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B. R. Baldwin · M. B. Mesarch · L. Nies

Broad substrate specificity of naphthalene- and biphenyl-utilizing bacteria

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Abstract Although aromatic compounds are most often present in the environment as components of complex mixtures, biodegradation studies commonly focus on the degradation of individual compounds. The present study was performed to investigate the range of aromatic substrates utilized by biphenyl- and naphthalene-degrading environmental isolates and to ascertain the effects of co-occurring substrates during the degradation of mono-aromatic compounds. Bacterial strains were isolated on the basis of their ability to utilize either biphenyl or naphthalene as a sole source of carbon. Growth and transformation assays were conducted on each isolate to determine the range of substrates degraded. One isolate, *Pseudomonas putida* BP18, was tested for the ability to biodegrade benzene, toluene, ethylbenzene and xylene isomers (BTEX) individually and as components of mixtures. Overall, the results indicate that organisms capable of growth on multi-ring aromatic compounds may be particularly versatile in terms of aromatic hydrocarbon biodegradation. Furthermore, growth and transformation assays performed with strain BP18 suggest that the biodegradation of BTEX and biphenyl by this strain is linked to a catabolic pathway with overlapping specificities. The broad substrate specificity of these environmental isolates has important implications for bioremediation efforts in the field.

Introduction

Many laboratory biodegradation studies focus on single substrates (Chang et al. 1993), neglecting two issues critical to addressing bioremediation in the field. First, environmental isolates will utilize or transform a range of aromatic hydrocarbons in addition to the original isolation substrate (Table 1). Secondly, aromatic hydrocarbons are almost invariably present as mixtures of pollutants, not individual compounds. The resulting substrate interactions include co-metabolism (Alvarez and Vogel 1991; Chang et al. 1993), enhanced biodegradation (Arvin et al. 1989), diauxie metabolism (Meyer et al. 1984) and competitive inhibition (Stringfellow and Aitken 1995) which cannot be determined from single compound degradation assays. The growth and transformation assays listed in Table 1 show that, when examined, polycyclic aromatic hydrocarbon (PAH)-degrading isolates utilize or transform a broad range of multi-ring aromatic compounds, but are rarely screened for the ability to degrade mono-aromatic hydrocarbons. Conversely, organisms isolated on mono-aromatic hydrocarbons tend to be relatively specific for growth on benzene, toluene or xylenes (BTX). Currently, little is known about the ability of PAH-degrading isolates to transform BTX, the contaminants of principal concern at petroleum-contaminated sites. The results of toluene-degradation assays conducted by Foght et al. (1990), however, suggest that PAH-degrading isolates may readily transform BTX even though these substrates do not necessarily support growth (Kästner et al. 1994).

In the present study, bacterial strains were isolated with either biphenyl or naphthalene as the sole source of carbon to determine the substrate specificity and the effects of co-occurring substrates. Each isolate was screened for the ability to metabolize BTX in addition to the isolation substrate. Strains capable of growth on multi-ring aromatic compounds appeared to have broad substrate specificity. One isolate, *Pseudomonas putida* BP18, was tested for the ability to transform BTX and ethylbenzene (E) individually and as components of a BTEX mixture. The presence of growth substrates allowed transformation of compounds not degraded individually.

B. R. Baldwin · M. B. Mesarch · L. Nies (✉)
Environmental and Hydraulic Engineering Area,
School of Civil Engineering, Purdue University,
West Lafayette, IN 47907-1284, USA
e-mail: nies@ecn.purdue.edu
Tel.: +1-765-4948327
Fax: +1-765-4961107

Table 1 Growth and transformation patterns of aromatic hydrocarbon-degrading bacterial strains. *Isolation substrate; +, compound supported growth; –, compound did not support growth; *Tr* transformed but did not necessarily support growth; *–Tr* compound was not transformed. Blank spaces indicate that the compound was not tested. Aromatic substrates: *B* benzene, *T* toluene, *X* xylenes, *N* naphthalene, *MN* methylnaphthalenes, *Bp* biphenyl, *Ac* acenaphene, *Fl* fluorene, *Ph* phenanthene, *An* anthracene, *Fla* fluoranthene, *Py* pyrene, *Ba* benz[a]anthracene, *Cy* chrysene

Isolate	Aromatic substrate													Reference
	B	T	X	N	MN	Bp	Ac	Fl	Ph	An	Fla	Py	Ba	Cy	
Alcaligenes denitrificans WW1				+	+			Tr	+	+	+*	Tr	Tr		Weissenfels et al. 1991
Pseudomonas paucimobilis EPA505				+	+	Tr		Tr	+	+	+*	Tr		Tr	Mueller et al. 1990
Pseudomonas putida OUS82	–Tr	–Tr	–Tr	+		Tr	Tr	Tr	+*	Tr		Tr			Kiyohara et al. 1994
Pseudomonas putida G7				+*					Tr	Tr					Sanseverino et al. 1993
Pseudomonas sp. NCIB10643		Tr		Tr		+			Tr	–Tr					Foght et al. 1990
Pseudomonas sp. D2		Tr		Tr		Tr			Tr	Tr					Foght et al. 1990
Pseudomonas sp. HL7b		Tr		Tr		Tr			Tr	Tr					Foght et al. 1990
Pseudomonas putida NCIB9816		+		+*		Tr			Tr	Tr					Foght et al. 1990
Pseudomonas putida NCIB8858		Tr		+*		–Tr			–Tr	–Tr					Foght et al. 1990
Pseudomonas putida NCIMB11767		+*	+	Tr		Tr									Heald and Jenkins 1996
Pseudomonas putida F1	+	+*				Tr									Furukawa et al. 1993
Pseudomonas putida ATCC33015		+*		Tr		–Tr			–Tr	–Tr					Foght et al. 1990
Pseudomonas putida NCIB12184p		+*		–Tr		–Tr			–Tr	–Tr					Foght et al. 1990
Pseudomonas putida	+	+		–Tr					–Tr	–Tr					Gibson et al. 1968

Materials and methods

Isolation of organisms and growth conditions

Biphenyl-degrading organisms (strains BP11, BP18) were isolated from polychlorobiphenyl (PCB)-contaminated sediments from the Hudson River, N.Y. and an inactive tertiary wastewater lagoon in Gary, Ind., respectively. Naphthalene-degrading organisms (strains N1, N4) were isolated from petroleum-contaminated soils from West Lafayette, Ind., and Parma Heights, Ohio, respectively. One-gram samples of each inoculum were diluted into 10 ml minimal medium (containing per litre: 2 g NH_4Cl, 1 g $NaH_2PO_4 \cdot H_2O$, 4.25 g $K_2HPO_4 \cdot 3H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$, 0.001 g $MnSO_4 \cdot H_2O$, 0.003 g $FeSO_4 \cdot 7H_2O$, 0.025 g $MgSO_4$ and 0.1 g yeast extract). Approximately 0.1 g biphenyl (aqueous solubility, C_{sat} = 7 mg l^{-1}; Mackay et al. 1992) or naphthalene (C_{sat} = 31 mg l^{-1}; Mackay et al. 1992) crystals was added to the medium to select for aromatic hydrocarbon-degrading organisms. Liquid cultures were capped with sterile cotton plugs and grown on a reciprocal shaker at 125 rpm in an incubator at 30 °C overnight. Biphenyl- and naphthalene-degrading organisms were obtained by streaking the liquid culture on to minimal medium agar plates (solidified with 20 g l^{-1} agar) and successively transferring individual colonies to new plates to obtain single isolates. All isolates were initially identified by BIOLOG in the laboratory of Dr. Pedro Alvarez, University of Iowa. *P. putida* BP18 was further identified by fatty acid methyl ester analysis at Microcheck, Inc. (Northfield, VT.), submitted to the American Type Culture Collection and given accession number 700807.

Growth on alternative substrates on solid medium

Single colonies of isolates were transferred to fresh minimal medium plates containing benzoate (10 mM) or crystals of biphenyl, naphthalene, phenanthrene or catechol in the lid. Growth was confirmed by comparison with control plates without substrate. All plates were incubated at 30 °C for 2 weeks unless growth was observed.

Growth on BTEX in liquid medium

BP18 was grown on agar plates with biphenyl serving as the carbon source. Single colonies were transferred to 20 ml yeast extract-free minimal medium to which individual BTEX compounds were added to a concentration of 30 mg l^{-1}. Experiments were performed in 125-ml serum bottles with Teflon-lined rubber septa. Additional experiments with *p*-xylene were performed at 23, 18 and 12 mg l^{-1}. All bottles were incubated at 30 °C on a reciprocal shaker at 125 rpm. Growth was determined by withdrawing samples of culture medium and measuring optical density (800 nm) with a UV-visible recording spectrophotometer (Shimadzu UV160U).

BTX transformation assays with environmental isolates

Initial assays were performed on all four isolates. Biomass was grown under the same conditions as those described above, using biphenyl, naphthalene or 10-mM benzoate as the sole carbon source for each isolate. Biomass was then filtered to remove any remaining biphenyl or naphthalene crystals and harvested by centrifugation at 4000 rpm for 30 min in a Sorvall RC-5B superspeed refrigerated centrifuge. Cells were resuspended in fresh, yeast extract-free minimal medium to an optical density of 1.0 at 640 nm. The cell suspension was then spiked with a mixture of benzene, toluene and total xylenes, giving a final concentration of 35 mg l^{-1} each. Transformation experiments were performed in triplicate with 20-ml samples of cell suspensions in sterile 125-ml crimp-sealed serum bottles with Teflon-lined rubber stoppers. Acid-killed

controls contained 1 ml of 50% (by vol.) hydrochloric acid. After incubation for 48 h at 30 °C, samples were analysed for BTX by gas chromatography (GC) analysis of headspace samples (0.5 ml withdrawn with a syringe and sterile needle) and compared with acid-killed controls.

BTEX transformation assays with *P. putida* BP18

BTEX transformation assays were conducted with cell suspensions of BP18 as above and were sampled periodically by withdrawing 1-ml liquid samples. Assays were run using both the individual BTEX compounds and with a mixture of all six BTEX compounds. Cells were spiked with 15–20 mg l^{-1} of each individual BTEX compound; for the BTEX mixture assay, cells were spiked with 9–12 mg l^{-1} each BTEX compound. BTEX concentrations were determined by withdrawing 1-ml liquid samples using a syringe with a sterile needle and injecting them into 5-ml vials (with Teflon-coated lids) that already contained 1 ml hexane. The vial was shaken for 1 min and the upper (hexane) layer was saved for GC analysis. This procedure routinely yielded an extraction efficiency of 90%.

GC analysis

BTEX concentrations were measured using a Hewlett-Packard 5890-series II gas chromatograph equipped with a cool on-column injection port, a flame-ionization detector, a 60-m DB-1 poly (dimethylsiloxane)-bonded capillary column (J&W Scientific) and Hewlett-Packard HP-CHEM analytical software. Helium (41.4 kPa, 5.5 ml min^{-1}) was used as the carrier gas. The injector temperature was maintained at 3 °C greater than the oven temperature. The oven-temperature programme was 10 min at 35 °C, an increase of 1 °C per min to 40 °C, maintenance for 1 min, an increase of 10 °C per min to 160 °C and maintenance for 10 min. The detector temperature was 210 °C. BTEX concentrations and retention times were determined by comparison with known standards. *p*-xylene and *m*-xylene co-eluted and could not be resolved in BTEX mixture assays.

Results

Initial growth and transformation assays

To varying degrees, each isolate utilized or transformed a range of aromatic compounds (Table 2). Overall, strains capable of growing on two- and three-ring aromatic substrates were also capable of utilizing or transforming smaller aromatic compounds. Two isolates (N1 and BP11), which utilized phenanthrene, utilized or transformed each smaller aromatic compound tested. Strains capable of growth on biphenyl (BP11, BP18 and N1) also utilized naphthalene for growth, but not all strains that could grow on naphthalene utilized biphenyl for growth (N4). Each strain grew on benzoate and catechol, which are key intermediates in the catabolism of aromatic compounds (Cerniglia 1992). BTX transformation assays revealed that each strain degraded BTX to below detectable concentrations. BP18 also transformed BTX following growth on benzoate.

Growth Assays with *P. putida* BP18

BP18 was able to utilize benzene, toluene and ethylbenzene at initial concentrations of 30 mg l^{-1} as sole sources of carbon. While none of the xylene isomers supported growth at 30 mg l^{-1}, accumulations of yellow and pink metabolites indicative of *meta* cleavage products were observed with *p*- and *m*-xylene, indicating partial transformation (Baggi et al. 1987). When the initial substrate concentration was reduced to between 10 mg l^{-1} and 20 mg l^{-1}, *p*-xylene supported growth, but neither *m*- nor *o*-xylene supported growth at the range of substrate concentrations attempted. Furthermore, the *o*-xylene concentration remained constant relative to the acid-killed controls during the course of the experiment and no evidence of metabolites was observed.

Single-compound transformation assays

Biphenyl-grown cell suspensions were spiked with individual BTEX compounds whose concentrations were monitored as functions of time. Each compound was transformed without any indication of a lag phase. Transformation rates, as estimated from pseudo-first-order rate constants, decreased from toluene ($k_{toluene}$ = 0.01 min^{-1}) ≅ *m*-xylene ≅ ethylbenzene > benzene ($0.5k_{toluene}$) > *p*-xylene ($0.3k_{toluene}$) > *o*-xylene ($0.2k_{toluene}$). The growth substrates were degraded to concentrations below the detection limit (Fig. 1A). Although they did not support growth, *m*- and *o*-xylene

Table 2 Growth and transformation patterns for naphthalene- and biphenyl-utilizing environmental isolates. The symbols used are the same as those in Table 1

Substrate	Isolate			
	Clavibacter sp. N1	*Pseudomonas* sp. BP11	*Pseudomonas putida* BP18	*Pseudomonas* sp. N4
Benzene	Tr	Tr	+	Tr
Toluene	Tr	Tr	+	Tr
Xylenes	Tr	Tr	+/Tr[a]	Tr
Naphthalene	+*	+	+	+*
Biphenyl	+	+*	+*	–
Phenanthrene	+	+	–	–

[a] BP18 grew on *p*-xylene at initial concentrations below 20 mg l^{-1}

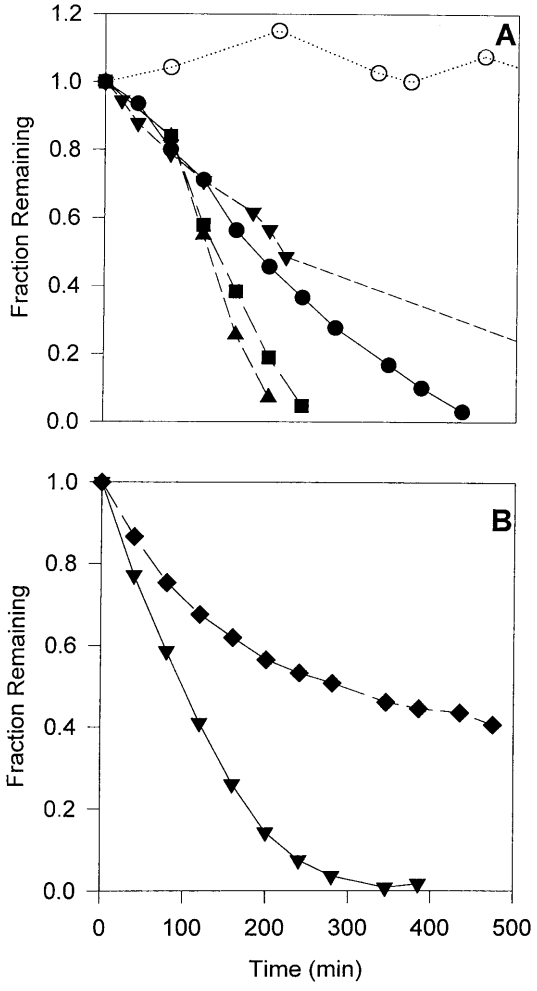

Fig. 1 **A** Transformation of growth-supporting substrates by biphenyl-grown BP18 cells. Benzene (●), toluene (■) and ethylbenzene (▲), *p*-xylene (▼) and a respresentative acid-killed control conducted with ethylbenzene (○). **B** Transformation of non-growth-supporting substrates by biphenyl-grown BP18 cells. ▼ *m*-Xylene, ◆ *o*-xylene. Initial concentrations were approximately 15–20 mg l^{-1}

were also transformed by biphenyl-grown cells. *o*-xylene, however, was still detected (Fig. 1B).

BTEX mixture transformation assays

Biphenyl-grown BP18 cells were spiked with a mixture of BTEX compounds to determine the effects of co-occurring growth and non-growth substrates. All compounds were transformed with a degradation sequence remaining essentially the same as that during single component assays (Fig. 2). The fact that *m*-xylene and *p*-xylene were transformed more slowly than benzene in the mixture may actually result from the inability to analytically resolve these two components when both were present. Unlike the single-compound assays, all components of the mixture including *o*-xylene were transformed to concentrations below the detection limit. Also, a lag period was evident before any appreciable biodegradation of *o*-xylene was observed.

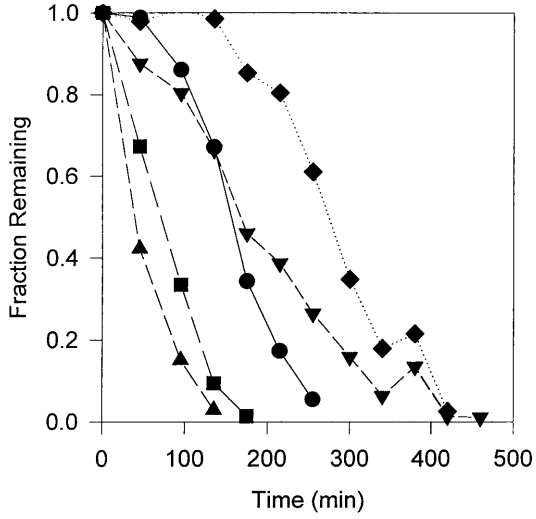

Fig. 2 Transformation of a mixture of benzene, toluene, ethylbenzene and xylene isomers by biphenyl-grown BP18 cells. Benzene (●), toluene (■), ethylbenzene (▲), *p*- and *m*-xylene (▼) and *o*-xylene (◆). Initial concentrations of each compound were between 9 mg l^{-1} and 12 mg l^{-1}

Discussion

Organisms isolated during this study which grew on multi-ring aromatic hydrocarbons were versatile in terms of the range of aromatic compounds serving as growth substrates. Combining the results of our growth and transformation assays (Table 2) with those available in the literature suggests that broad catabolic ability may be common among PAH-degrading strains (Table 1). Conversely, those strains isolated by growth on mono-aromatic compounds seem to be incapable of growth on higher molecular weight substrates and appear relatively specialized for mono-aromatic hydrocarbon degradation (Table 1). Each biphenyl- and naphthalene-utilizing strain was capable of transforming BTX following growth on the isolation substrate. Because successive cleavage of fused aromatic rings during biodegradation of multi-ring aromatic compounds eventually produces mono-aromatic compounds as intermediates, one might expect multi-ring degraders to be competent mono-aromatic-degraders, but reports differ. Kästner et al. (1994) found that although naphthalene-degrading isolates would grow on mono-aromatic compounds, other PAH-degrading isolates did not and these workers suggested that these strains are specialized for PAH utilization. While growth assays with BTX were not conducted with isolates other than BP18, the BTX transformation assays show that these isolates were at least capable of transforming mono-aromatic compounds. The results presented here are consistent with Foght et al. (1990), who suggested that aromatic hydrocarbon degradation may form a hierarchy in which isolates capable of biodegrading tricyclic aromatic compounds will also mineralize dicyclic and mono-aromatic compounds.

Growth and transformation assays conducted on BP18 suggest that the biodegradation of BTEX and biphenyl by this strain is linked to a catabolic pathway with overlapping specificities. Growth assays demonstrated that biphenyl, naphthalene, benzene, toluene, ethylbenzene, *p*-xylene and *m*-xylene each induced expression of at least one catabolic pathway which either supported growth or at least led to transformation. Whereas each substrate could have induced expression of a distinct pathway, biphenyl-grown cells transformed individual BTEX compounds without a lag phase, suggesting that either a constitutively expressed or a biphenyl-induced pathway led to their transformation. The lack of growth or metabolite production during growth assays with *o*-xylene indicated that its transformation is not due to a constitutively expressed pathway and that it is incapable of induction of any pathway leading to its transformation. Transformation of *o*-xylene by biphenyl-grown cells showed that it was probably co-oxidized by the biphenyl-induced pathway. In the BTEX mixture assay, *o*-xylene was transformed to below detectable concentrations, but this was probably due to induction of the pathway by the other mixture components such as ethylbenzene and toluene. Furthermore, the lag time observed before appreciable transformation of *o*-xylene may have resulted from competitive inhibition of *o*-xylene by the growth substrates since transformation of *o*-xylene did not begin until toluene and ethylbenzene were depleted (Fig. 2). The transformation of biphenyl, ethylbenzene (Smith and Ratledge 1989), benzene (Seto et al. 1995) and toluene (Heald and Jenkins 1996) by the same metabolic pathway has been demonstrated. Thus the broad spectrum of substrates and co-substrates transformed by BP18 may result from broad specificity of a single aromatic pathway. While organisms capable of growth on *o*-xylene have been isolated (Baggi et al. 1987), co-oxidation by other aromatic catabolic pathways, as shown by BP18, may significantly contribute to their removal in the field.

Although distinct pathways have been discovered for catabolism of single and multi-ring aromatic compounds, these pathways often proceed by a common method. The mechanistic similarity of these pathways suggests that each of these compounds could be degraded by a single set of enzymes with relaxed substrate specificities. Investigations of the specificity of catabolic enzymes and their regulation have shown that several classical aromatic catabolic pathways can accept alternative substrates and inducing compounds (Denome et al. 1993; Foght and Westlake 1990; Foght and Westlake 1996; Heald and Jenkins 1996; Kuhm et al. 1991; Menn et al. 1993; Sanseverino et al. 1993; Seto et al. 1995; Yang et al. 1994). Such experiments are important from a practical standpoint because biodegradation of the mixture of aromatic hydrocarbons at petroleum-contaminated sites will depend on the specificity of aromatic catabolic pathways as well as their regulation in the presence of multiple substrates. The induction and specificity of aromatic catabolic pathways may be particularly important for biodegradation of these compounds since co-oxidation may be the dominant mechanism for their removal. Biodegradation and bioremediation studies, however, often focus on a narrow range of hydrocarbons examined for their ability to support growth, potentially neglecting not only additional growth substrates but also co-oxidized substrates. The results of this study indicate that a more complete examination of the ability of PAH-degrading organisms to degrade mono-aromatic compounds is warranted. The broad substrate specificity of these organisms suggests that they may play an important role in biodegradation of mixtures of pollutants typically found at petroleum-contaminated sites.

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References

Alvarez PJ, Vogel TM (1991) Substrate interactions of benzene, toluene, and *para*-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries. Appl Environ Microbiol 57: 2981–2985

Arvin E, Jensen BK, Gundersen AT (1989) Substrate interactions during aerobic biodegradation of benzene. Appl Environ Microbiol 55: 3221–3225

Baggi G, Barbieri P, Galli E, Tollari S (1987) Isolation of a *Pseudomonas stutzeri* strain that degrades *o*-xylene. Appl Environ Microbiol 53: 2129–2132

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3: 351–368

Chang M-K, Voice TC, Criddle CS (1993) Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and *p*-xylene by two *Pseudomonas* isolates. Biotechnol Bioeng 41: 1057–1065

Denome SA, Stanley DC, Olson ES, Young KD (1993) Metabolism of dibenzothiophene and naphthalene in *Pseudomonas* strains: complete DNA sequence of a upper naphthalene catabolic pathway. J Bacteriol 175: 6890–6901

Foght JM, Westlake DWS (1990) Expression of dibenzothiophene-degradative genes in two *Pseudomonas* species. Can J Microbiol 36: 718–724

Foght JM, Westlake DWS (1996) Transposon and spontaneous deletion mutants for plasmid-borne genes encoding polycyclic aromatic hydrocarbon degradation by a strain of *Pseudomonas fluorescens*. Biodegradation 7: 353–366

Foght JM, Fedorak PM, Westlake DWS (1990) Mineralization of [^{14}C]hexadecane and [^{14}C]phenanthrene in crude oil: specificity among bacterial isolates. Can J Microbiol 36: 169–175

Furukawa KJ, Hirose J, Suyama A, Zaiki T, Hayashida S (1993) Gene components responsible for discrete substrate specificity in the metabolism of biphenyl (*bph* operon) and toluene (*tod* operon). J Bacteriol 175: 5224–5232

Gibson D, Koch JR, Kallio RE (1968) Oxidative degradation of aromatic hydrocarbons by microorganisms. I. Enzymatic formation of catechol from benzene. Biochemistry 7: 2653–2662

Heald SC, Jenkins RO (1996) Expression and substrate specificity of the toluene dioxygenase of *Pseudomonas putida* NCIMB 11767. Appl Microbiol Biotechnol 45: 56–62

Kästner M, Breuer-Jammali M, Mahro B (1994) Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). Appl Microbiol Biotechnol 41: 267–273

Kiyohara H, Torigoe S, Kaida N, Asaki T, Iida T, Hayashi H, Takizawa N (1994) Cloning and characterization of a chromosomal gene cluster, *pah*, that encodes the upper pathway for phenanthrene and naphthalene utilization by *Pseudomonas putida* OUS82. J Bacteriol 176: 2439–2443

Kuhm AE, Stolz A, Knackmuss H-J (1991) Metabolism of naphthalene by the biphenyl-degrading bacterium *Pseudomonas paucimobilis* Q1. Biodegradation 2: 115–120

Mackay D, Shiu WY, Ma KC (1992) Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals, vol II. Polynuclear aromatic hydrocarbons, polychlorinated dioxins, and dibenzofurans. Lewis, Boca Raton

Menn F-M, Applegate BM, Sayler GS (1993) NAH plasmid-mediated catabolism of anthracene and phenanthrene to naphthoic acids. Appl Environ Microbiol 59: 1938–1942

Meyer JS, Marcus MD, Bergman HL (1984) Inhibitory interactions of aromatic organics during microbial degradation. Environ Toxicol Chem 3: 583–587

Mueller JG, Chapman PJ, Blattmann BO, Pritchard PH (1990) Isolation and characterization of a fluoranthrene-utilizing strain of *Pseudomonas paucimobilis*. Appl Environ Microbiol 56: 1079–1086

Sanseverino J, Werner C, Fleming J, Applegate B, King JMH, Sayler GS (1993) Molecular diagnostics of polychlorinated aromatic hydrocarbon biodegradation in manufactured gas plant soils. Biodegradation 4: 303–321

Seto M, Kimbara K, Shimura M, Hatta T, Fukuda M, Yano K (1995) A novel transformation of polychlorinated biphenyls by *Rhodococcus* sp. Strain RHA1. Appl Environ Microbiol 61: 3353–3358

Smith MR, Ratledge C (1989) Catabolism of biphenyl by *Pseudomonas* sp. NCIB 10643 and *Nocardia* sp. NCIB 10503. Appl Microbiol Biotechnol 30: 395–401

Stringfellow WT, Aitken MD (1995) Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene-degrading Pseudomonads. Appl Environ Microbiol 61: 357–362

Weissenfels WD, Beyer M, Klein J, Rehm HJ (1991) Microbial metabolism of fluoranthrene: isolation and identification of ring fission products. Appl Microbiol Biotechnol 34: 528–535

Yang Y, Chen RF, Shiaris MP (1994) Metabolism of naphthalene, fluorene, and phenanthrene: preliminary characterization of a cloned gene cluster from *Pseudomonas putida* NCIB 9816. J Bacteriol 176: 2158–2164