.51±0.38 ab	2.06±0.55 b	2.98±0.63 a	2.18±0.49 ab
Benzoic acid	1.00±0.11 d	1.63±0.81 cd	3.26±0.76 b	6.99±0.30 a	2.63±0.49 bc
Palmitic acid	1.00±0.11 d	1.28±0.15 d	6.69±0.11 b	10.15±0.57 a	3.76±0.49 c
Stearic acid	1.00±0.11 d	1.18±0.16 d	3.11±0.34 c	4.91±0.26 a	4.01±0.53 b

Relative chemotaxis index (RCI) was calculated as the ratio of the accumulation value for the bacteria that entered the pipette to the corresponding value for the control pipette. An RCI of 2 or greater is considered significant for this method. Each treatment contained four replicates. Data were expressed as mean±standard error

stearic acid, and the control did not have any significant effect but benzoic acid and palmitic acid yielded a high induction effect on bacterial swarming. Diameters of bacterial circles corresponding to benzoic acid were 1.58 times than that of the control and the diameters of bacterial circles corresponding to palmitic acid were 1.98 times than that of the control (Fig. 3). In *Pseudomonas* sp. SB, ρ-hydroxybenzoic acid and palmitic acid showed significant induction of swarming motility compared with other organic acids, and the control and the swarming diameters of bacterial circles were 1.48 times and 1.27 times than that of the control (Fig. 3). In *Streptomyces* sp. KT, the diameters of bacterial circles of palmitic acid treatments were also significantly larger than that of the control (Fig. 3).

Colonization of *Klebsiella* sp. D5A in tall fescue rhizosphere amended with different organic acids

The ability of the organic acids in inducing recruitment of *Klebsiella* sp. D5A to the tall fescue root surface was also assessed (Fig. 4). Palmitic acid significantly increased the number of *Klebsiella* sp. D5A on the surface of the roots compared with the control. However, benzoic acid and stearic acid significantly decreased recruitment in this experiment. There was no significant difference between control and ρ-hydroxybenzoitic acid.

Petroleum removal as affected by organic acid amendment

Organic acids were added to contaminated soil to partly mimic the rhizosphere environment during

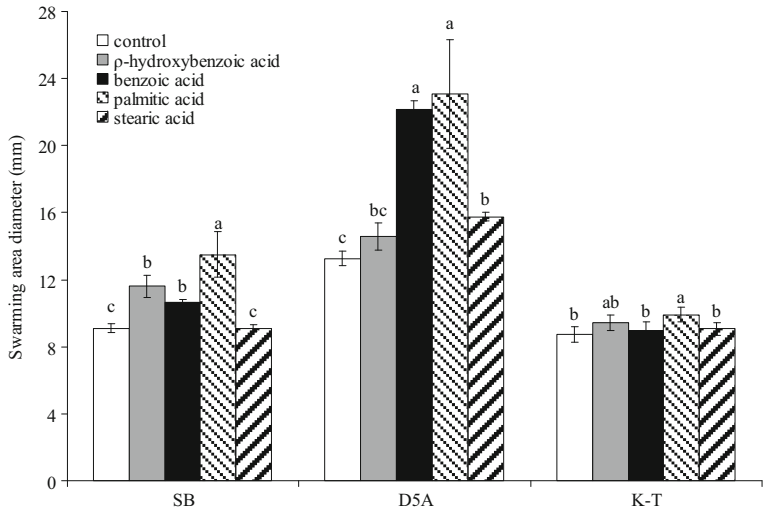

Fig. 3 Swarming area diameters of *Klebsiella* sp. D5A, *Pseudomonas* sp. SB, and *Streptomyces* sp. KT induced by different organic acids on agar plates

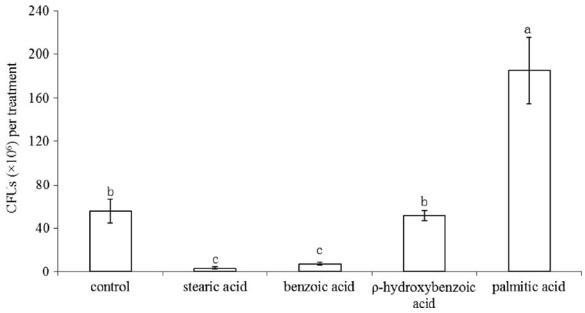

Fig. 4 Influence of different organic acids on colonization of tall fescue roots by *Klebsiella* sp. D5A

phytoremediation as a check for the key organic acid involved in the petroleum degradation process. Soil amended with ρ-hydroxybenzoic acid, benzoic acid, and palmitic acid significantly increased petroleum removal by 7.8, 14.9, and 19.2 %, respectively, compared with the control. However, stearic acid slightly inhibited petroleum removal (Fig. 5).

Impact of organic acids on the TPH degraders and biological function of the soil microbial community

The TPH degraders ($\times 10^7$ MPN g^{-1}) in soil amended with ρ-hydroxybenzoic acid, benzoic acid, palmitic acid, stearic acid, and control are 3.55, 5.35, 6.43, 1.04, and 1, respectively, after 4 weeks of the pot experiment. Of these, benzoic acid and palmitic acid addition significantly increased the counts of TPH degraders in the soil compared with the control. Furthermore, palmitic acid and ρ-hydroxybenzoitic acid also had significant positive effects compared with the other treatments on the substrate versatility of the soil bacterial community with a higher average well-color development of 31 carbon resources and some carbon guilds (Fig. 6a, b). The utilization pattern of amino acids described by AWCD was similar to that of 31 carbon sources with palmitic acid and ρ-hydroxybenzoitic acid showing significantly higher community level metabolic activity. In the amines/amides group, the AWCD followed the sequence palmitic acid > ρ-hydroxybenzoitic acid > benzoic acid > stearic acid > control at most monitoring time point. In the polymer groups, palmitic acid also showed the highest metabolic activity compared with other treatments. In the carboxylic and acetic acid group, ρ-hydroxybenzoitic acid showed higher AWCD to palmitic acid, followed by benzoic acid > stearic acid > control at most monitoring time points. All the treatments showed similar trends in changing AWCD in the carbohydrates group.

Discussion

Since numerous studies have focused on plant–bacteria interactions in the phytoremediation, there has been considerable interest in the role of root exudates in this process (Cheema et al. 2009; Segura and Ramos 2013). Many researches showed that the amendment of root exudates collected from plant roots could enhance microbial biodegradation of hydrocarbon in contaminated soils (Miya and Firestone 2001; Jeremy et al. 2004; Phillips et al. 2012; Xie et al. 2012). However, for each of these studies, no attempt was made to identify the role of individual compounds in the root exudate mixture (Martin et al. 2014). Organic acids, one of the important components of root exudates, may serve as both

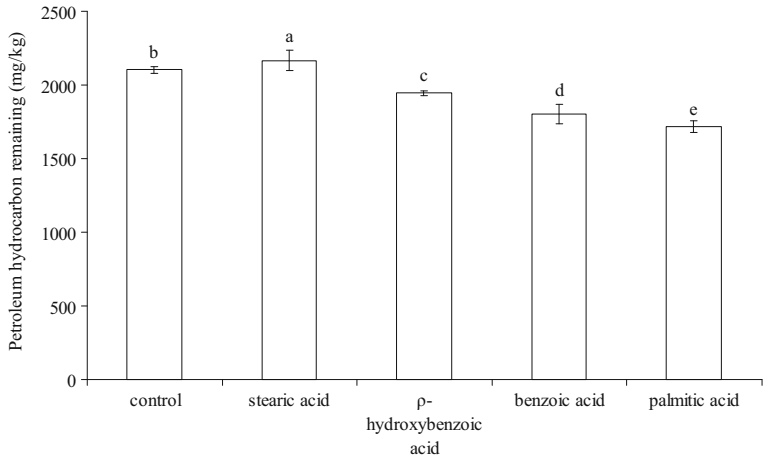

Fig. 5 Petroleum remaining in the soil 4 weeks following weekly addition of organic acids at a concentration of 500 mg kg^{-1}

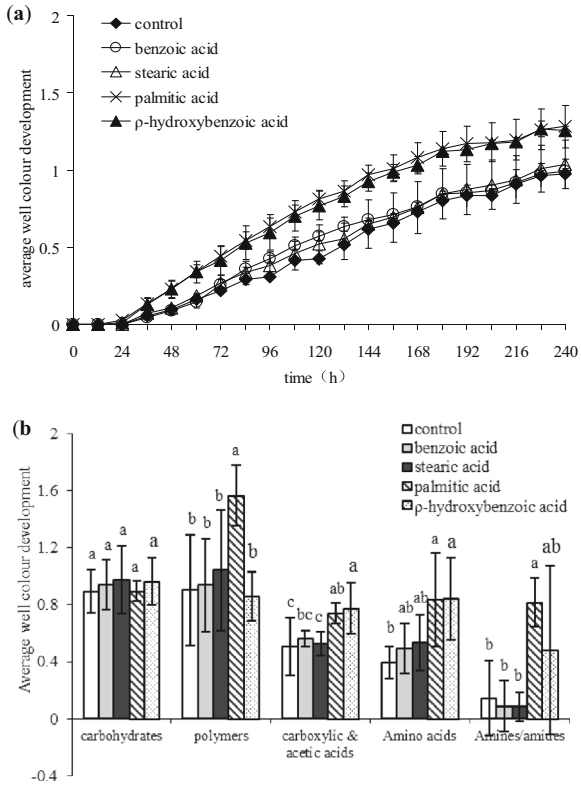

Fig. 6 Average well color development (AWCD) of **a** total carbon source and **b** carbon source guilds (carbohydrates, caboxylic and acetic acids, amino acids, amines/amides, and polymers) in Biolog Eco plate by soil microbial communities from soil amended with organic acids

nutrients and signal molecule for the soil microbes (Jones 1998). In this study, organic acids were collected from undisturbed conditions using continuous root exudate trapping system (CRETS) equipment and four major organic acids were identified as benzoic, palmitic, ρ-hydroxybenzoic, and stearic acids. CRETS equipment accumulated root exudates in the resin, thus, making it possible to collect root exudates at any time without damaging the plants. Moreover, this equipment could mimic the soil medium with a solid culture matrix and overcame the drawbacks of collecting root exudates from hydroponic nutrient solution (Tejeda-Agredano et al. 2013) or ground root tissue (Yi and Crowley 2007) which cannot represent root exudates of plants growing in soil. To the best of our knowledge, this is the first time that the root exudates of tall fescue have been collected and identified in undisturbed conditions. Nevertheless, the XAD-4 resin used in the CRETS equipment can only adsorb hydrophobic or partially hydrophobic organic acids and water-soluble organic molecules such as most of the amino acids pass through the column without substantial retention. Some of these may also play an important role in plant–-bacteria interactions. A more complete answer to the role of the root exudates in the phytoremediation process will require the development of more advanced techniques.

The organic acids identified were then used to examine their effects on functional bacteria related to petroleum degradation such as the biosurfactant producing bacterium *Pseudomonas* sp. SB, the PGPR *Klebsiella* sp. D5A, and the petroleum-degrading bacterium *Streptomyces* sp. KT (Liu et al. 2013, 2014). Chemotaxis toward specific root exudate compounds is a key factor in efficient root colonization (de Weert et al. 2002), and the successful application of functional bacteria assisting phytoremediation is largely dependent on the colonization of beneficial bacteria on root surfaces (Lugtenberg et al. 2001). Ortega-Calvo et al. (2003) also reported that chemotactic transport induced by root exudates could increase the rates of pollutant dissipation by soil microbial populations. We found that all four organic acids have significant effects on *Klebsiella* sp. D5A and the maximal chemotactic response was observed at 30 mM palmitic acid (Table 1). Ling et al. (2011) also used the capillary chemotaxis assay to detect chemotactic responses to different organic acids on *Paenibacillus polymyxa* SQR-21 and found that *P. polymyxa* SQR-21 exhibits positive, concentration-dependent chemotactic behavior within a dose range of 5–30 mM when treated with malic acid and citric acid.

Swarming motility is also an important bacterial motility mechanism that allows bacteria to move rapidly over surfaces and is thought to be a successful strategy developed by flagellated microorganisms to ensure their rapid expansion in the natural environment, where microbial activities are often associated with solid surfaces (Senesi et al. 2002). In this study, the swarming ability of *Pseudomonas* sp. SB was notably increased by ρ-hydroxybenzoic acid and palmitic acid, *Klebsiella* sp. D5A by benzoic acid and palmitic acid, and *Streptomyces* sp. KT by palmitic acid, respectively (Fig. 3). It is worth noting that the swarming experiment in this study not only examined the mobility of bacteria, but also evaluated the bacterial growth rate which reflects their survival competitiveness as affected by organic acids in the rhizosphere (Dennis et al. 2010). The ability of the organic acids in inducing recruitment of *Klebsiella* sp. D5A to the tall fescue root surface was also assessed. The results showed that plant recruitment of *Klebsiella*

sp. D5A was significantly promoted by palmitic acid and this result is consistent with those of the assays for chemotaxis (Table 1) and swarming (Fig. 3). Ling et al. (2011) have reported that malic acid and citric acid secreted by watermelon can induce the chemotaxis and swarming of PGPR and showed a result of an increase in population of the rhizosphere and biofilm formation on the root surface.

Subsequent pot experiments were also carried out to study the influence of organic acids amendment on the petroleum removal rate in the soil. The results indicated that soil amended with ρ-hydroxybenzoic acid, benzoic acid, and palmitic acid significantly increased petroleum removal by 7.8, 14.9, and 19.2 %, respectively. Wei et al. (2014) have also reported that the addition of palmitic acid amplified the rhizosphere effect on soil microbial populations and diesel removal. Palmitic acid is a type of lipophilic organic acid and is considered to be a key component enhancing pyrene biodegradation and selectively enriching the whole PAH degrader community (Meng and Zhu 2010). Our study also showed that benzoic acid and palmitic acid amendment resulted in an MPN enhancement of approximately 5 and 6 times, respectively, relative to the control. ρ-Hydroxybenzoic acid is a type of flavonoid and has been reported to be catabolized by *Pseudomonas putida* as a nutrient source (Pillai and Swarup 2002). However, in this study, stearic acid showed repression effects on the functional bacteria associated with petroleum degradation and on the mineralization of petroleum. The explanation may be that the biodegradation activity of soil microbial communities is influenced by a myriad of chemical, physical, and biological factors and is unlikely to respond to root exudate inputs in a manner comparable to bacterial isolates (Phillips et al. 2012). In some cases, the root exudates even provide alternative, more attractive, substrates for the microbial population than the polluting compounds, which therefore may reduce the degradation rates of hydrocarbons.

Palmitic acid and ρ-hydroxybenzoic amendment not only significantly enhanced petroleum removal, but also showed higher bacterial community metabolic activity of utilization of all carbon sources, especially in the carbon source groups of amine/amides, carboxylic and acetic acids, polymers, and amino acids. Thus, the petroleum-degrading microbial communities are accompanied by an increased capacity to utilize these types of carbon source. The higher utilization of polymers in Biolog Eco™ plates can indicate the presence of microorganisms related to the metabolism of recalcitrant complex molecules (Ros et al. 2014). Likewise, the high consumption of carboxylic acids at the end of the process might indicate, according to Kreitz and Anderson (1996), the presence of bacteria that metabolize mainly organic substances such as hydrocarbons. Changes in enzymatic activities and microbial community structure require further study. The nutrient balance for optimal hydrocarbon biodegradation in soil systems is similar to any other biological system. In petroleum-contaminated soil systems, nitrogen is a nutrient that is frequently deficient compared with carbon because carbon is a major constituent of hydrocarbons and this may lead to higher utilization of organic nitrogen (amine/amides and amino acids) in Biolog Eco™ plates.

Conclusions

In this study, root exudates of tall fescue were collected through CRETS equipment and the four main organic acids were identified to be palmitic, benzoic, ρ-hydroxybenzoic, and stearic acids. Of these, palmitic acid could significantly promote petroleum degradation associated functional bacterial growth and PGPR colonization on the roots of *F. arundinacea* L. The results of the pot experiment also showed that amendment with palmitic acid significantly enhanced the degradation of petroleum and microbial activity and diversity in petroleum-contaminated soil. These findings partly explain the mechanisms by which tall fescue enhances petroleum removal and also give direction for plant selection and application of organic acids to facilitate petroleum phytoremediation.

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