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Benzo[*a*]pyrene co-metabolism in the presence of plant root extracts and exudates: Implications for phytoremediation

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Bacterial benzo[a]pyrene cometabolism, a plant-microbe interaction affecting polycyclic aromatic hydrocarbon phytoremediation was demonstrated with Sphingomonas yanoikuyae JAR02 that utilized plant root extracts and exudates as primary substrates.

Abstract

Benzo[*a*]pyrene, a high molecular weight (HMW) polycyclic aromatic hydrocarbon (PAH) was removed from solution by *Sphingomonas yanoikuyae* JAR02 while growing on root products as a primary carbon and energy source. Plant root extracts of osage orange (*Maclura pomifera*), hybrid willow (*Salix alba*×*matsudana*), or kou (*Cordia subcordata*), or plant root exudates of white mulberry (*Morus alba*) supported 15–20% benzo[*a*]pyrene removal over 24 h that was similar to a succinate grown culture and an unfed acetonitrile control. No differences were observed between the different root products tested. Mineralization of ^{14}C-7-benzo[*a*]pyrene by *S. yanoikuyae* JAR02 yielded 0.2 to 0.3% $^{14}CO_2$ when grown with plant root products. Collectively, these observations were consistent with field observations of enhanced phytoremediation of HMW PAH and corroborated the hypothesis that co-metabolism may be a plant/microbe interaction important to rhizoremediation. However, degradation and mineralization was much less for root product-exposed cultures than salicylate-induced cultures, and suggested the rhizosphere may not be an optimal environment for HMW PAH degradation by *Sphingomonas yanoikuyae* JAR02.

Keywords: Rhizodegradation; High molecular weight polycyclic aromatic hydrocarbons; PAH; Benzo[*a*]pyrene

1. Introduction

Enhanced degradation of high molecular weight (greater than four fused rings, HMW) polycyclic aromatic hydrocarbons (PAH) during phytoremediation (Aprill and Sims, 1990; Banks et al., 1999; Binet et al., 2000; Paquin et al., 2002) has motivated investigations of controlling plant/microbe interactions. Rhizosphere processes are implicated for improved degradation due to the high K_{ow} of the contaminants that precludes uptake and translocation within plants (Burken and Schnoor, 1998). Previously suggested hypotheses that support improved HMW PAH degradation within the rhizosphere compared to bulk soils include (i) prolific microbial growth (population increase; (Gunther et al., 1996; Shimp et al., 1993)), (ii) catabolic enzyme induction (Gilbert and Crowley, 1997; Harvey et al.,

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2002), (iii) co-metabolism of high-molecular weight PAHs (Gunther et al., 1996; Nichols et al., 1997; Shimp et al., 1993), and (iv) improved bioavailability (Harvey et al., 2002). Presently, three reports provide data to support these proposed mechanisms. First, Ortega-Calvo et al. (2003) demonstrated chemotaxis of PAH-degrading rhizosphere bacteria (*Pseudomonas alcaligenes*, *P. stutzeri*, and *P. putida*) to naphthalene, phenanthrene, and root exudates. Interestingly, the bacteria were repelled by anthracene and pyrene. The attraction of competent bacteria to the root zone may improve bioavailability and increase PAH degradation in the rhizosphere. Repression of the phenanthrene-degrading activity of *Pseudomonas putida* following exposure to root extracts and exudates (Rentz et al., 2004) suggested that enzyme induction may not occur during rhizodegradation of PAHs. Related studies by Kamath et al. (2004) observed repression of PAH-catabolic genes on a per cell basis and demonstrated greater naphthalene degradation by cultures grown on root products compared to naphthalene, supporting the notion that prolific microbial growth provides improved degradation in the rhizosphere. However, these reports did not investigate HMW PAH.

HMW PAHs do not serve as a carbon and energy sources for microbial populations during degradation (Juhasz and Naidu, 2000). In particular, initial ring attack with mono- or di-oxygenase enzymes is accomplished by bacteria that may or may not directly benefit from the reactions. Once hydroxylated, however, PAHs become increasingly soluble and can be attacked by enzymes from additional members of the bacterial community (Kanaly et al., 2000, 2002). Successive enzymatic steps may eventually yield central metabolites that a bacterium could use for energy. Within the web of PAH degradation, the initial ring-hydroxylating organisms may control the rate of degradation, because mass transfer (low solubility) limits many remediation efforts (Cerniglia, 1992; Juhasz and Naidu, 2000).

Providing appropriate primary carbon sources for growth and energy may improve in situ biodegradation of HMW PAH. Selection of an appropriate carbon source must consider possible negative responses, such as enzyme repression or diauxic growth. In addition, many microbial communities are adapted to low nutrient conditions and degradation ceases upon the addition of excess carbon sources provided by plant rhizospheres (Ciccillo et al., 2002). Previous study has shown successful stimulation of HMW PAH degradation with the addition of salicylate (Chen and Aitken, 1999), an inducer of PAH-degrading enzyme systems. However, full field scale application may prove to be too costly.

The use of plants as a method to "inject" substrates to contaminated soil has been hypothesized by previous researchers (Aprill and Sims, 1990; Fletcher and Hedge, 1995). Carbon is provided to soil bacteria through root exudation and root turnover; a summary of potential root zone carbon sources is given in Table 1. Soil experiments with ^{14}C-pyrene (4 fused rings) and root exudates demonstrated increases in mineralization of 10 to 15% over unamended soils and co-metabolism was implied (Yoshitomi and Shann, 2001). However, pyrene can be used as a sole carbon and energy source for bacteria (Bouchez et al., 1997). Co-metabolism of HMW PAH during rhizoremediation has not been demonstrated using a well defined system.

To test the hypothesis that PAH-degrading microbes can cometabolize HMW PAHs using plant root compounds as a primary substrates, benzo[*a*]pyrene degradation by *Sphingomonas yanoikuyae* JAR02 grown using plant root extracts and exudates was examined. The objectives of this study were to (i) demonstrate co-metabolism of benzo[*a*]pyrene using plant root extracts or exudates as a primary carbon and energy source and (ii) determine the extent of benzo[*a*]pyrene degradation and mineralization. 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 originating from whole living plants in the root zone. Because of the relatively low total organic carbon (TOC) concentrations of root exudates, we used root extracts in some experiments. Root extracts provide higher TOC concentrations and simulate products of root sloughing and root turnover that can contribute 7 to 27% of the total plant mass per year (Shimp et al., 1993) to the rhizosphere. Leigh et al. (2002) recently demonstrated that root turnover provides a large source of carbon in rhizoremediation.

2. Materials and methods

2.1. Chemicals

Benzo[*a*]pyrene, phenanthrene, and acetonitrile were purchased from Sigma (St. Louis, MO), and were HPLC grade or better. ^{14}C-7-benzo[*a*]pyrene was purchased

Table 1
Chemical compounds observed in plant root exudates and extracts

Compound	Examples	Reference
Sugars	Glucose, xylose, mannitol	Pandya et al. (1999)
	maltose, oligosaccharides	Curl and Truelove (1986)
Amino acids	Glutamate, isoleucine, methionine tryptophan	Pandya et al. (1999)
Aromatics	Benzoate, phenols, *l*-carvone	Hegde and Fletcher (1996)
	limonene, *p*-cymene	Tang and Young (1982)
		Gilbert and Crowley (1997)
Organic acids	Acetate, citrate, malate, propionate	Curl and Truelove (1986)

from Sigma (St. Louis, MO) with a radiochemical purity of ~98.0%. All other reagents were ACS grade or better.

2.2. Isolation, maintenance, and identification of *Sphingomonas yanoikuyae JAR02*

S. yanoikuyae JAR02 was used in this study as a model HMW PAH-degrader (Rentz, 2004). Previous studies demonstrated the ability of *S. yanoikuyae* to colonize plant roots (White et al., 1996). Takeuchi et al. (1995) reclassified *Chromobacterium lividum*, isolated from the roots of *Psychotria nairobiensis* and *Ardisia crispa*, as *S. yanoikuyae* following introduction of the *Sphingomonas* genus in 1990 by Yabuuchi et al. (1990). Their report also identified related *Sphingomonas* strains that were isolated from the roots of *Rosa, Prunus*, and *Mali* plants. Previous studies identified *Chromobacterium lividum* from the root vegetables green onion and carrot (Koburger and May, 1982). The genus *Sphingomonas* is also closely related to the genus *Rhizomonas*, well known bacteria associated with plants (Van Bruggen et al., 1993).

L9 minimal media and LB rich media were previously described (Rentz et al., 2004). *Sphingomonas yanoikuyae* JAR02 was described previously (Rentz, 2004) and maintained on L9 media plates (1.9% Noble agar) with phenanthrene supplied in the vapor phase (30 °C). Sublimation of phenanthrene was improved by spraying a 10% (wt/vol) acetone solution onto filter paper and letting the acetone evaporate. Filter papers with fine particles of phenanthrene were placed in plate lids and plates were incubated upside down.

2.3. Plants used and root product preparation

Eight plant species were used in this study. Hybrid poplar (*Populus deltoides*×*nigra* DN34) and willow (*Salix alba*×*matsudana*) were used due to their 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*), white mulberry (*Morus alba*), and red mulberry (*Morus rubra*) were investigated because the plants produce a large portion of phenolic compounds (Monache et al., 1984) that are potential inducers to PAH degradation. Kou (*Cordia subcordata*) is a tropical plant that was examined to extend the potential for PAH phytoremediation to warm regions.

Description of plant source material and procedures for preparation of root extracts and poplar and oat exudates was described previously (Rentz et al., 2004). White mulberry (*Morus alba*) and red mulberry (*Morus rubra*) seeds were purchased from Angelgrove Seeds (Harbour Grace, NF, Canada). Briefly, water soluble root extract was prepared from well rinsed wet roots that were cut into small pieces and pulverized with a mortar and pestle. DI (deionized) water was added to the crushed roots and macerated with a hand-held homogenizer. The resulting plant cell suspension was pelleted and the supernatant saved as root extract. Homogenation was repeated, root extract fractions pooled and then filtered using successively smaller pore sizes. Finally, a 0.2 micron Nalgene filter unit was employed for filter-sterilization, and root products were stored at 4 °C.

Root exudates were collected by first soaking 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 to 5 mm diameter) and 0.10 strength modified Hoagland's solution (Epstein, 1972); aluminum foil covered the bottom and sides of 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 micron Nalgene filter units for sterilization, and stored at 4 °C.

2.4. Growth on root products

LB medium was aseptically inoculated with a single colony of *S. yanoikuyae* JAR02 from a minimal plate supplied phenanthrene as a sole carbon source. The culture of *S. yanoikuyae* JAR02 was incubated overnight, centrifuged at 8000 r.p.m. (14000×*g*) for 8 min and re-suspended in L9 media. Triplicate flasks (per carbon source) containing 100 ml L9 medium with willow (84.2 mg C l^{-1}) root extract, poplar (5.7 mg C l^{-1}), oat (7.6 mg C l^{-1}), or red mulberry (43.1 mg C l^{-1}) root exudates, or succinate (96 mg C l^{-1}) were inoculated. All cultures were incubated at 28 °C with shaking and growth was monitored spectroscopically (600 nm). Conversion to biomass concentration (W, mg l^{-1}) was calculated by the following correlation to optical density, measured at 600 nm (OD_{600}): $W = 364.74*OD_{600} + 6.7*OD_{600}^2$ (Koch, 1994).

2.5. Co-metabolism of benzo[a]pyrene

50 ml flasks contained 15 ml 2X L9 media and 30 μl of a 1630 μg l^{-1} benzo[*a*]pyrene solution (acetonitrile). Filter-sterilized root products, succinate or salicylate from 100X stock solutions were added as carbon sources to achieve similar total organic carbon concentrations (triplicate flasks per carbon source). Sterile DI water was added to bring the final solution volume to 30 ml. A killed control contained 750 μl 20% H_3PO_4. Flasks were

inoculated using an overnight culture of JAR02 in LB medium (as above) that was centrifuged at 8000 r.p.m. (14000×*g*) for 8 min and resuspended in L9 media. Cultures were incubated with shaking at 30 °C in a dark incubator (to limit photo-oxidation).

Two samples were removed from each culture every 4 h using sterile pipettes. A 0.8 ml sample was placed in an autosample vial, culture growth was halted with 25 μl 20% H_3PO_4, and 200 μl acetonitrile was added to improve analysis of benzo[*a*]pyrene concentrations. An additional 1.0 ml sample was transferred to a cuvette and OD_{600} was measured.

2.6. Benzo[a]pyrene mineralization

250 ml amber bottles contained 25 ml 2× L9 media and 1 ml of a 1.0 μCi ml^{-1} ^{14}C 7-benzo[*a*]pyrene solution in N,N-dimethylformamide. Filter sterilized root products or succinate and salicylate, 100X stock solutions, were added as carbon sources to achieve similar total organic carbon concentrations (triplicate flasks per carbon source). Sterile DI water was added to bring the final solution volume to 50 ml. A killed control contained 1.25 ml 20% H_3PO_4. Each bottle contained a culture tube with 5 ml of 1N NaOH solution to serve as a CO_2 trap. Bottles were inoculated using an overnight culture of JAR02 in LB medium that was centrifuged at 8 000 r.p.m. (14000×*g*) for 8 min and resuspended in L9 media. Cultures were incubated with shaking at 30 °C in a dark incubator (to limit photo-oxidation).

Aqueous samples (700 μL) were removed from the culture to assess growth (OD_{600}). A 100 μl sample was removed from the NaOH trap, transferred to a scintillation vial with 10.0 ml Ultima gold liquid scintillation liquid, and analyzed on a Beckman (Fullerton, CA) model LS 6000IC liquid scintillation system. At the conclusion of the experiments, radiolabled products were recovered with ethyl acetate extraction (2 volumes) of acidified supernatants (pH ~ 2.5); cells were removed via centrifugation at 8000 r.p.m. (14000×*g*) for 8 min. Ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated under vacuum at 30 °C in the dark. Pelleted cells (neutral fraction) were extracted with 30 ml of acetone and these extracts were evaporated under vacuum at 30 °C in the dark. Samples were dissolved in acetone or acetonitrile and stored at 4 °C prior to analysis.

2.7. Analytical methods

Total organic carbon (TOC) was measured using a Shimadzu TOC analyzer 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}).

Benzo[*a*]pyrene concentrations were analyzed using an Agilent 1100 series HPLC equipped with an Agilent 1100 FLD. Chemical separation was achieved using a Supelcosil − PAH 5-micron column (150 mm×4.6 mm), a mobile phase of 10% water and 90% acetonitrile, a 1.0 ml min^{-1} flow rate, and an injection volume of 100 μl. The fluorescent detector was set with excitation at 264 nm and emission at 412 nm. The detection limit for benzo[*a*]pyrene was approximately 32.0 p.p.t. (32.0 ng l^{-1}).

^{14}C-labeled metabolites of benzo[*a*]pyrene degradation were observed using Agilent 1100 series HPLCs. Chemical separation was achieved using an Alltech 150 mm×2.1 mm Zorbax 80A extend C-18 (5 μm) column guarded with a 12.5 mm×2.1 mm Agilent C-18 guard column (5 μm). The MeOH/water mobile phase at a flow rate of 0.3 ml min^{-1} contained 2 mmol ammonium acetate with the following solvent ratios ($MeOH/H_2O$, minutes): 47.5/52.5, 0; 95/5, 20; 95/5, 33; 47.5/52.5, 38 (linear gradients were used). A 50 μl sample was injected for ^{14}C analysis that was detected using a Packard (Meridian, CT) model Radiomatic™ 525TR flow scintillation analyzer. Ultima-flo scintillation liquid was used at a 1.0 ml min^{-1} flowrate. Data collection and processing was completed with Packard FLO-ONE software.

3. Results and discussion

3.1. Growth with root products

Growth of *Sphingomonas yanoikuyae* JAR02 using a variety of plant root extracts and root exudates as a sole carbon and energy source was observed (Figs. 1 and 3–4). Increases in cell mass varied from 0.004 ΔOD_{600} (poplar exudate) to 0.165 ΔOD_{600} (osage orange extract) and were related to the total organic carbon content of the root products used. For cultures reaching stationary phase growth (Fig. 1; willow, oat and mulberry) a correlation between maximum change in OD_{600} and initial root product TOC was determined using a linear best fit (Fig. 2). The *r*-square value of 0.994 suggested a significant correlation that was corroborated with a *P*-value (Student's *t*-test; Hogg, 1987) of 0.05 (significant at 95% confidence level) for the slope of the best fit line. The correlation between maximum growth and initial TOC suggested similar ratios of carbon sources were bioavailable within the various root products for the growth of *S. yanoikuyae* JAR02.

3.2. Co-metabolism of benzo[a]pyrene

Benzo[*a*]pyrene was removed from solution by *Sphingomonas yanoikuyae* JAR02 during growth on

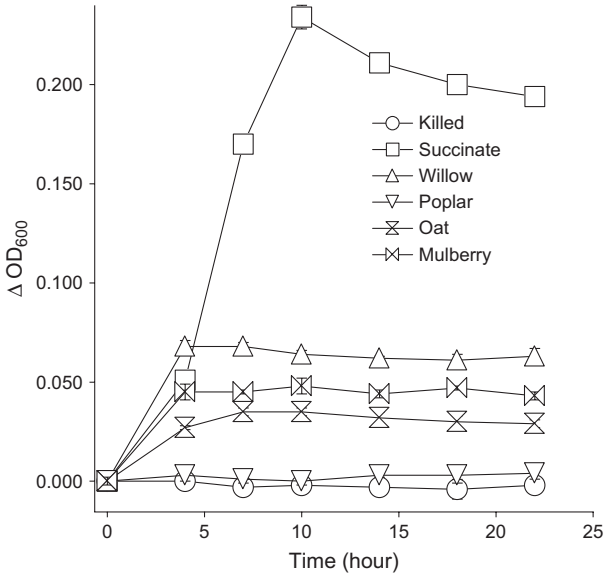

Fig. 1. Growth of *S. yanoikuyae* JAR02 on willow (84.2 mg C l^{-1}) root extracts, poplar (5.7 mg C l^{-1}), oat (7.6 mg C l^{-1}), or red mulberry (43.1 mg C l^{-1}) root exudates, or succinate (96 mg C l^{-1}). Phosphoric acid was used for the killed control. Bars represent one standard deviation from the mean of three replicates and are not shown if smaller than symbol.

root products as a sole carbon and energy source (Figs. 3–4). For root exudates or root extract grown cells, 15 to 20% removal of benzo[*a*]pyrene was observed. Degradation was not concurrent with growth and the rate of degradation was similar to that of cells growing exponentially on succinate. These results suggest that degradation of benzo[*a*]pyrene occurred by constitutively expressed PAH enzymes. Interestingly, similar amounts of benzo[*a*]pyrene loss occurred for cultures that grew to different culture densities. Cells exposed to willow

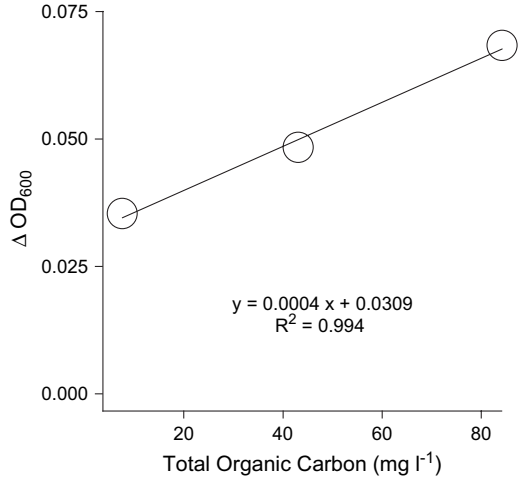

Fig. 2. Correlation between maximal *S. yanoikuyae* JAR02 culture growth and initial root product total organic carbon concentrations shown in Fig. 1.

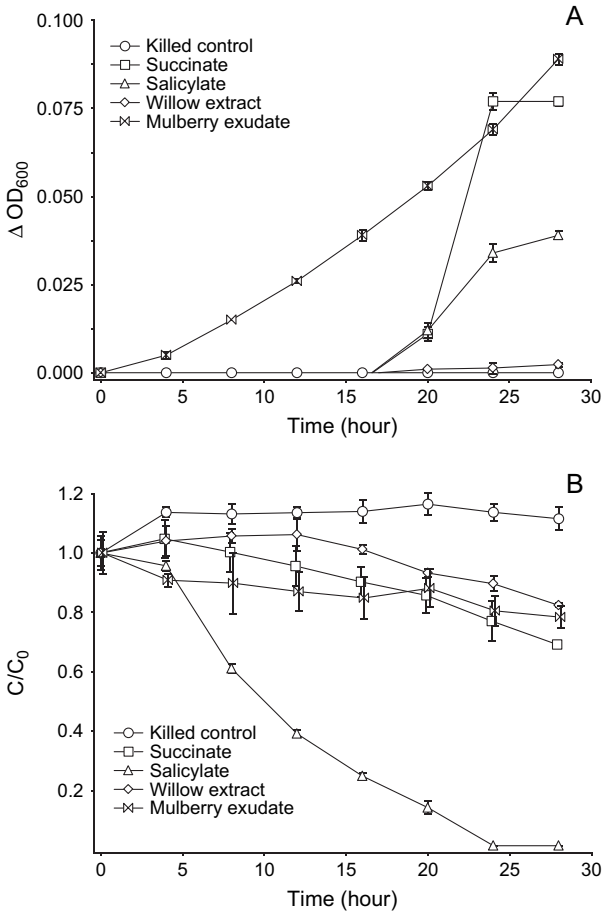

Fig. 3. *Sphingomonas yanoikuyae* JAR02 growth (A) and benzo[*a*]pyrene removal (B) for cultures fed succinate (40 mg C l^{-1}), salicylate (24 mg C l^{-1}), willow (84.2 mg C l^{-1}) extract, or white mulberry (60.1 mg C l^{-1}) exudates. Phosphoric acid was used for the killed control. Initial OD_{600} was ~0.034 and initial benzo[*a*]pyrene concentration was ~1100 μg l^{-1}. Bars represent one standard deviation from the mean of three replicates.

extract as a carbon and energy source demonstrated no growth over 24 h and alternately, cells grown using osage orange root extracts grew with a ΔOD_{600} of ~0.160. Observation of significant benzo[*a*]pyrene loss for cells showing limited growth and significant growth suggested degradation was not necessarily coupled to growth. This was corroborated with the acetonitrile control (Fig. 5), where the presence of no carbon substrate supported no growth, yet 20% benzo[*a*]pyrene loss was observed.

Removal of benzo[*a*]pyrene from solution was significantly less for cultures exposed to root products that for a salicylate induced culture (Fig. 3). Repression of PAH degradation was previously reported for bacteria exposed to root extracts, exudates, and root derived substrates (Kamath et al., 2004; Rentz et al., 2004), and could explain the minimal 15–20% benzo[*a*]pyrene degradation that was observed here. When grown with root products, *S. yanoikuyae* JAR02 was provided with

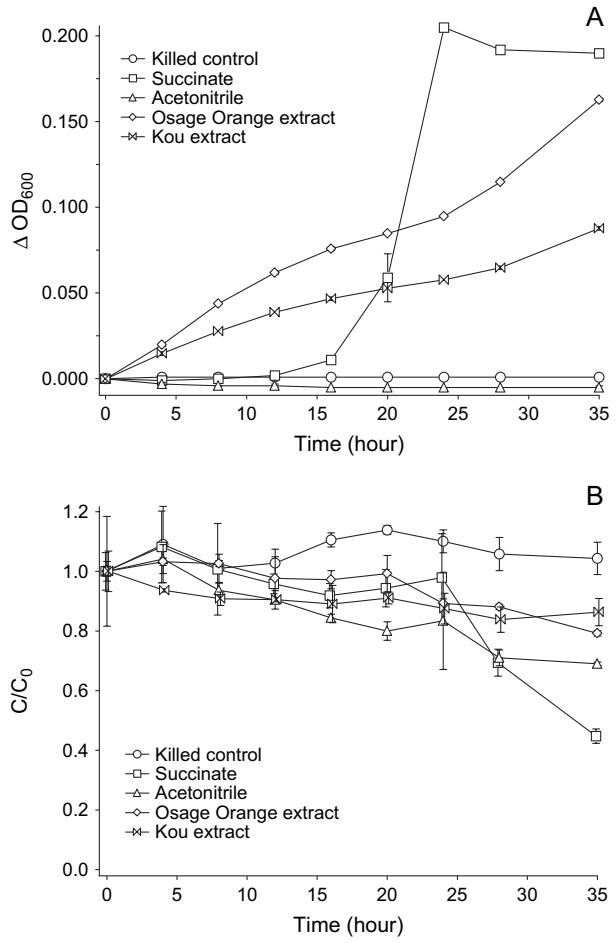

Fig. 4. *Sphingomonas yanoikuyae* JAR02 growth (A) and benzo[*a*]pyrene removal (B) for cultures fed succinate (96 mg C l^{-1}), osage orange (195 mg C l^{-1}), or kou (175 mg C l^{-1}) root extracts. The acetonitrile control contained 0.1% (vol/vol) acetonitrile, the concentration used for introduction of benzo[*a*]pyrene to solution. Phosphoric acid was used for the killed control. Initial OD_{600} was ~0.028 and initial benzo[*a*]pyrene concentration (C_0) was ~1300 μg l^{-1}. The largest standard deviation for the initial samples was for the killed control. Bars represent one standard deviation from the mean of three replicates.

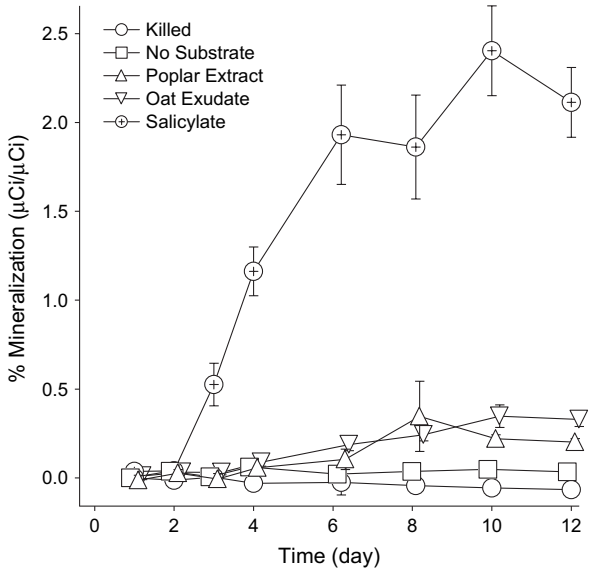

Fig. 5. Mineralization of ^{14}C-7-benzo[*a*]pyrene by *Sphingomonas yanoikuyae* JAR02 grown using root products as a primary substrate. The killed and no substrate controls exhibited no growth and the maximum ΔOD_{600} for the cells grown using poplar extract (78 mg C l^{-1}), oat exudate (36 mg C l^{-1}), and salicylate (75 mg C l^{-1}) was 0.09±0.01, 0.11±0.1, and 0.31±0.01, respectively. Bars represent one standard deviation from the mean of three replicates and are not shown if smaller than symbol.

numerous labile substrates (Rentz et al., 2004; Table 1) that may have limited expression of catabolic genes.

3.3. Benzo[a]pyrene mineralization

S. yanoikuyae JAR02 mineralized benzo[*a*]pyrene when grown using poplar root extracts or oat root exudates as a primary substrate (Fig. 5). In these cases, 0.2±0.01% and 0.3±0.01% $^{14}CO_2$ production was observed for poplar extract and oat exudates, respectively. Mineralization of benzo[*a*]pyrene on this order, 0.2%, was reported for several fungi (Bezalel et al., 1996; Rafin et al., 2000). As with benzo[*a*]pyrene removal from solution, mineralization was much less for root product exposed cultures than those exposed to the positive control, salicylate. In each experiment, nearly all $^{14}CO_2$ production occurred while the bacteria were in stationary phase growth (data not shown).

No radio-labeled metabolites were observed in extracts (ethyl acetate or acetone) from cultures grown using oat exudates or poplar extracts (data not shown), compared to salicylate induced cultures that produced a polar metabolite that accounted for 10% of initial applied radioactivity (Rentz et al., 2004). The minimal 0.2 to 0.3% mineralization may not have yielded appropriate quantities for detection. In addition, the mineralization observed may have resulted from oxidation of radio-labeled impurities (~2.0%). These results suggested that significant degradation of benzo[*a*]pyrene by *S. yanoikuyae* JAR02 requires an inducer, such as salicylate that may not be available or active in the rhizosphere of plants during HMW PAH degradation (Kamath et al., 2004; Rentz et al., 2004).

4. Conclusions

Growth of *Sphingomonas yanoikuyae* JAR02 on plant root exudates and root extracts was observed along with removal of benzo[*a*]pyrene from solution through constitutively expressed degradative enzymes, corroborating field observations of increased PAH degradation in the rhizosphere (Aprill and Sims, 1990; Banks et al., 1999; Binet et al., 2000; Paquin et al.,

2002). Limited mineralization of ^{14}C-7-benzo[*a*]pyrene provided corroborating evidence. This suggests initial ring hydroxylating microorganisms could use plant root products as a sole carbon and energy source for co-metabolism of HMW PAHs during phytoremediation. Within the rhizosphere, initial ring hydroxylating microorganisms could grow to significant population sizes if they can compete with other members of the rhizosphere microbial community for carbon sources. However, significant growth of initial ring hydroxylating bacteria must overcome repression of PAH catabolic genes (Kamath et al., 2004; Rentz et al., 2004) to degrade HMW PAHs in the rhizosphere.

Degradation and mineralization of benzo[*a*]pyrene was much less for root product grown cultures than for cultures exposed to salicylate, and suggested that an inducer was required for significant degradation by *S. yanoikuyae* JAR02. Limitations of HMW PAH rhizoremediation include the need for competent PAH-degrading microbial populations, as HMW PAH and plant root products may not serve as inducers of PAH degradation (Kamath et al., 2004; Rentz et al., 2004). In soils containing naphthalene, phenanthrene, fluoranthene, or pyrene co-contamination, competent microbial populations will likely be present (Siciliano et al., 2001). In these soils, selection of PAH-degrading microbes by degradable PAHs could lead to HMW PAH co-metabolism. Aged soils containing only HMW PAHs may not be effectively bioremediated using rhizosphere processes. These soils would produce increased numbers of bacteria in the root zone, but the absence of a bioavailable selective pressure for PAH-degraders may limit degradation.

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