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Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates

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Summary

The phenanthrene-degrading activity (PDA) of *Pseudomonas putida* ATCC 17484 was repressed after incubation with plant root extracts of oat (*Avena sativa*), osage orange (*Maclura pomifera*), hybrid willow (*Salix alba* × *matsudana*), kou (*Cordia subcordata*) and milo (*Thespesia populnea*) and plant root exudates of oat (*Avena sativa*) and hybrid poplar (*Populus deltoides* × *nigra* DN34). Total organic carbon content of root extracts ranged from 103 to 395 mg l^{-1}. Characterization of root extracts identified acetate (not detectable to 8.0 mg l^{-1}), amino acids (1.7–17.3 mg l^{-1}) and glucose (1.6–14.0 mg l^{-1}), indicating a complex mixture of substrates. Repression was also observed after exposure to potential root-derived substrates, including organic acids, glucose (carbohydrate) and glutamate (amino acid). Carbon source regulation (e.g. catabolite repression) was apparently responsible for the observed repression of *P. putida* PDA by root extracts. However, we showed that *P. putida* grows on root extracts and exudates as sole carbon and energy sources. Enhanced growth on root products may compensate for partial repression, because larger microbial populations are conducive to faster degradation rates. This would explain the commonly reported increase in phenanthrene removal in the rhizosphere.

Introduction

Enhanced polycyclic aromatic hydrocarbon (PAH) degradation during phytoremediation has stimulated interest regarding the responsible mechanisms (Aprill and Sims, 1990; Gunther *et al*., 1996; Reilley *et al*., 1996; Banks *et al*., 1999; Binet *et al*., 2000; Liste and Alexander, 2000; Miya and Firestone, 2000). The hydrophobic nature of PAHs prevents significant uptake and translocation within plants (Edwards, 1986; Burken and Schnoor, 1998), so these contaminants generally remain in the root zone. This suggests that plant roots stimulate microbial populations for enhanced biodegradation. Potential plant–microbe interactions affecting PAH degradation include: (i) prolific microbial growth (population increase; Shimp *et al*., 1993; Gunther *et al*., 1996); (ii) catabolic enzyme induction (Gilbert and Crowley, 1997; Harvey *et al*., 2002); (iii) co-oxidation of high-molecular-weight PAHs (Shimp *et al*., 1993; Gunther *et al*., 1996; Nichols *et al*., 1997); and (iv) improved bioavailability (Harvey *et al*., 2002). However, limited research has evaluated these mechanisms, which are difficult to discern in complex natural systems.

Aerobic bacterial PAH degradation is initiated by monooxygenase or dioxygenase enzymes, which often exhibit broad substrate specificity (Cerniglia, 1984; Gibson and Subramanian, 1984). Previous research has shown that salicylate and *o*-phthalate, downstream metabolites within PAH-degrading pathways, can induce oxygenase enzymes that initiate PAH degradation (Shamsuzzaman and Barnsley, 1974; Doddamani and Ninnekar, 2000). Chen and Aitken (1999) successfully used salicylate to stimulate mineralization of PAHs in soil. Some aromatic root components, structural analogues of known inducers, have been identified in plants important for phytoremediation (Table 1; Hedge and Fletcher, 1996). However, induction of PAH-degrading enzymes by plant root products has not been thoroughly demonstrated in the literature. Gilbert and Crowley (1997) demonstrated induction of polychlorinated biphenyl (PCB) degradation in *Arthrobacter* sp. strain B1B, a Gram-positive organism, using spearmint root products, and identified *l*-carvone as the compound responsible. Interestingly, *l*-carvone was not a growth substrate for *Arthrobacter* sp. strain B1B, and it inhibited growth of the bacteria on fructose.

Global carbon source regulation (e.g. catabolite repression) of xenobiotic-degrading enzyme systems has been demonstrated in *Pseudomonas putida* (Stulke and Hillen, 1999; Cases and de Lorenzo, 2001). Tricarboxylic acid (TCA) cycle intermediates (e.g. succinate, pyruvate and citrate), carbohydrates (e.g. glucose and gluconate) and amino acids repressed phenol-degrading activity (Muller

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Table 1. Aromatic components reported in the literature for plants used in this study.

Plant name			
Common	Genus	Reported aromatics	Reference
Willow	*Salix*	Salicylates	Ruuhola and Julkunen-Tiitto (2000)
Osage orange	*Maclura*	Flavanones, xanthones	Monache *et al*. (1984)
Milo	*Thespesia*	Gossypols	Bell *et al*. (1975)
Kou	*Cordia*	Naphthoquinones	Bieber *et al*. (1990); Ioset *et al*. (2000)
Oat	*Avena*	Cinnamic acid, coumarins	Pomilio *et al*. (2000)
Poplar	*Populus*	Hydroxycinnamic acids	Kurkin *et al*. (1994)

et al., 1996; Sze and Shingler, 1999). McFall *et al*. (1997) observed repression of chloro-substituted aromatics degradation by fumarate, another TCA cycle intermediate. Reduced expression of alkane- and toluene-degrading genes has also been shown for cultures growing on rich media (Ramos *et al*., 1997; Yuste and Rojo, 2001). With respect to phenanthrene degradation, Tian *et al*. (2002) showed no repression of *Pseudomonas medocina* by glucose. Conversely, Keuth and Rehm (1991) observed repression of phenanthrene degradation by *Arthrobacter polychromogenes*, a Gram-positive microorganism, at glucose concentrations of 4 g l^{-1} but not at 0.45 g l^{-1}.

A few investigations probing plant–microbe interactions with respect to PAH degradation used contaminated soil (Miya and Firestone, 2000; Yoshitoma and Shann, 2001; Siciliano *et al*., 2003). These studies were representative of contaminated sites, but system complexity did not allow for the identification of specific plant–microbe interactions and their effect on catabolic gene expression. Miya and Firestone (2000) observed greater percentages of phenanthrene-degrading bacteria in rhizosphere soil than in bulk soils and suggested that the rhizosphere selected for PAH degraders. Siciliano *et al*. (2003) observed a higher frequency of catabolic genes in tall fescue rhizosphere than in bulk soils, suggesting that gene transfer or another mechanism of selection exists in the rhizosphere. However, the presence of contaminants in these experimental systems probably provided a strong selective pressure for competent strains (Siciliano *et al*., 2001).

To evaluate plant–microbe interactions with respect to phenanthrene-degrading activity (PDA), we studied *Pseudomonas putida* exposed to plant root products (extracts and exudates). The objectives of this study were to (i) examine the PDA of *P. putida* exposed to plant root products; (ii) characterize the chemical composition of root extracts; and (iii) identify root extract constituents responsible for observed effects on PDA. Both soluble root exudates and soluble root extracts (from ground roots) were used in this research for different purposes. Soluble root exudates mimic the products leaked from whole living plants in the root zone. Because of the relatively low total organic carbon (TOC) concentrations of root exudates, root extracts were used in some experiments. Root extracts provide higher TOC concentrations and simulate products of root sloughing and root turnover that can contribute 7–27% of the total plant mass per year (Shimp *et al*., 1993) to the rhizosphere. Leigh *et al*. (2002) demonstrated recently that root turnover provides a large source of carbon in rhizoremediation.

Six plant species were used in this study. Hybrid poplar (*Populus deltoides* × *nigra* DN34) and willow (*Salix alba* × *matsudana*) were used because of broad-reaching root systems and common use for phytoremediation. Slender oat (*Avena sativa*) was a representative grass species, previously used to investigate rhizosphere degradation of PAHs (Miya and Firestone, 2000). Osage orange (*Maclura pomifera*) was investigated because the plant contains a large portion of phenolic compounds (Monache *et al*., 1984) that are potential inducers of PAH degradation. Milo (*Thespesia populnea*) and kou (*Cordia subcordata*) are tropical plants that were examined to extend the potential for PAH phytoremediation to warm regions.

Pseudomonas putida ATCC 17484 was used in this study as a model PAH degrader known to be induced by salicylate (Shamsuzzaman and Barnsley, 1974). Previous studies have demonstrated the ability of *P. putida* to colonize plant roots. Kuiper *et al*. (2001; 2002) isolated a naphthalene-degrading *P. putida* strain from the rhizosphere of the grass *Lolium multiflorum*, whereas Bakker *et al*. (2002) modified another *P. putida* strain for improved activity against soil-borne pathogens in the wheat rhizosphere.

Results and discussion

Growth of P. putida *on root extracts*

Growth of *P. putida* was demonstrated using root extracts as a sole carbon and energy source (Fig. 1A), supporting the hypothesis that enhanced biodegradation in the rhizosphere results, in part, from proliferation of competent microorganisms. *P. putida* was grown on three root extracts, willow, kou and milo, with initial TOC concentrations of 84.2, 175.0 and 51.7 mg l^{-1} respectively. Each

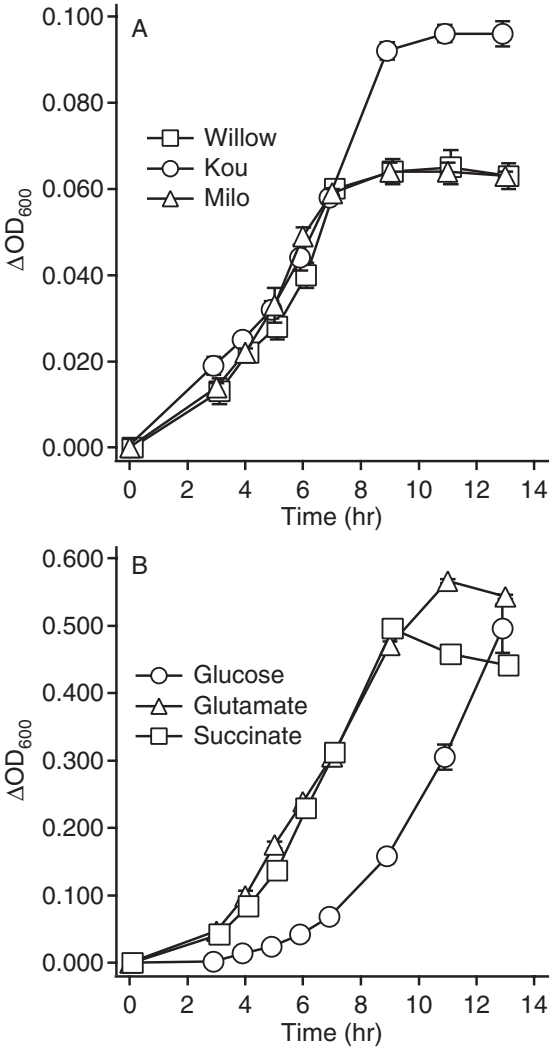

Fig. 1. Growth of *P. putida* on root extracts of similar carbon concentrations.
A. Willow (84 mg C l^{-1}), milo (52 mg C l^{-1}) and kou (175 mg C l^{-1}) root extracts were used as carbon sources.
B. Glucose (144 mg C l^{-1}), glutamate (120 mg C l^{-1}) and succinate (96 mg C l^{-1}) were used as carbon sources.
Bars represent one standard deviation from the mean of three replicates and are not depicted when smaller than the symbol.

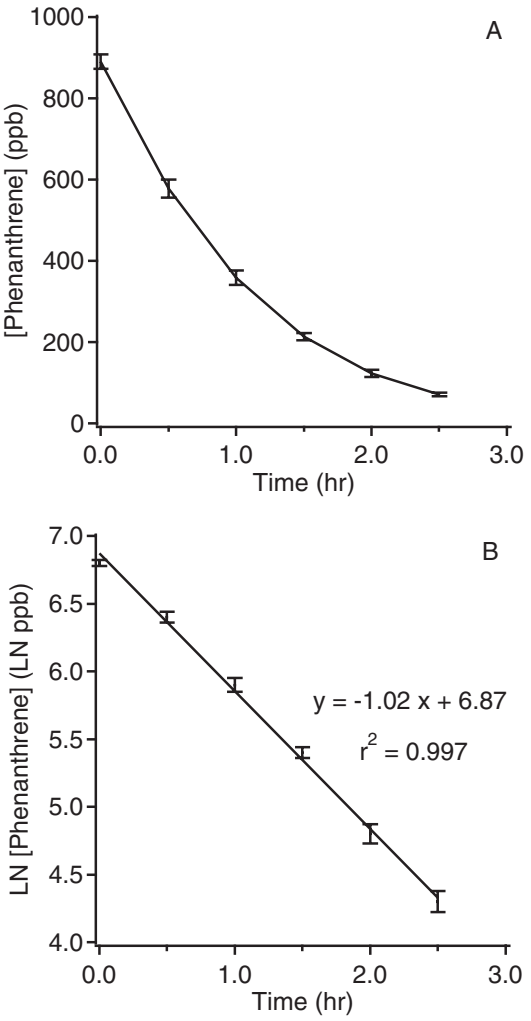

Fig. 2. An example phenanthrene-degrading activity (PDA) assay representative of more than 50 assays performed in triplicate. *P. putida* was exposed to kou root extract for 4 h before the assay.
A. Phenanthrene disappearance plotted versus time.
B. Natural log-transformed phenanthrene concentrations plotted versus time with a linear least squares regression best-fit line. The slope of the line represented the pseudo-first-order degradation rate coefficient (−1.02 h^{-1}). PDA was defined as the pseudo-first-order degradation rate coefficient normalized to initial OD_{600}. Bars represent one standard deviation from the mean of three replicates.

culture experienced increases in OD_{600} between 0.064 ± 0.003 (milo) and 0.096 ± 0.003 (kou) during a 13 h period. Cultures of *P. putida* were also grown on glucose, glutamate and succinate with initial theoretical TOC concentrations of 144.0, 120.0 and 96.0 mg l^{-1} respectively (Fig. 1B). Growth of these cultures ranged from 0.496 ± 0.036 (glucose and succinate) to 0.543 ± 0.003 (glutamate) OD_{600} over the 13 h period. Growth efficiency of *P. putida* on root extracts was 16% (kou) to 36% (milo) of glucose-grown cells (conservatively low estimate, as the glucose-fed culture had not reached stationary phase), indicating that a small portion of root extract TOC was used as a carbon and energy source. Growth of *P. putida* on root extracts and on model carbon sources exhibited typical exponential growth initially, followed by transition to stationary phase (Fig. 1).

Phenanthrene-degrading activity

Expression of *P. putida* PDA was evaluated using a whole-cell assay in phosphate buffer to preclude confounding effects resulting from growth. Removal of phenanthrene from solution by *P. putida* followed an exponential decay model (Fig. 2A); no phenanthrene was removed from an

acid (H_3PO_4)-killed control (Fig. 4B). Natural log-transformed phenanthrene concentrations were plotted with time, and the slope (h^{-1}) was determined (Fig. 2B; Walpole and Myers, 1978). The slope of the semi-log plot represented the pseudo-first-order degradation rate coefficient. PDA was defined as the degradation rate coefficient normalized by the measured initial optical density (OD_{600}, optical density remained relatively constant throughout the 3 h assay).

$$\text{PDA}\,(\text{OD}_{600}^{-1}\,\text{h}^{-1}) = -\text{slope}(\text{h}^{-1})/\text{initial}\,\text{OD}_{600}$$

Repression by plant root products

Hybrid willow, osage orange, milo and kou root extracts repressed PDA of *P. putida* 17484 after a 4 h exposure period (Fig. 3), an observation that conflicted with the hypothesis that plant roots enhance bacterial enzyme induction. PDA levels were lower for cells exposed to root extract than for cells exposed to similar TOC-based concentrations of succinate, an identified repressor of aromatic hydrocarbon catabolic pathways in *P. putida* (Stulke and Hillen, 1999). In contrast, salicylate, a known inducer of phenanthrene degradation (Shamsuzzaman and Barnsley, 1974), stimulated phenanthrene degradation and PDA (Figs 3 and 5–7). Assuming a maximum observed PDA (naphthalene-induced; not shown) of 115.2 $OD_{600}^{-1}\,h^{-1}$, the activity of root extract-exposed bacteria ranged from 12% (kou) to 21% (willow) of the maximum.

Oat and hybrid poplar root exudates also repressed the PDA of *P. putida* after an 8.0 h exposure period (Fig. 4) with patterns of repression that were similar to those observed for the plant root extracts of oat, hybrid willow,

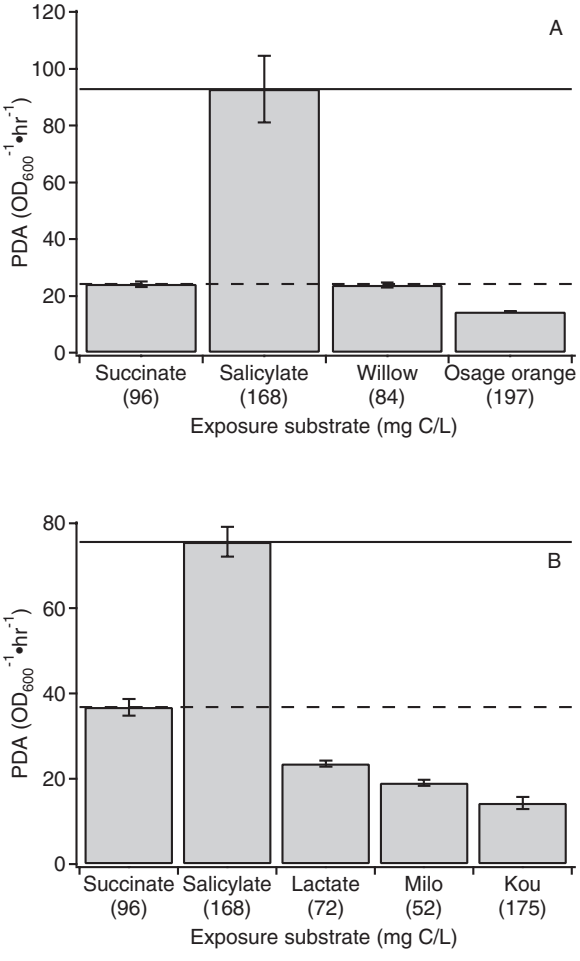

Fig. 3. Repression of *P. putida* phenanthrene-degrading activity (PDA) exposed to root extracts, succinate (repressor control, dashed line) or salicylate (inducer control, solid line) for 4 h. Individual plots represent independent experiments (A and B). Bars represent one standard deviation from the mean of three replicates.

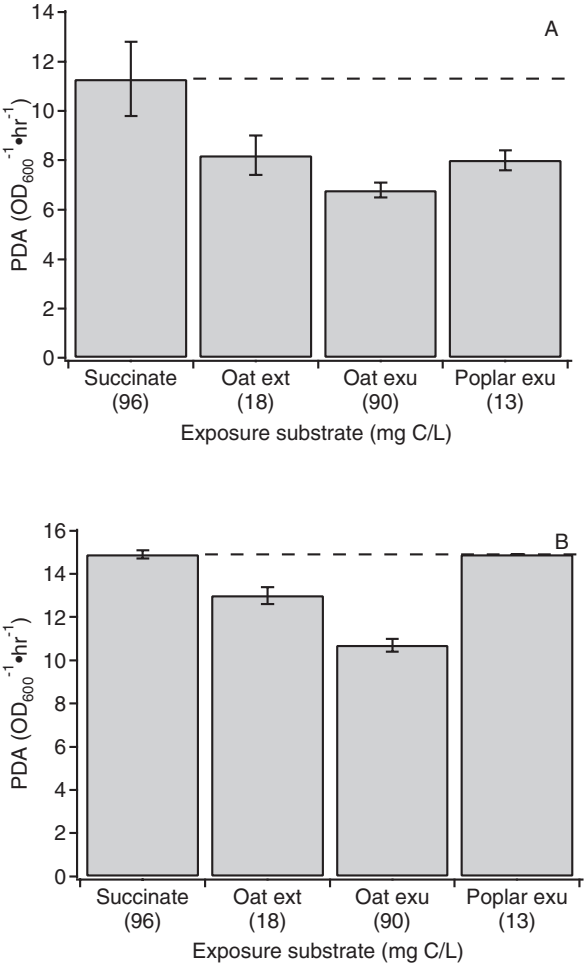

Fig. 4. Repression of phenanthrene-degrading activity (PDA) of *P. putida* exposed to oat extract (ext), oat exudates (exu), poplar exudates (exu) or succinate (repressor control, dashed line) for 8.5 h. Individual plots represent independent experiments (A and B). Bars represent one standard deviation from the mean of three replicates.

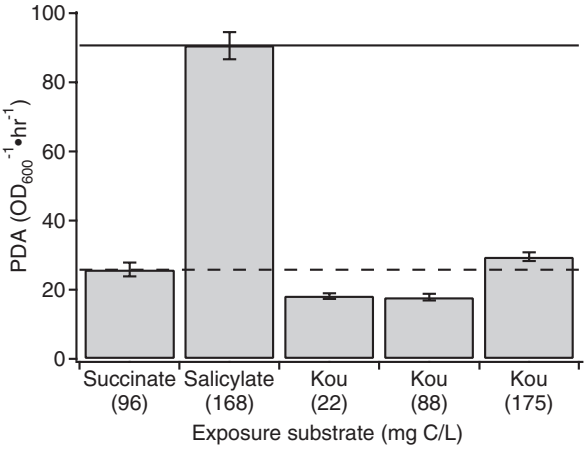

Fig. 5. Repression of phenanthrene-degrading activity (PDA) of *P. putida* exposed to varying concentrations of kou root extract for 4 h. Effects of succinate (repressor control, dashed line) and salicylate (inducer control, solid line) are also depicted. Bars represent one standard deviation from the mean of three replicates.

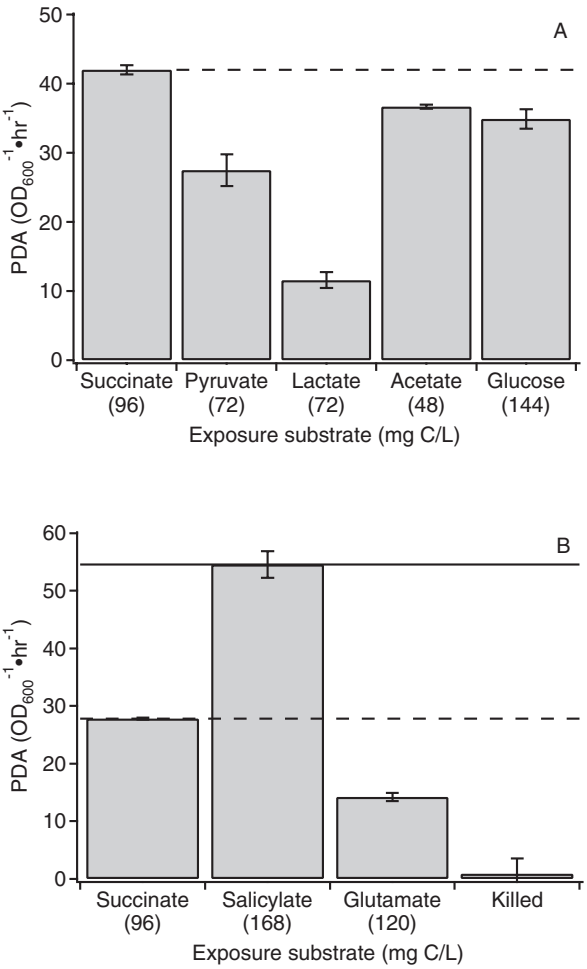

Fig. 6. Phenanthrene-degrading activity (PDA) of *P. putida* exposed to surrogate repressor chemicals, succinate (repressor control, dashed line) or salicylate (inducer control, solid line) for 4 h. Individual plots represent independent experiments (A and B). Bars represent one standard deviation from the mean of three replicates.

osage orange, milo and kou (Figs 3 and 4). Observation of repression using root exudates, an environmentally relevant rhizospheric carbon substrate, corroborated the results with plant root extracts, which are representative of root sloughing and root turnover. This suggests that the rhizosphere may be rich in alternative carbon sources that exert catabolite repression. In addition, the concentration of root product carbon did not appear significantly to affect observed PDA. The PDA of *P. putida* was repressed to similar levels with varied kou root extract concentrations of 22, 88 and 175 mg l^{-1} (Fig. 5).

Repression of PDA was also observed after exposure to acetate, lactate, pyruvate, glucose (carbohydrate) and glutamate (amino acid) added at 2.0 mmol (Figs 3B and 6), suggesting that global carbon source regulation (e.g. catabolite repression) was responsible for the observed repression by root extracts and exudates. PDA repression was also observed when naphthalene (crystal) was added to induce the cultures (Fig. 7). Once again, PDA was lower for lactate- and willow-exposed cells than for succinate-exposed cells. The activity of milo-exposed cells was greater than that of succinate-exposed controls, but was statistically lower than that of salicylate-induced cells ($P < 0.05$). These results indicate that root extracts repressed the expression of phenanthrene degradation in the presence of the inducer naphthalene. Although this is the first report of catabolite repression with respect to phenanthrene degradation, many catabolic pathways within *P. putida* are subject to global carbon source regulation, including alkanes (Yuste and Rojo, 2001), chloroaromatics (McFall *et al*., 1997), phenol (Muller *et al*., 1996; Sze and Shingler, 1999) and toluene (Ramos *et al*., 1997).

Chemical characterization of root extracts

Quantification of root extract composition provided insight into potential repressors and additional support for the use of root extracts as representative rhizospheric carbon sources. Acetate (organic acid), amino acids and glucose (carbohydrate) were quantified in plant root extracts (Table 2) to demonstrate the presence of potential repressors. Acetate was observed in willow (1.3% TOC) and milo (0.7% TOC) root extracts. Amino acids, particularly aspartic acid and glutamic acid, were identified in all root extracts, and the fraction of TOC ranged from 0.2% (kou) to 3.9% (willow). Sze and Shingler (1999) demonstrated silencing of an aromatic response regulator (controls the expression of catabolic genes) within *P. putida* using amino acid concentrations of the order of 0.001–0.1% (1–100-mg l^{-1}). The amino acid concentrations measured here were well within this range, although it is unknown

Table 2. Partial chemical composition detected in root extracts by TOC, HPLC and enzymatic assay analysis (detection limits are given in parenthesis in mg l^{-1}, dl).

	Willow	Osage orange	Kou	Milo
TOC (mg C l^{-1})[a]	168.4 ± 3.6	394.8 ± 11.5	350.0 ± 3.5	103.5 ± 4.4
Amino acids (mg l^{-1})[b]				
Aspartic acid (0.33)	10.64	5.62	0.45	0.87
Glutamic acid (0.37)	2.95	2.34	0.60	0.74
Histidine (0.39)	0.59	0.44	~ dl	0.55
Serine (0.26)	~ dl	~ dl	~ dl	~ dl
Arginine (0.44)	0.53	0.52		~ dl
Glycine (0.19)	1.30	0.44		
Threonine (0.30)	0.50	0.42	0.34	~ dl
Alanine (0.22)				
Tyrosine (0.45)				
Methionine (0.37)	0.44	0.45		~ dl
Valine (0.29)				
Phenylalanine (0.41)	~ dl			
Isoleucine (0.33)				
Leucine (0.33)				
Lysine (0.37)				
Total (mg l^{-1})	17.3	10.4	1.67	3.00
% TOC	3.87	1.00	0.19	1.16
Organic acids (mg l^{-1})[c]				
Acetate (1.0)	7.4			8.0
% TOC	1.3			0.7
Carbohydrates (mg l^{-1})[d]				
Glucose (0.5)	2.42 ± 0.17	14.0 ± 0.88	1.57 ± 0.13	4.98 ± 0.75
% TOC	0.58	1.41	0.61	0.57

a. Shimadzu TOC analyser (dl ~1.0 mg C l^{-1}).
b. High-performance liquid chromatography (HPLC) with online *o*-phthalaldehyde derivatization using fluorescence detection.
c. HPLC with UV (210 nm) detection.
d. Sigma glucose (HK) assay kit.

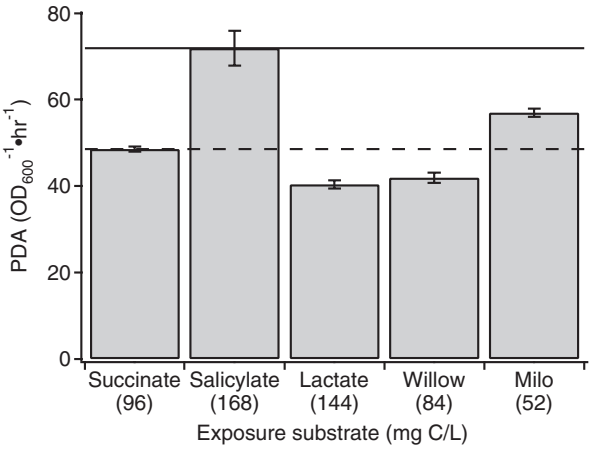

Fig. 7. Repression of phenanthrene-degrading activity (PDA) of *P. putida* exposed to a naphthalene crystal (to induce PDA) in addition to root extracts, lactate (surrogate repressor), succinate (repressor control, dashed line) or salicylate (inducer control, solid line) for 4 h. Bars represent one standard deviation from the mean of three replicates.

whether this type of regulation controls phenanthrene degradation in the strain tested. Glucose was also identified in each of the root extracts, comprising 0.57% (milo) to 1.41% (osage orange) of TOC. Total characterized TOC (0.8%, kou; 5.8%, willow) was of the same order of magnitude as the range of estimated utilized root extract carbon (discussed above), but additional uncharacterized organic substrates were probably present in the root extracts.

The TOC of the root extracts used here was similar to values reported by other researchers for root exudates and within the range of root exudates collected here for poplar (25 mg l^{-1}) and oat (180 mg l^{-1}). Miya and Firestone (2001) reported a TOC concentration of 54 mg l^{-1} (M. K. Firestone, personal communication) for slender oat root exudate (14 days collection), which is similar to the concentrations used here. The acetate content of root extracts was also similar to root exudates reported by others. Here, willow and kou extracts contained acetate concentrations of ≈0.14 and 0.38 mg C g^{-1} root, respectively, values less than or equal to those observed in the root exudates of lupin (0.32–1.5 mg C g^{-1} root; Lucas García *et al.*, 2001) and wheat (0.70 mg C g^{-1} root; Fan *et al.*, 2001).

Initial qualitative analysis of root extracts indicated that phenolic compounds comprised a minor portion of separable acids (Bruker Daltonics, Esquire$_{3000}$*plus*™ mass spectrometer). Others have observed root phenolic contents ranging from 0.02% to 0.40% (g phenolic g^{-1} root) for root exudates and 2.2–4.7% for ethanol-extracted roots

(Hedge and Fletcher, 1996; Leigh *et al*., 2002). The low proportion of phenolic compounds within plant root products suggested that easily degradable substrates such as organic acids, amino acids and sugars might be more readily accessible as substrates, masking potential induction mechanisms by phenolics.

The characterization of plant root extracts used here indicated a complex medium containing several chemicals known to be repressors of catabolic pathways within *P. putida* (Stulke and Hillen, 1999). Different plant root extracts contained varying amounts of amino acids, organic acids, carbohydrates and aromatic compounds, but similar patterns of repression were observed. It is unclear whether an individual component or the additive effect of the complex substrate mixture led to the observed repression.

Implications for phytoremediation of PAHs in the field

The observation of PDA repression with plant root extracts and exudates does not necessarily contrast with field observations of enhanced PAH degradation in the rhizosphere (Aprill and Sims, 1990; Gunther *et al*., 1996; Reilley *et al*., 1996; Banks *et al*., 1999; Binet *et al*., 2000; Liste and Alexander, 2000; Miya and Firestone, 2000). The availability of simple carbon sources (e.g. organic acids and amino acids) promotes the growth of rhizosphere bacterial populations that are reported to be four- to 100-fold greater than surrounding bulk soils (Gunther *et al*., 1996; Jordahl *et al*., 1997; Nichols *et al*., 1997; Chaineau *et al*., 2000; Miya and Firestone, 2000; Siciliano *et al*., 2003). Assuming (conservatively) that bulk soil degraders are optimally induced with an arbitrary activity of 1, repression of the order reported here (12–21% of full activity with naphthalene) would require competent population increases greater than five- to ninefold to demonstrate improved contaminant removal. This value is well within the range reported above. These calculations assume that phenanthrene-degrading microbial populations comprise similar percentages of total heterotrophic bacteria in rhizosphere and bulk soils, and are probably a conservative estimate. Miya and Firestone (2000) reported a greater percentage of phenanthrene degraders of total heterotrophs in the rhizosphere of oat plants compared with bulk soils. In addition, the most probable number (MPN) method used selected for bacteria capable of growth on phenanthrene and did not account for bacteria that could co-metabolize phenanthrene. Such MPN methods may underestimate the concentration of potential phenanthrene-degrading organisms (Wrenn and Venosa, 1996; Johnson *et al*., 2002).

Our results indicate that root-derived substrates repress *P. putida* ATCC 17484 PDA; however, the extent of repression will probably differ among different microbial strains and plant species. We also showed that *P. putida* grows on root products as a sole carbon and energy source, which is consistent with the notion that microbial populations within the rhizosphere are greater than in surrounding bulk soils. Thus, the positive effect of root products on microbial growth may offset partial repression mechanisms and enhance phenanthrene removal.

Experimental procedures

Chemicals

Phenanthrene, *o*-phthalaldehyde, acetonitrile and amino acid standards were purchased from Sigma and were high-performance liquid chromatography (HPLC) grade or better. All other reagents were ACS grade or better.

Growth media and bacterium

L9 minimal media contained (per l) Na_2HPO_4 (7.1 g), KH_2PO_4 (3.0 g), NH_4Cl (1.1 g), $MgSO_4{\cdot}7H_2O$ (247 mg), $ZnCl_2$ (0.131 mg), $CoCl_2{\cdot}6H_2O$ (0.099 mg), $Na_2MoO_4{\cdot}2H_2O$ (0.078 mg), H_2Bo_3 (0.076 mg), $MnCl_2{\cdot}4H_2O$ (0.074 mg), $CuSO_4{\cdot}5H_2O$ (0.049 mg), $NiCl_2{\cdot}6H_2O$ (0.048 mg) and $CuCl_2$ (0.028 mg). L9 media plates were solidified with 1.9% Noble (Difco) agar. LB rich media contained (per l) tryptone (10.0 g), NaCl (10.0 g) and yeast extract (5.0 g). LB media plates were solidified with 1.9% Bacto (Difco) agar.

Pseudomonas putida ATCC 17484 was obtained from the American Type Culture Collection (ATCC) and maintained on L9 minimal plates supplemented with a naphthalene crystal. Naphthalene was used as a carbon and energy source to maintain PAH-degrading plasmids (30°C; Shamsuzzaman and Barnsley, 1974).

Root product preparation

Six-month-old kou (*Cordia subcordata*) and milo (*Thespesia populnea*) plants were obtained from Future Forests Nursery. Two-year-old osage orange (*Maclura pomifera*) trees were purchased from Green Plant Market. Hybrid willow (*Salix alba* × *matsudana*) and hybrid poplar (*Populus deltoides* × *nigra* DN34) cuttings were purchased from Hramor Nursery. Willow cuttings were grown at the University of Iowa Oakdale Campus greenhouse in potting soil (Shultz) for 3 months according to the method of Rentz *et al*. (2003). Wild oats (*Avena sativa* AGR118) seeds were purchased from Keepsmilin.com.

Water-soluble root extract was prepared as follows. All soil and sand was rinsed from roots with at least 10 volumes of water. Roots were then cut into small pieces and pulverized with a mortar and pestle. The crushed roots were transferred to 50 ml Sarstedt centrifuge tubes, 40 ml of DI water was added, and maceration was continued using a Biospec Products hand-held homogenizer. The resulting plant cell suspension was centrifuged at 10 000 r.p.m. for 10 min, and the supernatant was transferred to a clean flask. The pellet was resuspended with 40 ml of DI water, homogenized once more and centrifuged as above. The combined supernatants were

then filtered using successively smaller pore sizes. Whatman GF/C glass filters (1.2 μm) were used with a magnetic Gelman Sciences filter apparatus, and then Osmonics/MSI membranes (0.45 μm) were used with a Buchner funnel. Finally, a Nalgene filter unit (0.20 μm) was used for filter sterilization, and root products were stored at 4°C.

Oat exudates were collected by first soaking oat seeds with shaking in 70% ethanol for 10 min and rinsed with DI water. The seeds were then sterilized in a 2% bleach solution for 10 min with shaking and rinsed with sterile DI water. Sterile seeds were transferred to autoclaved 500 ml glass jars containing glass beads (2–5 mm diameter) and 0.10 strength-modified Hoaglund's solution; aluminium foil covered the jar to the level of the growth medium. Plants were grown under fluorescent lights with a 16 h day. Growth medium was collected, filtered through 0.2 μm Nalgene filter units for sterilization and stored at 4°C.

Hybrid poplar cuttings were grown in 0.5 strength Hoaglund's solution for 1 month (fluorescent light, 16 h day). After 1 month of growth, nutrient solution was collected and filtered through 0.2 μm Nalgene filter units for sterilization and stored at 4°C.

Growth on root extracts

An overnight culture of *P. putida* in LB medium was centrifuged at 8000 r.p.m. for 8 min and resuspended in L9 media. Triplicate flasks containing 100 ml of L9 medium with root extracts (Willow, 84.2 mg C l^{-1}; Milo, 51.7 mg C l^{-1}; Kou, 175.0 mg C l^{-1}) or 2.0 mmol of glucose, glutamate or succinate were inoculated. The cultures were incubated at 28°C with shaking, and growth was monitored by optical density at 600 nm (OD_{600}). Growth efficiency was determined by the ratio $OD_{600\ MAXIMUM}$/TOC and was normalized to glucose-grown cells.

Phenanthrene-degrading activity assay: exposure experiments

An overnight culture of *P. putida* ATCC 17484 in LB medium was centrifuged at 8000 r.p.m. for 8 min and resuspended in L9 media; the cells were washed to eliminate confounding repression by a rich medium. Triplicate flasks containing 100 ml of 2× L9 medium were supplemented with 2 mmol of sodium succinate and inoculated with the washed *P. putida* cell suspension. Cultures were incubated at 28°C with shaking, and OD_{600} was monitored. When an approximate OD_{600} of 0.100 was reached, one culture was split. Fifteen millilitres of the succinate culture was transferred to each of five flasks containing 15 ml of root extract or 15 ml of sterile DI water supplemented with model carbon compounds using 100× stock solutions. Succinate and salicylate were used as negative and positive controls, respectively, for exposure experiments. The cultures were incubated at 28°C with shaking for 4 h (8.5 h and 10 h for exudates), and growth was monitored spectroscopically (OD_{600}).

Exposed cultures were centrifuged at 10 000 r.p.m. for 10 min and resuspended to comparable OD_{600} (≈ 0.100) in phosphate buffer containing (per l) Na_2HPO_4 (7.1 g), KH_2PO_4 (3.0 g) and chloramphenicol (25 mg) to inhibit *de novo* synthesis of proteins, and phenanthrene (1.12 mg) supplied as a 100× concentrated stock solution in acetonitrile. Triplicate aliquots of ≈9.5 ml were distributed to 50 ml flasks and incubated at 28°C with shaking. Initial OD_{600} was recorded. Samples (500 μl) were removed at 30 min intervals and transferred to microcentrifuge tubes where 500 μl of acetonitrile was added. Cells were spun down at 13 200 r.p.m. for 3 min, and the supernatant was transferred to autosample vials that were stored at 4°C before analysis.

Analytical methods

Total organic carbon (TOC) was measured using a Shimadzu TOC analyser with a detection limit less than 1.0 p.p.m. (1.0 mg C l^{-1}). A Spectronic Genesys 5 spectrophotometer was used to determine optical density at a wavelength of 600 nm (OD_{600}).

Glucose concentrations were determined using the Sigma glucose (HK) assay kit according to the manufacturer's specifications. The detection limit for glucose was 0.5 p.p.m. (0.5 mg l^{-1}).

Acetate was analysed using an Agilent 1100 HPLC equipped with an Agilent 1100 UV detector (210 nm). Separation was achieved using an Alltech OA-1000 column (300–6.5 mm) with a 0.01 N sulphuric acid mobile phase (0.8 ml min^{-1}) at 35°C. The detection limit was ≈1.0 p.p.m. (1.0 mg l^{-1}).

Amino acids concentrations (Table 2) were determined using automated, online derivatization using *o*-phthalaldehyde (OPA; Henderson *et al.*, 2000) with fluorescence detection utilizing an Agilent 1100 series HPLC, autosampler (1313A) and fluorescence detector. A Supecosil C8 column (150–4.6 mm, 5 μm) was used for separation with mobile phases: A, 50 mM sodium acetate, 5% THF B, methanol (2.0 ml min^{-1}). At 0 min, phase B was 10%, at 15 min, phase B was 65% and, at 20 min, phase B returned to 10%. The fluorescence detector was set with excitation at 340 nm and emission at 450 nm. For analysis, 900 μl of root extract was combined with 100 μl of 1 N HCl in an autosample vial. Detection limits for amino acids were ≈0.5 p.p.m. (0.5 mg l^{-1})

Phenanthrene concentrations were analysed using an Agilent 1100 series HPLC equipped with an Agilent 1100 FLD. Chemical separation was achieved using a Supelcosil – PAH 5 μm column (150 mm × 4.6 mm), a mobile phase of 25% water and 75% acetonitrile, a 1.0 ml min^{-1} flow rate and an injection volume of 100 μl. The fluorescent detector was set with excitation at 280 nm and emission at 365 nm. The detection limit for phenanthrene was ≈2.0 p.p.b. (2.0 μg l^{-1}).

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