Biodegradation (2008) 19:859–881
DOI 10.1007/s10532-008-9189-z

ORIGINAL PAPER

Genomic analysis of polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1

Seong-Jae Kim · Ohgew Kweon · Richard C. Jones · Ricky D. Edmondson · Carl E. Cerniglia

Received: 23 January 2008 / Accepted: 28 March 2008 / Published online: 18 April 2008

Abstract *Mycobacterium vanbaalenii* PYR-1 is well known for its ability to degrade a wide range of high-molecular-weight (HMW) polycyclic aromatic hydrocarbons (PAHs). The genome of this bacterium has recently been sequenced, allowing us to gain insights into the molecular basis for the degradation of PAHs. The 6.5 Mb genome of PYR-1 contains 194 chromosomally encoded genes likely associated with degradation of aromatic compounds. The most distinctive feature of the genome is the presence of a 150 kb major catabolic region at positions 494 ~ 643 kb (region A), with an additional 31 kb region at positions 4,711 ~ 4,741 kb (region B), which is predicted to encode most enzymes for the degradation of PAHs. Region A has an atypical mosaic structure made of several gene clusters in which the genes for PAH degradation are complexly arranged and scattered around the clusters. Significant differences in the gene structure and organization as compared to other well-known aromatic hydrocarbon degraders including *Pseudomonas* and *Burkholderia* were revealed. Many identified genes were enriched with multiple paralogs showing a remarkable range of diversity, which could contribute to the wide variety of PAHs degraded by *M. vanbaalenii* PYR-1. The PYR-1 genome also revealed the presence of 28 genes involved in the TCA cycle. Based on the results, we proposed a pathway in which HMW PAHs are degraded into the β-ketoadipate pathway through protocatechuate and then mineralized to CO_2 via TCA cycle. We also identified 67 and 23 genes involved in PAH degradation and TCA cycle pathways, respectively, to be expressed as proteins.

Keywords Degradation · Genomic analysis · *Mycobacterium vanbaalenii* PYR-1 · Polycyclic aromatic hydrocarbons · Proteome analysis

S.-J. Kim and O. Kweon contributed equally to this work.

S.-J. Kim · O. Kweon · C. E. Cerniglia (✉)
Division of Microbiology, National Center for Toxicological Research/U.S. FDA, Jefferson, AR 72079, USA
e-mail: carl.cerniglia@hhs.fda.gov

R. C. Jones · R. D. Edmondson
Division of Systems Toxicology, National Center for Toxicological Research/U.S. FDA, Jefferson, AR 72079, USA

Present Address:
R. C. Jones
NextGen/PRS, Ann Arbor, MI 48108, USA

Present Address:
R. D. Edmondson
Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR 72305, USA

Abbreviations

CYPs	Cytochrome P450 monooxygenases
HMW	High-molecular-weight
JGI	Joint Genome Institute

KEGG	Kyoto Encyclopedia of Genes and Genomes
ORFs	Open reading frames
PAHs	Polycyclic aromatic hydrocarbons
RHOs	Ring-hydroxylating oxygenases

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of the most ubiquitous classes of organic compounds commonly detected in the environment. They are often formed as a result of incomplete combustion of organic matter (IARC 1983). Over the years, extensive efforts have been devoted to develop bioremediation strategies since many PAHs and their metabolic intermediates transformed by mammalian enzyme systems are toxic, mutagenic, and carcinogenic (Penning et al. 1999; Bolton et al. 2000). High-molecular-weight (HMW) PAHs, with four or more fused benzene rings, are particularly troubling contaminants since they are generally less soluble, highly hydrophobic, and are known to be more difficult to be degraded and to persist in the environment (Kanaly and Harayama 2000).

Mycobacterium vanbaalenii PYR-1 was originally isolated in 1986 from oil-contaminated estuarine sediment exposed to petrogenic chemicals in the watershed of Redfish Bay, Texas (Khan et al. 2002; Kim et al. 2005a). It was the first bacterium reported to degrade the HMW PAH pyrene (Heitkamp and Cerniglia 1988). *M. vanbaalenii* PYR-1 has the ability to degrade many other PAHs, including some of the HMW PAHs such as fluoranthene, 1-nitropyrene, benzo[*a*]pyrene, benz[*a*]anthracene, and 7,12-dimethylbenz[*a*]anthracene (Heitkamp and Cerniglia 1988, 1989; Kelley et al. 1991; Kelley and Cerniglia 1995; Moody et al. 2003, 2004, 2005). Due to its versatile PAH degradability, this bacterium has been studied for the purpose of elucidating PAH degradation pathways along with other *Mycobacterium* spp. isolated from PAH contaminated soils (Grosser et al. 1991; Boldrin et al. 1993; Schneider et al. 1996; Vila et al. 2001; López et al. 2005). Attempts have also been made to apply strain PYR-1 to remediate PAHs-contaminated soils (Heitkamp and Cerniglia 1989; Ramirez et al. 2001; MacLeod and Daugulis 2003). However, despite years of research, many critical aspects related to PAH metabolism by mycobacteria, including the metabolite gaps in HMW PAH degradation pathways; the enzymatic and molecular basis and regulatory mechanisms, remain unknown.

In recent years, the catabolism of aromatic compounds in several other bacterial species, such as *Pseudomonas*, *Burkholderia*, *Acinetobacter*, and *Rhodococcus*, have been evaluated at the genomic level (Jimenez et al. 2002; Barbe et al. 2004; Chain et al. 2006; McLeod et al. 2006). The genome-based sequence information has provided a considerable body of knowledge with respect to the degradation of aromatic compounds. However, those genomes were mostly involved in the degradation of monocyclic aromatic compounds or low-molecular-weight PAHs and no reports have described HMW PAH degradation. It has been shown that genes and catabolic processes for the degradation of aromatic compounds in mycobacteria are not closely related to their counterparts from other microorganisms (Habe and Omori 2003). For example, oligonucleotide primers designed to specify dioxygenase genes from mycobacterial species have low homologies to those classical *nahAc*, *phnAc*, and *bphA1* genes from PAH-degrading *Pseudomonas*, *Sphingomonas*, and *Burkholderia* species (Hamann et al. 1999; McLellan et al. 2002; Brezna et al. 2003; Hall et al. 2005; Zhou et al. 2006). This suggests either different origins for the aromatic catabolic genes by *M. vanbaalenii* PYR-1 or significant genetic divergence from these other bacterial isolates. Previously, we have identified parts of genes and enzymes involved in aromatic hydrocarbon degradation by strain PYR-1 (Khan et al. 2001; Kim et al. 2004a, b, 2006; Stingley et al. 2004; Brezna et al. 2006). Recently, we studied strain PYR-1 grown on PAHs using functional genomic approaches (Kim et al. 2007; Kweon et al. 2007), which established the groundwork for the PYR-1 genome analysis in relation to PAH degradation. In the current study, we analyzed the PYR-1 complete genome sequence to understand the molecular background for PAH metabolism. We have particularly focused on the aromatic catabolic genes and TCA cycle genes that are responsible for the complete degradation of PAHs. In addition, PYR-1 proteome profiles were examined to see whether the genome analysis is supported at the protein level.

Materials and methods

Genome analysis with respect to PAH degradation

The complete *M. vanbaalenii* PYR-1 genome sequence is available at the Joint Genome Institute (JGI) (http://img.jgi.doe.gov) and NCBI (accession no. CP000511). The automatically generated genes by JGI were reanalyzed with respect to PAH metabolism. BLASTP searches were conducted with the non-redundant protein database (Altschul et al. 1997). Functionally characterized proteins or most similar proteins of strain PYR-1 were taken into account for reassignment of functions. Since many genes for enzymes functionally identical to those in the aromatic degradation pathway have been characterized in other organisms, such as genes for ring-hydroxylating oxygenases, we were able to search putative genes for enzymes in the PAH pathway from the PYR-1 genome. Paralog groups were identified using JGI system based on BLASTP hits, with cutoff values of $<10^{-5}$ (E-value). We also picked ORFs of interest by keywords and BLAST searches using the PYR-1 JGI database. Selected ORFs were further analyzed for the conservation of the molecular structures in comparison with other aromatic degrading enzymes. Missing genes were also checked in the JGI initial annotation and start/stop positions of some genes were revised using the Artemis comparison tool (Carver et al. 2005).

Reconstruction of the TCA cycle

The *M. vanbaalenii* PYR-1 genome was examined for sequence similarity to enzymes participating in the TCA cycle. Initially, biochemical reactions in the TCA cycle that have been proposed to be operative in this organism were collected from the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/pathway/map/) (Kanehisa et al. 2006). ORFs predicted by KEGG were thoroughly analyzed and verified for the respective reaction steps. Genes were additionally assigned using BLAST tool, especially when gaps in the pathway were found. Similarity searches were based on the same cutoff values of $<10^{-5}$ (E-value).

Proteome data normalization

Previously, PYR-1 proteomes from pyrene- and fluoranthene-supplemented media were identified in two independent studies using sorbitol as a control medium for each PAH induction condition (Kim et al. 2007; Kweon et al. 2007). Briefly, proteins were separated by SDS-PAGE, excised into 40 equal bands and each band analyzed by nano liquid chromatography coupled to tandem mass spectrometry. The product ion data were searched against the *M. vanbaalenii* PYR-1 translated genome sequence and the database search results were collated into non-redundant lists. The total number of peptides per protein (spectral count) was used for approximate relative quantitation between samples. The sum of the spectral count per lane from all identified proteins was used for normalization to compensate for analytical drift in the system; arbitrarily we normalized with respect to the control lane.

Alignments and phylogenetic analyses

Pairwise and multiple alignments were performed using CLUSTALX version 1.83 (Thompson et al. 1997) with all parameters set to their default values. The phylogenetic tree was constructed for 47 oxygenase sequences by the Neighbor-Joining method (Saitou and Nei 1987) then visualized with TREEVIEW (Page 1996). The reliability of the tree obtained was evaluated by 1,000 bootstrap replications.

Results and discussion

Overview of the genome analysis in relation to PAH metabolism

The recent complete sequencing of the *M. vanbaalenii* PYR-1 genome and four other closely related *Mycobacterium* spp. conducted by the JGI/Integrated Microbial Genome (IMG) has enabled genomic studies that could provide the molecular basis of metabolic versatility as well as deeper insight into unique biology of the genus *Mycobacterium*. The 6.5 Mb (6,491,865 bp) genome of strain PYR-1 has been shown to contain 5,979 predicted protein coding sequences in a single circular chromosome with an average G + C content of 67%. The PYR-1 genome sequence confirmed the existence of the previously reported catabolic genes involved along with their relative positions on the genome. Additional paralogs

Table 1 ORFs identified in the PYR-1 genome containing the genes involved in the PAHs degradation in *M. vanbaalenii* PYR-1

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
0012		Cytochrome P450 monooxygenase				MonD	36	*Streptomyces cinnamonensis* ATCC15413	AF440781
0245		Cytochrome P450 monooxygenase				ORF42	33	*Streptomyces globisporus* C-1027	AY048670
0246		Cytochrome P450 monooxygenase				**NikQ**	41	*Streptomyces tendae* Tue901	AJ250581
0317		Cytochrome P450 monooxygenase				**PksS**	33	*Bacillus subtilis* subsp. subtilis 168	EF546698
0373		Cytochrome P450 monooxygenase				**MorA**	37	*Mycobacterium chlorophenolicum* PCP-1	AY960119
0401		Cytochrome P450 monooxygenase				**CypEA**	54	*Streptomyces tubercidicus* I-1529	AY549197
0462	PhtR	Putative transcriptional regulator	3.5	5.4	3.8	PadR2	68	*Rhodococcus* sp. RHA1	AB154537
0463	**PhtAa**	Phthalate 3,4-dioxygenase, α subunit	0.0	9.5	0.0	**PhtA1**	75	*Terrabacter* sp. DBF63	AB084235
0464	**PhtAb**	Phthalate 3,4-dioxygenase, β subunit	0.0	5.4	0.0	**PhtA2**	68	*Terrabacter* sp. DBF63	AB084235
0466	**PhtB**	3,4-Dihydroxy-3,4-dihydrophthalate dehydrogenase	0.0	17.6	0.0	**PhtB**	64	*Terrabacter* sp. DBF63	AB084235
	PhtAc	Phthalate dioxygenase ferredoxin subunit	1.5	2.7	1.9	**PhtAc**	69	*Arthrobacter keyseri* 12B	AF331043
0467	**PhtAd**	Phthalate dioxygenase reductase subunit	3.0	17.6	1.9	**PhtAd**	58	*Arthrobacter keyseri* 12B	AF331043
0468	PhdI	1-Hydroxy-2-naphthoate dioxygenase	3.0	14.9	2.8	**PhdI**	45	*Nocardioides* sp. KP7	D89987
0469	PhdJ	*trans*-2'-Carboxybenzalpyruvate hydratase-aldolase	4.0	9.5	1.9	**PhdJ**	56	*Nocardioides* sp. KP7	D89988
0470	PhdF	Ring-cleavage dioxygenase	0.0	18.9	7.6	PhdF	83	*Nocardioides* sp. KP7	AB031319
0472	PhdG	Hydratase-aldolase	10.0	21.6	7.6	PhdG	87	*Nocardioides* sp. KP7	AB031319
0483	**NidB2**	Ring-hydroxylating dioxygenase β subunit	0	8.1	0.0	**PdoB1**	98	*Mycobacterium* sp. S65	AJ494744
0484		Succinate dehydrogenase/fumarate reductase	2	31.1	0.0	SdhA	27	*Methanothermobacter thermautotrophicus*	AJ000941
0485		4Fe-4S ferredoxin				**OorD**	34	*Helicobacter pylori* NCTC 11637	AF021094
0486	NidD	Aldehyde dehydrogenase	11.5	46.0	6.6	PhdH	85	*Nocardioides* sp. KP7	AB031319
0487	**NidB**	Pyrene hydroxylating dioxygenase β subunit				**PdoB1**	100	*Mycobacterium* sp. S65	AJ494744
0488	**NidA**	Pyrene hydroxylating dioxygenase α subunit	0	13.5	0.0	**PdoA1**	98	*Mycobacterium* sp. S65	AJ494744
0489	ORF22	Putative short-chain alcohol dehydrogenase	0	9.5	0.0	NidC	99	*Mycobacterium* sp. S65	AF546904
0490	ORF23	Putative zinc-containing alcohol dehydrogenase	2.5	12.2	0.0	NidH	99	*Mycobacterium* sp. S65	AF546904
0492	ORF25	Ring hydroxylating oxygenase α subunit	0	5.4	0.0	**AhdA1e**	43	*Sphingomonas* sp. P2	AB091693

Table 1 continued

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
0493	ORF26	Ring-hydroxylating oxygenase β subunit	0	6.8	0.0	**AhdA2e**	38	*Sphingomonas* sp. P2	AB091693
0495	ORF28	Protocatechuate 3,4-dioxygenase alpha subunit				**PcaG**	41	*Terrabacter* sp. DBF63	AP008980
0521		Epoxide hydrolase I	6.5	13.5	6.6	**Ephx1**	36	*Oryctolagus cuniculus*	M21496
0522		2-Carboxybenzaldehyde dehydrogenase	9	29.8	10.4	**PhdK**	60	*Nocardioides* sp. KP7	D89989
0524	NidR	Transcriptional regulator				**SlyA**	31	*Salmonella typhimurium*	P40676
0525	**NidA3**	Fluoranthene hydroxylating dioxygenase α subunit	30.5	35.2	61.7	**PdoA**	97	*Terrabacter* sp. HH4	DQ118530
0526	**NidB3**	Fluoranthene hydroxylating dioxygenase β subunit	23.5	33.8	18.0	**PdoB**	98	*Terrabacter* sp. HH4	DQ118530
0527	ORF4	Putative alcohol dehydrogenase	11.5	9.5	6.6	PdoH	41	*Mycobacterium* sp. S65	AF546905
0532		Phthalate dioxygenase ferredoxin subunit				**PhtAc**	63	*Arthrobacter keyseri* 12B	AF331043
0533		Ring hydroxylating oxygenase α subunit	10	9.5	14.2	**DfdA1**	38	*Terrabacter* sp. YK3	AB075242
0534		Ring hydroxylating oxygenase β subunit	12.5	10.8	10.4	**BnzA2**	38	*Rhodococcus opacus* B-4	AB193045
0536		3-Hydroxyisobutyrate dehydrogenase	2	0.0	3.8	**MmsB**	32	*Pseudomonas aeruginosa* PAO	P28811
0537		Transcriptional regulator	1.5	1.4	0.0	OrfX	30	*Delftia tsuruhatensis* AD9	AY940090
0538		*Meta*-cleavage product hydrolase	32.5	24.4	32.3	FlnE	44	*Terrabacter* sp. DBF63	AB095015
0539		Ring hydroxylating oxygenase α subunit	39.5	47.4	59.8	**DbfA1**	38	*Terrabacter* sp. DBF63	AB054975
0540		Ring hydroxylating oxygenase β subunit	38	23.0	36.1	**DbfA2**	39	*Terrabacter* sp. DBF63	AB054975
0541		Phthalate dihydrodiol dehydrogenase	34	29.8	31.3	**OphB**	35	*Burkholderia cepacia* DBO1	AF095748
0542		Catalytic subunit of *meta*-cleavage enzyme	11.5	13.5	10.4	**CarBb**	26	*Nocardioides aromaticivorans* IC177	AB244528
0543		3,4-Dihydroxyphthalate 2-decarboxylase	5	10.8	3.8	**PhtC**	74	*Arthrobacter keyseri* 12B	AF331043
0544		Dihydrodiol dehydrogenase	0	8.1	3.8	**PhdE**	76	*Nocardioides* sp. KP7	AB031319
0545		Ring-cleavage dioxygenase				PhdF	83	*Nocardioides* sp. KP7	AB031319
0546		Ring-hydroxylating dioxygenase α subunit	0	81.2	38.9	**PdoA2**	99	*Mycobacterium* sp. 6PY1	AJ494743
0547		Ring-hydroxylating dioxygenase β subunit	0	37.9	19.9	**PdoB2**	99	*Mycobacterium* sp. 6PY1	AJ494743
0549		3Fe-4S ferredoxin				**PhdC**	55	*Nocardioides* sp. KP7	AB031319
0558		Hydratase-aldolase	0	28.4	15.2	NarC	27	*Nocardioides* sp. KP7	AB031319
0559		Regulatory protein				PcaR	62	*Terrabacter* sp. DBF63	AP008980
0560		Protocatechuate 3,4-dioxygenase β subunit	13	16.2	9.5	**PcaH**	71	*Terrabacter* sp. DBF63	AP008980
0561		Protocatechuate 3,4-dioxygenase α subunit	8.5	4.1	6.6	**PcaG**	55	*Terrabacter* sp. DBF63	AP008980
0562		β-Carboxy-*cis*,*cis*-muconate cycloisomerase	32	41.9	35.1	PcaB	45	*Terrabacter* sp. DBF63	AP008980

Table 1 continued

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
0563		γ-Carboxymuconolactone decarboxylase/ β-ketoadipate enol-lactone hydrolase	9	18.9	10.4	**PcaL**	39	*Rhodococcus opacus* 1CP	AF003947
0564		β-Ketoadipate CoA-transferase α subunit	8	2.7	7.6	**PcaI**	65	*Terrabacter* sp. DBF63	AP008980
0565		β-Ketoadipate CoA-transferase β subunit	5	4.1	2.8	**PcaJ**	65	*Terrabacter* sp. DBF63	AP008980
0591		2,3-Dihydroxybiphenyl 1,2-dioxygenase				**PcbC**	35	*Pseudomonas* sp. DJ-12	D44550
0592		2-Hydroxy-6-phenyl-6-oxo-2,4-dienoic acid hydrolase				**HbpD**	39	*P. azelaica* HBP1	U73900
0593		3-(3-Hydroxy-phenyl)propionate hydroxylase				**MhpA**	35	*Escherichia coli*	P77397
0594		Bacterial transcription regulator, TetR family				**CprB**	72	*Streptomyces coelicolor* M130	AB000385
0595		2-Hydroxypent-2,4-dienoate hydratase				**AphE**	47	*Comamonas testosteroni* TA441	AB029044
0596		Acetaldehyde dehydrogenase (Acylating)				AmnH	54	*Pseudomonas* sp. AP-3	AB020521
0597		4-Hydroxy-2-oxovalerate aldolase				**CmtG**	70	*Pseudomonas putida* F1	U24215
0600		Cytochrome P450 monooxygenase	2.5	0.0	2.8	**Ema7**	66	*Streptomyces* sp. IHS-0435	AY549186
0675		Cytochrome P450 monooxygenase				SpiL	28	*Sorangium cellulosum* So ce90	AM407731
0682		Cytochrome P450 monooxygenase				**Cyp230**	47	*Streptomyces tubercidicus* R-922	AY549204
0735		Superoxide dismutase	11	12.2	9.5	**SodC**	64	*Mycobacterium avium* subsp. paratuberculosis	AF326234
0779		Cytochrome P450 monooxygenase				**P450RhF**	35	*Rhodococcus* sp. NCIMB 9784	AF459424
0858		Cytochrome P450 monooxygenase				**CYP4C15**	27	*Orconectes limosus*	AF091117
0908		Ring hydroxylating oxygenase α subunit				**PsbAb**	29	*Rhodopseudomonas palustris* No.7	AB022919
0909		Ferredoxin component				**PhlF**	40	*Pseudomonas putida* H	X80765
0916		3-Ketoadipyl-CoA thiolase	0	1.4	0.0	**DitO**	45	*P. abietaniphila* BKME-9	AF119621
1001		3-Chlorobenzoate-3,4/4,5-dioxygenase				**CbaA**	28	*Conidiobolus coronatus* BR60	U18133
1038		Cytochrome P450 monooxygenase				SpiL	38	*Sorangium cellulosum* So ce90	AM407731
1290		Reductase component	4	0.0	2.8	**PadAd1**	41	*Rhodococcus* sp. RHA1	AB154536
1301		Cytochrome P450 monooxygenase				**Cyp108**	40	*Pseudomonas* sp.	M91440
1302		Cytochrome P450 monooxygenase				**Cyp108**	40	*Pseudomonas* sp.	M91440

Table 1 continued

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
1848	**CYP151**	Cytochrome P450 monooxygenase				**MorA**	86	*Mycobacterium* sp. RP1	AJ310142
2001		Cytochrome P450 monooxygenase				**PksS**	31	*Bacillus subtilis* subsp. subtilis 168	EF546698
2007		Cytochrome P450 monooxygenase				**PicK**	34	*Streptomyces venezuelae* ATCC15439	AF079139
2012		Ring hydroxylating oxygenase				KshA	29	*Rhodococcus erythropolis*	AY083508
2021		Cytochrome P450 monooxygenase				**Cyp108**	39	*Pseudomonas* sp.	M91440
2031		Cytochrome P450 monooxygenase				ORF R12	45	*Mycobacterium abscessus* 390R	AF513500
2039	**PQR**	Phenanthrene quinone reductase							
2234		Cytochrome P450 monooxygenase				MonD	45	*Streptomyces cinnamonensis* ATCC15413	AF440781
2239		Cytochrome P450 monooxygenase				**Ema7**	28	*Streptomyces* sp. IHS-0435	AY549186
2422		Cytochrome P450 monooxygenase				**P450RhF**	44	*Rhodococcus* sp. NCIMB 9784	AF459424
2869		Ring hydroxylating oxygenase				KshA	59	*R. erythropolis* SQ1	AY083508
2889		Regulatory proteins, IclR							
2891		2,3-Dihydroxybiphenyl 1,2-dioxygenase				**BphC1**	70	*R. erythropolis* TA421	D88013
2893		Reductase component				**TdnB**	35	*Frateuria* sp. ANA-18	AB089795
2894		Cyclohexanone monooxygenase				TrkA	62	*Rhodococcus* sp. RHA1	YP708237
2897		Reductase component				**KshB**	61	*Rhodococcus erythropolisv* SQ1	AAL96830
2898		2-hydroxy-6-phenylhexa-2,4-dienoate hydrolase				**BphD**	39	*P. putida* KF715	M33813
2912		2-hydroxy-6-oxohepta-2,4-dienoate hydrolase				**EtbD2**	31	*Rhodococcus* sp. RHA1	AB154536
2984	**KatG**	Catalase peroxidase				**KatG**	68	*Mycobacterium tuberculosis*	DQ056357
3012		Cytochrome P450 monooxygenase	1	0.0	1.9	MonD	45	*Streptomyces cinnamonensis* ATCC15413	AF440781
3029		Cytochrome P450 monooxygenase	3.5	2.7	3.8	MonD	45	*Streptomyces cinnamonensis* ATCC15413	AF440781
3104		Cyclohexanone monooxygenase	2	1.4	3.8	TrkA	66	*Mycobacterium avium 104*	YP881381
3108		Cytochrome P450 monooxygenase	1.5	2.7	1.9	SpiL	33	*Sorangium cellulosum* So ce90	AM407731
3118		*O*-Methyltransferase				TcmP	34	*Streptomyces glaucescens*	M80674

Table 1 continued

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
3157		4,5-Dihydroxyphthalate decarboxylase				TauA	39	*Aurantimonas sp. SI85-9A1*	ZP01226528
3187		Cytochrome P450 monooxygenase				ABR68807	35	*Streptomyces virginiae* IBL14	EF646281
3201		Catalase	6	9.5	4.7	**KatE**	51	*Mycobacterium avium*	L41246
3208		Peroxidase/catalase 1	13	16.2	12.3	**KatGI**	83	*Mycobacterium fortuitum*	Y07865
3280	**COMT**	Catechol *O*-methyltransferase	6	9.5	7.6	**COMT**	38	*Sus scrofa*	Q99028
3427		Cyclohexanone monooxygenase				**HhapE**	32	*P. fluorescens* ACB	AF355751
3495		Ferredoxin component				NirD	43	*Rhodobacter capsulatus*	AY273169
3781		Cytochrome P450 monooxygenase				**PiKC**	34	*Streptomyces venezuelae* ATCC15439	AF079139
3784		Ring hydroxylating oxygenase				KshA	25	*R. erythropolis* SQ1	AY083508
3974		Cytochrome P450 monooxygenase				**PiKC**	34	*Streptomyces venezuelae* ATCC15439	AF079139
3992		Cytochrome P450 monooxygenase				MonD	31	*Streptomyces cinnamonensis* ATCC15413	AF440781
4141	**CYP150**	Cytochrome P450 monooxygenase				ABR68807	29	*Streptomyces virginiae* IBL14	EF646281
4160		Cytochrome P450 monooxygenase				**NikQ**	30	*Streptomyces tendae* Tue901	AJ250581
4175		Cytochrome P450 monooxygenase				BioI	34	*Bacillus subtilis* OK2	AB088066
4180		Cytochrome P450 monooxygenase				ABR68807	29	*Streptomyces virginiae* IBL14	EF646281
4184		Ring-hydroxylating oxygenase α subunit				**PhdA**	30	*Nocardioides* sp. KP7	AB031319
4186		Reductase component				KshB	36	*R. erythropolis* SQ1	AY083509
4190		Ring hydroxylating oxygenase α subunit				Dio	32	*Arthrobacter globiformis* NRRL B-2979	AF329477
4206		3-Ketoadipyl-CoA thiolase				ro03951	50	*Rhodococcus* sp. RHA1	CP000431
4213		Ring hydroxylating oxygenase α subunit				**DntAc**	36	*Burkholderia* sp. DNT	U62430
4221		Cytochrome P450 monooxygenase				CYP189	65	*Mycobacterium ulcerans* Agy99	YP904524
4234		Cytochrome P450 monooxygenase				MoxA	**44**	*Nonomuraea recticatena*	AB180844
4235		Ferredoxin component				**Fd230**	54	*Streptomyces tubercidicus* R-922	AY549204
4236		Regulatory protein, TetR				**PltZ**	31	*Pseudomonas* sp. M18	AY394844
4237		2,5-Dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase				LinX3	37	*Sphingomonas paucimobilis* B90	AY150580

Table 1 continued

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
4238		2-Hydroxy-6-oxohepta-2,4-dienoate hydrolase				**EtbD1**	34	*Rhodococcus* sp. RHA1	AB004320
4239		Reductase component				**Azr**	26	*Bacillus subtilis* ATCC6633	AB071366
4244		Extradiol dioxygenase				**BphC3**	54	*R. rhodochrous* K37	AB117721
4245		Monooxygenase				**MhpA**	47	*C. testosterone* TA441	AB024335
4246		*meta*-Cleavage compound hydrolase				flnE	41	*Terrabacter* sp. DBF63	AP008980
4247		Regulatory proteins, IclR family							
4249		Regulatory protein, TetR family							
4317		Cytochrome P450 monooxygenase				ABR68807	33	*Streptomyces virginiae* IBL14	EF646281
4319		Ring hydroxylating oxygenase α subunit				**DbfA1**	26	*Paenibacillus* sp. YK5	AB201843
4390		Extradiol dioxygenase				**BphC**	25	*S. xenophaga* BN6	U22355
4391		4-Hydroxy-2-oxovalerate aldolase				**BphF**	65	*Pseudomonas* sp. KKS102	D16407
4392		Acylating aldehyde dehydrogenase				**TodI**	66	*P. putida* F1	U09250
4393		2-Hydroxypenta-2,4-dienoate hydratase				**TodG**	52	*P. putida* F1	U09250
4402		β-Ketoadipate CoA-transferase β subunit				**PcaJ**	72	*Terrabacter* sp. DBF63	AP008980
4403		β-Ketoadipate CoA-transferase α subunit				**PcaI**	64	*Terrabacter* sp. DBF63	AP008980
4404		Hydroxyquinol 1,2-dioxygenase				**ChqB**	73	*Pimelobacter simplex* 3E	AY822041
4405		Maleylacetate reductase				**TcbF**	63	*Pseudomonas* sp. P51	P27101
4406		Chlorohydroquinone monooxygenase				**ChqA**	75	*Pimelobacter simplex* 3E	AY822041
4411		Bacterial transcriptional regulator				**KdgR**	35	*Pectobacterium carotovorum Carotovorum* 71	AF103871
4412		Dihydrodiol dehydrogenase				**BphB**	60	*Rhodococcus* sp. RHA1	D32142
4413		Extradiol dioxygenase				**BphC**	41	*Burkholderia xenovorans* LB400	X66122
4414		*Meta*-cleavage compound hydrolase				**BphD**	53	*R. erythropolis* TA421	D88016
4415		Ring-hydroxylating dioxygenase α subunit				**TcbAa**	48	*Pseudomonas* sp. P51	U15298
4416		Ring-hydroxylating dioxygenase β subunit				**BphE**	46	*Comamonas testosterone* B-356	U47637
4417		Ferredoxin component				**DfdA3**	60	*Terrabacter* sp. YK3	AB075242
4465		Cytochrome P450 monooxygenase				ORF8	33	*Stigmatella aurantiaca* Sg a15	AJ421825
4589		β -Ketoadipyl-CoA thiolase	13	21.6	18.0	**Thl**	43	*Clostridium pasteurianum* W5	DQ195208
4611		Epoxide hydrolase				**LimA**	42	*R. erythropolis* DCL14	Y18005

Table 1 continued

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
4677		3-Ketoadipyl-CoA thiolase	13	17.6	13.3	**Thl**	41	*Clostridium pasteurianum* W5	DQ195208
4764		Epoxide hydrolase				MT0142	34	*M. tuberculosis* CDC1551	AE000516
4880		Cytochrome P450				**CinA**	29	*Citrobacter braakii*	AF456128
4906		Monooxygenase				**MhpA**	26	*E. coli* W3110	D86239
4907		2-Keto-4-pentenoate hydratase				Orf21	34	*R. erythropolis* TA421	D88014
4908		Extradiol dioxygenase				**DntD**	36	*Burkholderia cepacia* DNT	AF076848
4909		Transcriptional regulator, TetR family				ro00766	60	*Rhodococcus* sp. RHA1	CP000431
4910		Ring-hydroxylating dioxygenase α subunit				**BphA1**	33	*Bacillus* sp. JF8	AB113649
4911		Transcriptional regulator, TetR family				DitK	28	*P. abietaniphila* BKME-9	AF119621
4913		Transcriptional regulator, MarR family							
4962		Ring-hydroxylating dioxygenase α subunit				**PhtAa**	30	*M. vanbaalenii* PYR-1	AY365117
4979		Cytochrome P450 monooxygenase				MycG	31	*Micromonospora griseorubida* A11725	D16098
4983		Cytochrome P450 monooxygenase				BioI	29	*Bacillus subtilis* OK2	AB088066
4984		Cytochrome P450 monooxygenase				**MorA**	34	*Mycobacterium sp. RP1*	CAC84231
4998		Epoxide hydrolase	1.5	2.7	0.0	**Ephx1**	36	*Mus musculus*	AK018249
5043		Putative glutathione *S*-transferase	1	0.0	3.8	SAMR0828	65	*Streptomyces ambofaciens* ATCC 23877	AM238664
5144		Cytochrome P450 monooxygenase				SanQ	39	*Streptomyces ansochromogenes* 7100	AF322179
5151		Cytochrome P450 monooxygenase				MonD	36	*Streptomyces cinnamonensis* ATCC15413	AF440781
5159		Cytochrome P450 monooxygenase				PlaO3	36	*Streptomyces* sp. Tu6071	DQ230532
5161	CYP51	Cytochrome P450 monooxygenase	2.5	2.7	1.9	CYP51	38	*Solanum chacoense*	AY552551
5170		Cytochrome P450 monooxygenase				BioI	30	*Bacillus subtilis* OK2	AB088066
5211		Ring-hydroxylating dioxygenase				KshA	57	*R. erythropolis* SQ1	AY083508
5217		Cytochrome P450 monooxygenase				**NikQ**	36	*Streptomyces tendae* Tue901	AJ250581
5225		Ring-hydroxylating dioxygenase				KshA	60	*Rhodococcus erythropolis*	AY083508
5234		4-Hydroxy-2-oxovalerate aldolase	13.5	14.9	7.6	**BphI**	50	*Burkholderia xenovorans* LB400	X76500
5235		Acylating acetaldehyde dehydrogenase	4	4.1	4.7	**HpdG**	61	*Rhodococcus* sp. RHA1	AB085906
5236		2-Hydroxypenta-2,4-dienoate hydratase	10	10.8	12.3	**HpdE**	58	*Rhodococcus* sp. RHA1	AB085906

Table 1 continued

Locus Tag[a]	Gene product[b]	Functional description	Normalized peptide counts[c]			Homologous gene products			
			Control	Pyrene	Fluoranthene	Matching protein[d]	% Identity[e]	Organism	Accession no.
5258		Cytochrome P450 monooxygenase	9.5	12.2	11.4	MonD	36	*Streptomyces cinnamonensis* ATCC15413	AF440781
5282		3-Ketoadipyl-CoA thiolase	2.5	4.1	2.8	**PaaJ**	45	*Azoarcus evansii* KB740	AF548005
5305		Monooxygenase				**C1-hpah**	37	*Acinetobacter baumannii*	AY566613
5306		2,3-Dihydroxybiphenyl 1,2-dioxygenase				**BphC5**	79	*Rhodococcus* sp. RHA1	AB030672
5307		*Meta*-cleavage compound hydrolase	2.5	6.8	2.8	**BphD**	44	*P. putida* KF715	M33813
5309		Reductase component				**TdnB**	34	*Frateuria* sp. ANA-18	AB089795
5481		Cytochrome P450 monooxygenase				**NikQ**	39	*Streptomyces tendae* Tue901	AJ250581
5493		Catalase				KatE	56	*Micrococcus luteus*	AJ438208
5525		Cytochrome P450 monooxygenase				BioI	39	*Bacillus subtilis* OK2	AB088066
5579		Cytochrome P450 monooxygenase				ABR68807	38	*Streptomyces virginiae* IBL14	EF646281
5671		Superoxide dismutase				**SodN**	51	*Streptomyces seoulensis*	AF047528
5712		Cytochrome P450 monooxygenase				SpiL	31	*Sorangium cellulosum* So ce90	AM407731

[a] Mvan indicates the locus tag number assigned by JGI to each ORF in the *M. vanbaalenii* PYR-1 complete genome. Proteins that were previously shown to be expressed are in bold

[b] Protein name assigned previously

[b,d] Proteins in boldface type were functionally characterized

[c] Normalized peptide counts were from combined calculation of the previous proteome studies. The sum of the total peptides for all proteins from both studies were evaluated and normalized with respect to the control samples

[e] Percent identity was based on alignments with BlastP hits from the nonredundant NCBI protein database

of most of the genes were also identified. As listed in Table 1, strain PYR-1 possesses 194 chromosomally-encoded genes likely to be associated with metabolism of aromatic hydrocarbons. Most of these genes identified by JGI were reannotated with respect to aromatic hydrocarbon degradation. We added to the annotation list a gene (*phtAc*), located between Mvan_0466 and 0467, which encodes a ferredoxin subunit of ring-hydroxylating oxygenase.

As shown in Fig. 1, most predicted catabolic genes of the PYR-1 genome were localized in two regions at positions 494–643 kb (region A) and 4,711–4,741 kb (region B) with some others distributed all over the chromosome. When the identified ORFs were compared against the entire non-redundant protein database from NCBI, many had highest sequence similarity to proteins associated with the aromatic hydrocarbon degradation of members of *Mycobacterium* and other nocardioform actinomycetes such as *Terrabacter*, *Arthrobacter*, *Nocardioides*, *Streptomyces*, and *Rhodococcus*. However, besides its PAH degrading activity, comparison between the catabolic genes of *M. vanbaalenii* PYR-1 and other PAH degrading Gram positive bacteria revealed significant differences in the degree of sequence similarity. The overall organization of the genes was also found to be significantly different. The highest BLAST hit to other well known gram-negative aromatic hydrocarbon degraders, such as *Sphingomonas*, *Burkholderia*, *Comamonas*, and *Pseudomonas*, gave only 32 annotations.

Within the 150 kb stretch of region A, all of the genes involved in the complete pathway of PAH degradation were identified. These include multiple isozymes for each metabolic step in the degradation pathways. The region appears to be specialized in the degradation of HMW PAHs since it possesses all the catabolic genes (Mvan_0462-0600) required for complete PAH degradation including those of the β-ketoadipate pathway. The structural genes required for aromatic degradation in *Pseudomonas* spp. and other gram-negative bacteria are usually well organized in a particular cluster (Harayama et al. 1987). However, as shown in Fig. 1, the organization of region A in strain PYR-1 is an atypical mosaic structure of complexly arranged catabolic genes, in which genes involved in particular degradation processes are not arranged into a single cluster but dispersed throughout several different gene clusters. For instance, the genes (Mvan_0487/0488) encoding ring-hydroxylating oxygenase (RHO) are located 53 kb upstream of Mvan_0470 and 17 kb downstream of Mvan_0544, which encode the enzymes required for the next two steps of PAH ring-hydroxylation, dihydrodiol dehydrogenase and ring-cleavage dioxygenase, respectively.

There is a considerable amount of experimental information about the proteome profiles of strain PYR-1 induced by several kinds of PAHs (Kim et al. 2004a, 2007; Kweon et al. 2007). Thus we tried to relate the genome sequence-based analysis for *Mycobacterium* to the experimental proteome data to determine if the predicted genes are translated as protein. From the proteome data, we confirmed that 67 of the 194 genes are expressed as proteins involved in PAH degradation pathways (Table 1).

Genes involved in the upper pathway of PAH degradation

Degradation of HMW PAHs in *M. vanbaalenii* PYR-1 proceeds via multiple routes, which usually channel into a limited number of central metabolic intermediates, such as protocatechuate. For example, strain PYR-1 degrades pyrene and fluoranthene into the TCA cycle via protocatechuate with at least two and four different degradation routes, respectively (Kim et al. 2005b, 2007; Kweon et al. 2007). These degradation pathways, however, can be generalized based on the type of metabolic products and enzyme reactions as shown in Fig. 2. Detoxification reactions are also included in the generalized degradation pathways.

Initially, the degradation of PAH begins with aromatic ring hydroxylation (Fig. 2). Analyses of metabolites performed with whole cells or enzymes of strain PYR-1 indicate that the hydroxylation of PAHs is initiated by both mono- and dioxygenation reactions. In this step, one or two atoms of dioxygen are incorporated into substrates forming dihydrodiol compounds with *trans* and *cis* configurations, respectively (Heitkamp et al. 1988; Kelley et al. 1990). Two groups of oxygenases, RHOs and cytochrome P450 monooxygenases (CYPs) have been found in *M. vanbaalenii* PYR-1 for the aerobic metabolism of PAHs. Analysis of the genome sequence predicted the existence of a total of 21 genes encoding RHO (Table 1). Among them, eight RHOs (seven in region A and one in region B) exist as α and β subunit pairs of RHO. Six gene pairs in region A, Mvan_0463/0464 (*phtAaAb*), 0487/0488 (*nidAB*), 0525/0526

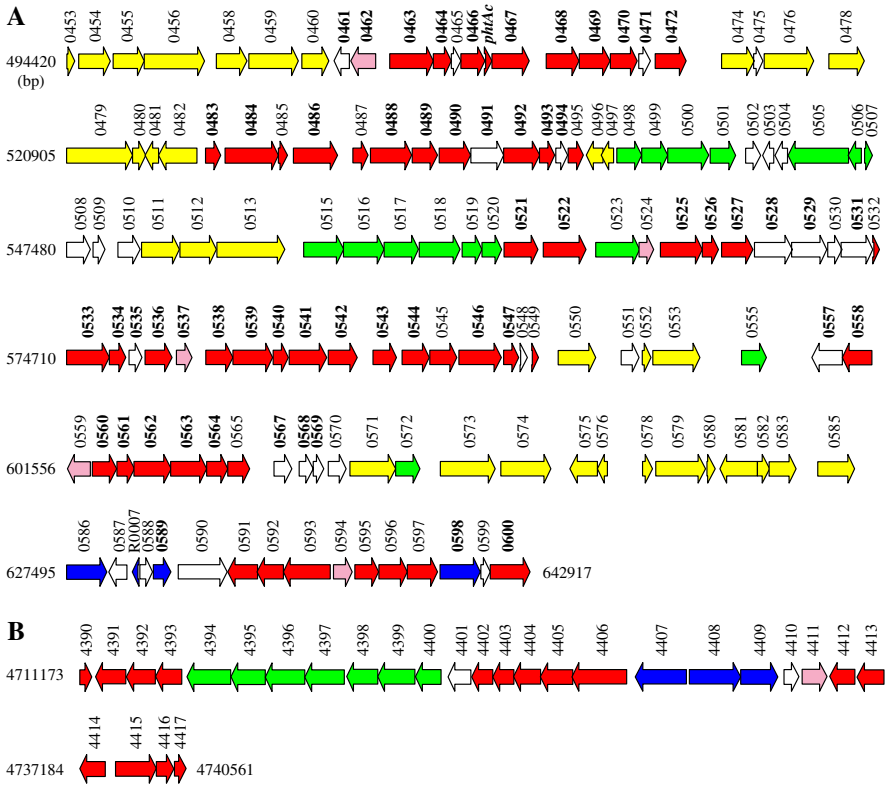
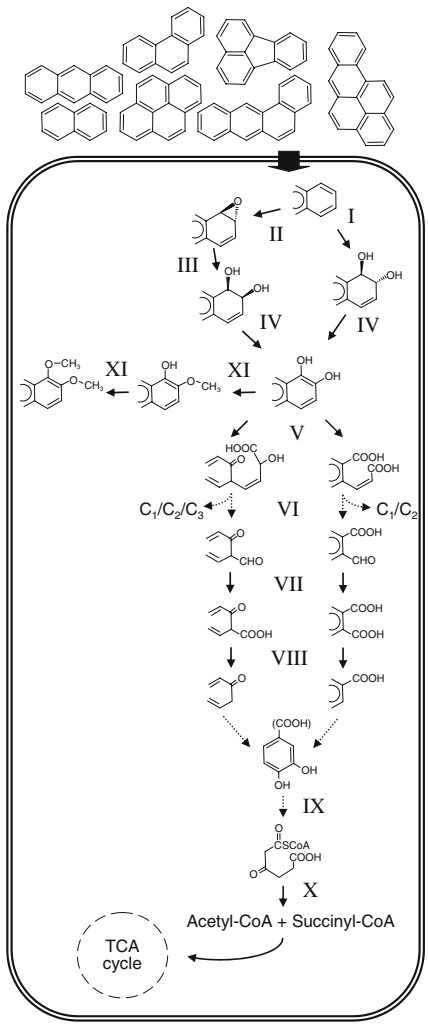


Fig. 1 Schematic diagram showing genetic information derived from the catabolic regions A and B on the PYR-1 genome. Genes and predicted ORFs are indicated by arrows and the arrowheads indicate the directions of transcription. Numbers above the arrows indicate the ORF (Mvan_0000) from the PYR-1 genome, which are as described in the text and listed in Table 1. Genes whose expression was identified in the proteome analyses are in boldface number. The ORFs are grouped on the basis of (putative) function as follows: red, ORFs involved in PAH catabolism; pink, ORFs involved in transcriptional regulation; blue, ORFs with predicted function other than PAH degradation; yellow, ORFs involved in DNA mobilization; green, ORFs predicted to be involved in membrane transport system; white, ORFs with no predicted function or encoding hypothetical proteins

(*nidA3B3*), 0533/0534, 0539/0540, and 0546/0547, have been previously identified to be involved in aromatic hydrocarbon degradation (Khan et al. 2001; Stingley et al. 2004; Kim et al. 2006, 2007; Kweon et al. 2007). Sequence analysis indicated that Mvan_0546/0547 also likely transforms other PAHs including phenanthrene, naphthalene, and biphenyl because it contains a considerable sequence similarity to other enzymes having such substrate specificities (Larkin et al. 1999; Mukerjee-Dhar et al. 2005). The seventh RHO pair in region A, Mvan_0492/0493 is similar to the salicylate hydroxylase from *Sphingomonas* sp. P2 (Pinyakong et al. 2003). This enzyme is known to catalyze the monooxygenation reaction of salicylate to produce catechol. The eighth RHO pairs, Mvan_4415/4416 in region B, were matched to the proteins involved in the oxidation of chlorophenol/biphenyl. On the genome of strain PYR-1, the paralogs to oxygenase subunits are not always located in pairs; 13 paralogs of oxygenase subunits were identified to be positioned as an independent ORF. Most of these orphan oxygenases also appeared to be associated with PAH degradation (Table 1).

One interesting observation in Table 1 is the responsiveness and extent of fold changes in abundance of some proteins with respect to PAH induction, which can be correlated to regulation and function of some enzymes; some of the genes were not as responsive to fluoranthene as compared to the cells induced by pyrene and vice versa. For example, Mvan_0525 (NidA3), encoding an α subunit of RHO, were about two-fold more abundant during growth with fluoranthene than with pyrene. This proteomic data not only confirms the involvement of the gene



- Transport system

I. Ring-hydroxylating oxygenase: 21
II. P450 monooxygenase: 50
III. Epoxide hydrolase: 4

IV. Dihydrodiol dehydrogenase: 5

V. Ring-cleavage dioxygenase: 10

VI. *meta*-Cleavage product hydrolase: 8
Hydratase-aldolase: 3
Hydratase: 4
Aldolase:2
Alcohol dehydrogenase: 7

VII. Aldehyde dehydrogenase: 2

VIII. Decarboxylase: 2

IX. Protocatechuate 3,4-dioxygenase α subunit: 2
Protocatechuate 3,4-dioxygenase β subunit: 1
β-Carboxy-*cis*,*cis*-muconate cycloisomerase: 1
γ-Carboxymuconolactone decarboxylase/
β-ketoadipate enol-lactone hydrolase: 1
β-Ketoadipate CoA-transferase α subunit: 2
β-Ketoadipate CoA-transferase β subunit: 2

X. β-Ketoadipyl CoA thiolase : 5

XI. Catechol *O*-methyltransferase: 2

Fig. 2 Overview of degradative pathway for HMW PAHs. Solid arrows indicate one-step reactions and dashed arrows show two or more steps. Roman numbers represent enzymatic steps in the pathway. Enzyme names are shown with the number of genes identified in the PYR-1 genome

products in fluoranthene degradation as demonstrated previously (Khan et al. 2001; Stingley et al. 2004; Kim et al. 2006, 2007; Kweon et al. 2007) but also indicates that the enzyme is likely the major enzyme for the degradation of fluoranthene in strain PYR-1. On the other hand, Mvan_0488 (NidA), encoding another RHO α subunit, was only found in the pyrene-induced PYR-1 cells, which also supports our previous findings that the enzyme plays an important role in pyrene degradation by strain PYR-1 (Khan et al. 2001; Stingley et al. 2004; Kim et al. 2006, 2007; Kweon et al. 2007).

The success of strain PYR-1 in degrading various aromatic compounds appears to be based on the possession of multiple copies of RHO. Figure 3 shows a phylogenetic tree for the 21 strain PYR-1 and 25 other bacterial RHO α subunits. They are widely distributed in the tree, which reflects a considerable degree of sequence diversity. The relatively large deviations in sequence identities (28–59% at the level of amino acids) between these 21 oxygenases within strain PYR-1 implicate different substrate specificities and are thought to constitute the genomic potential which significantly increases the catabolic degradation ability and efficiency of *M. vanbaalenii* PYR-1.

Ring-hydroxylating oxygenase is a multi component enzyme system which often consists of a terminal oxygenase(s) and an electron transfer component(s) (Mason and Cammack 1992; Gibson and Parales 2000). In the genome analysis, *phtAc*/*phtAd* were identified as electron transfer components, whose function has been implicated in electron transfer coupled with ring hydroxylation reaction (Kim et al. 2006, 2007; Kweon et al. 2007). Multiple

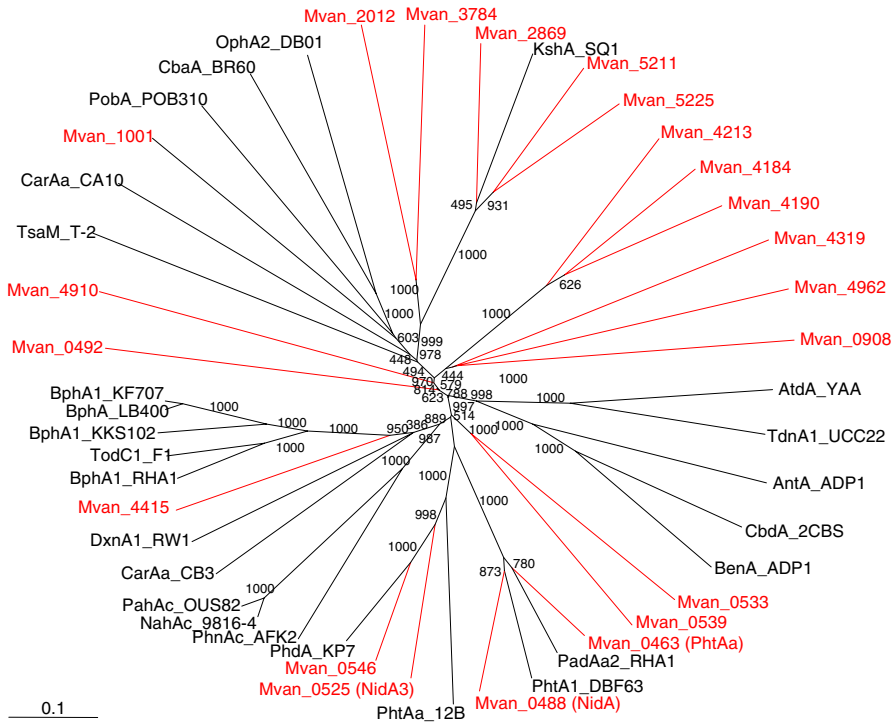


Fig. 3 Phylogenetic tree of 21 ring-hydroxylating oxygenases of strain PYR-1 obtained from alignment with 25 related proteins from other microorganisms. The PYR-1 genes are in red. The numbers on branches refer to the percentage confidence, estimated by a bootstrap analysis with 1,000 replications. Scale bar indicates percentage divergence. GenBank accession numbers for the sequences are as follows: PhtA1_*Terrabacter* sp. DBF63, AP008980; PhtAa_*Arthrobacter keyseri* 12B, AF331043; PhdA_*Nocardioides* sp. KP7, AB017794; PhnAc_*Alcaligenes faecalis* AFK2, AB024945; NahAc_*Pseudomonas* sp. 9816-4, U49496; PahAc_*P. putida* OUS82, AB004059; CarAa_*Sphingomonas* sp. CB3, AF060489; DxnA1_*Sphingomonas* sp. RW1, X72850; BphA1_*Rhodococcus* sp. RHA1, D32142; TodC1_*P.putida* F1, J04996; BphA1_*Pseudomonas* sp. KKS102, D17319; BphA_ *Burkholderia xenovorans* LB400, M86348; BphA1_*P. pseudoalcaligenes* KF707, AF049345; TsaM_*Comamonas testosterone* T-2, AF303942; CarAa_*Pseudomonas* sp. CA10, D89064; PobA_*P. pseudoalcaligenes* POB310, X78823; CbaA_*Comamonas testosteroni* BR60, U18133; OphA2_*Burkholderia cepacia* DBO1, AF095748; KshA_*R. erythropolis* SQ1, AY083508; AtdA3_*Acinetobacter* sp. YAA, D86080; TdnA1_*P. putida* UCC22, D85415; AntA_*A. calcoaceticus* ADP1, AF071556; CbdA_*B. cepacia* 2CBS, X79076; BenA_*A. calcoaceticus* ADP1, AF009224

paralogs to ferredoxin (Mvan_0532/0549/0909/3495/4235/4417) and ferredoxin reductase (Mvan_1290/2893/2897/4186/4239/5309) were also found in separate regions.

The involvement of CYPs in the initial monooxygenation of PAHs has been demonstrated in *M. vanbaalenii* PYR-1 (Heitkamp et al. 1988; Kelley et al. 1990). The enzyme along with epoxide hydrolase produces *trans*-dihydrodiols. By genome data mining and analysis, we identified 50 paralogs to CYPs dispersed throughout the genome, most of which had unknown specific functions. Of these CYPs, only those encoded by Mvan_1848 and 4141, belong to classes CYP151 and CYP150, respectively, have been experimentally characterized as catalyzing the hydroxylation of dibenzothiophene, 7-methylbenz[*a*]anthracene, and pyrene (Brezna et al. 2006). Among other CYPs, two ORFs, Mvan_0779 and Mvan_2422, showed similarity to the published gene P450RhF from *Rhodococcus* sp. NCIMB 9784. The gene was shown to be involved in aromatic degradation (Karlson et al. 1993). Although disproportionately high numbers of CYP-encoding genes relative to genome size are typical of actinomycetes, the presence of 50 paralogs to CYP is a distinguishing feature of the PYR-1 genome. For example, the genome sequences of *Rhodococcus* sp. RHA1 (McLeod et al. 2006), *M. tuberculosis* H37Rv (Cole et al. 1998), *Streptomyces avermitilis* (Lamb et al. 2003), and *S. coelicolor* A3 (Bentley et al. 2002) revealed 25, 20, 33, and 18 CYPs,

respectively. The number of CYP paralogs is 57 in the human genome (Guengerich et al. 2005). Although we could not assign specific functions for most CYPs based on the current data, their abundance is also thought to contribute to the wide range of PAHs degraded by *M. vanbaalenii* PYR-1. Four paralogs (Mvan_0521/4611/4764/4998) to epoxide hydrolase were also found on the genome. We identified in the proteome list that 6 and 2 genes encoding CYP and epoxide hydrolase, respectively, were expressed as protein.

The second step in the upper pathway for the metabolism of PAHs is the dehydrogenation of the dihydrodiol to form *ortho* or *meta* dihydroxylated intermediates catalyzed by dihydrodiol dehydrogenase (Fig. 2). Analysis of the genome sequence identified 5 paralogs to dihydrodiol dehydrogenase, which rearomatizes dihydrodiols produced from initial ring-hydroxylation to form catechol derivatives. Among them, the expression of four dihydrodiol dehydrogenases (Mvan_0466/0541/0544/2848) was previously shown to be associated with PAH degradation (Kim et al. 2006, 2007; Kweon et al. 2007). The fifth paralog (Mvan_4412) seems to be essential for the biphenyl/monocyclic aromatic degradation. In the case of ring-cleavage oxygenase, which adds molecular oxygen to break carbon–carbon bonds of the aromatic ring, the genome appeared to encode a total of 10 paralogs. Of these, we previously proposed that 4 ring-cleavage oxygenases (Mvan_0468/0470/0542/0545) were related to pyrene and fluoranthene degradation (Kim et al. 2007; Kweon et al. 2007). The rest have been assigned with putative function in relation to homology to other documented proteins. As demonstrated in the studies with *Rhodococcus* (Sakai et al. 2002; Gonçalves et al. 2006), the presence of multiple ring-cleavage dioxygenase isozymes in strain PYR-1 could also improve its catabolic ability.

We also recognized sets of genes possibly coding for the enzymes, hydratase–aldolase, hydrolase, alcohol dehydrogenase, aldehyde dehydrogenase, decarboxylase, hydratase, and aldolase, by BLAST searches based on sequence similarity. They are involved in a series of reactions catalyzing ring-cleavage products to protocatechuate. These genes were also often found in clusters with other aromatic degrading genes or scattered around the genome. Redundancy to some extent was observed for some of these genes, which could increase strain PYR-1's catabolic potential. For example, 8 paralogs of *meta*-cleavage product hydrolase (Mvan_0538/0592/2898/2912/4238/4246/4414/5307) were identified in the genome. The hydrolase enzyme has been reported to be inefficient and could be a metabolic bottleneck in transforming *meta*-cleavage products in PAH degradation (Seah et al. 2000).

Genes involved in the β-ketoadipate pathway

As in many other aromatic-degrading microorganisms (Harwood and Parales 1996; Jimenez et al. 2002), *M. vanbaalenii* PYR-1 is able to transform the diverse structures of many aromatic compounds to one of the common aromatic intermediates, protocatechuate. In region A of the PYR-1 genome, the *pca* genes encoding the six enzymes involved in the β-ketoadipate pathway, were arranged as *pcaHGBLIJ* (Mvan_0560 to 0565) (Table 1, Fig. 1) with an additional *pcaIJ* gene set (Mvan_4402/4403) encoding β-ketoadipate CoA transferase α and β subunits in region B. The pathway has been previously proposed for the degradation of phthalate, phenanthrene, pyrene and fluoranthene in strain PYR-1 and many other actinobacteria (Eulberg et al. 1998; Habe et al. 2005; Patrauchan et al. 2005). The cluster found in the PYR-1 genome lacks the gene encoding β-ketoadipyl CoA thiolase (*pcaF*) catalyzing the last step of the pathway, transforming β-ketoadipyl CoA to succinyl CoA and acetyl CoA. We identified five probable paralogs (Mvan_0916/4206/4589/4677/5282) for the enzyme, which occur as independent ORFs located separately. Most of these genes encoding β-ketoadipate protocatechuate pathway enzymes were previously shown to be expressed in the proteome analysis (Table 1) (Kim et al. 2007; Kweon et al. 2007).

Additional catabolic functions found in the 31 kb region B

The 31 kb region B also contains an abundance of other genes (Mvan_4390-4417) showing significant similarity to those encoding proteins involved in the degradation of the biphenyl and chloro-substituted phenols. In this region, genes encoding α and β subunits of ring-hydroxylating oxygenase (Mvan_4415/4416), probably involved in the initial oxidation of biphenyl, were identified clustering closely with a series of other genes for biphenyl degradation such as 2,3-dihydroxy-1-phenylcyclohexa-4,6-diene (dihydrodiol) dehydrogenase (Mvan_4412), 2,3-dihydroxybiphenyl

1,2-dioxygenase (Mvan_4413), and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate (*meta*-cleavage compound) hydrolase (Mvan_4414). The genes *bphEGF* encoding 2-hydroxypenta-2,4-dienoate hydratase, acetaldehyde dehydrogenase (acylating), and 4-hydroxy-2-oxovlerate aldolase were localized in a different region (Mvan_4413 to 4416). These enzymes convert 2-hydroxypenta-2,4-dienoic acid, the intermediate of biphenyl degradation, to acetyl-coenzyme A. Copies of these genes (*bphEFG*) were additionally found in 3 separate clusters; Mvan_0595/0596/0597 in region A, Mvan_4393/4392/4391 in region B, and Mvan_5236/5235/5234 in an independent location. However, implication of these enzymes in the degradation of biphenyl has to be confirmed experimentally. We also screened the genome sequence to see whether enzymes involved in the degradation of benzoate are present. However, no obvious genes encoding benzoate 1,2-dioxygenase or enzymes involved in the aerobic benzoyl-CoA catabolic pathway (Gescher et al. 2006) appeared to be present in the genome.

Genes involved in detoxification

M. vanbaalenii PYR-1 has a large number of determinants associated with protection against PAH substrates and metabolites. In prior studies, we provided evidence for the existence of enzyme(s) involved in the *O*-methylation of anthracene, phenanthrene, fluoranthene, and pyrene, which produce monomethoxy- and dimethoxy-compounds (Kelley et al. 1993; Moody et al. 2001). Recently, a catechol-*O*-methyltransferase was detected in the cytosol of strain PYR-1 (Kim et al. 2004b). This enzyme was proposed to play a role in the detoxification of compounds with hydroxyl groups by generating less reactive methoxy-derivatives (Haggblom et al. 1988). We identified Mvan_3118 to be the catechol-*O*-methyltransferase (Kim et al. 2004b) and Mvan_3118 was further assigned based on sequence similarity to catalyze the *O*-methylation reaction. PAH *o*-quinones, which are known to be genotoxic, can be produced from PAH dihydrodiols by the catalysis of dihydrodiol dehydrogenase followed by spontaneous oxidation. However, PAH *o*-quinones can be reduced by a quinone reductase to form PAH catechol compounds, which can be metabolized by ring-cleavage enzymes and proceed into the downstream degradation pathway (Kim et al. 2003). We successfully matched the N-terminal sequence of the purified quinone reductase enzyme (Kim et al. 2003) to the gene Mvan_2039, which was initially annotated by JGI as an NADPH-dependent FMN reductase. In addition, paralogs to catalase peroxidase (Mvan_2984/3201/3208/5493) and superoxide dismutase (Mvan_0735/5671) were identified on the genome. Catalase peroxidase has been proposed to have a detoxification function in strain PYR-1 (Wang et al. 2000). The enzyme together with superoxide dismutase has been associated with the dioxygenase-mediated uncoupling reaction of aromatic oxidation that results in the release of H_2O_2 (Fiorenza and Ward 1997; Jouanneau et al. 2006). A paralog to glutathione *S*-transferase (Mvan_5043) was also included in the list since the enzyme has been proposed to have a detoxification function in relation to PAH catabolism (Lloyd-Jones and Lau 1997; Vuilleumier and Pagni 2002; Gilmartin et al. 2003).

Genes potentially related to genetic mobile elements/membrane transport system

The catabolic gene clusters responsible for aromatic degradation often involve membrane transport genes responsible for the uptake of aromatic compounds into the cell and mobile genetic elements that play a major role in the effective genetic acquisition of the accessory functions in many microorganisms. It is interesting to note that a high number of genes associated with membrane transport systems and insertion sequences flank PAH catabolic genes in the PYR-1 genome. We identified in region A a total of 34 ORFs showing similarities to enzymes associated with DNA transposition and integration (Fig. 1). The roster of these mobile genetic elements includes proteins encoding IS*3*/IS*911* transposase (Mvan_0453), IS*21* transposase (0482), mutator-type transposase (0454), recombinase (0455), transposase B/C-like protein (0479/0489), and many other transposable elements. These multiple mobile genetic elements are most likely involved in the transposition of catabolic modules that resulted in gene rearrangement leading to complicated mosaic gene structure. A total of 23 ORFs involved in membrane transport systems were also identified in the region A and B, which include genes similar to ABC transport systems, efflux pump proteins, and other membrane associate proteins. These enzymes are thought to be involved in the uptake of a multitude of organic

compounds (Hearn et al. 2003; Dos Santos et al. 2004; Kurbatov et al. 2006). These two features probably contribute to strain PYR-1's flexibility when surviving in oligotrophic environments and competing successfully with other organisms. However, although it is tempting to speculate on their function, it is beyond the scope of this study to determine whether and how such genes of *M. vanbaalenii* PYR-1 are involved in the catabolism of aromatic compounds.

Citric acid cycle

The *M. vanbaalenii* PYR-1 genome was also examined for the existence of genes encoding enzymes participating in the TCA cycle, which is required for the metabolism of the end products of the *β*-ketoadipate pathway, succinyl-CoA and acetyl-CoA, which feed directly into the cycle. We took the KEGG database (Kanehisa et al. 2006) as starting point for the elucidation of TCA cycle genes since this database has linked some of the PYR-1 genome sequence to the metabolic pathways. However, in the KEGG database, only 10 ORFs were proposed to be related to the TCA cycle and some of the metabolic steps were not assigned any ORFs. For example, among the eight reaction steps of the TCA cycle, genes for aconitase, 2-oxoglutarate dehydrogenase, and fumarase were missing. Therefore, we manually looked for additional gene candidates which resulted in the identification of a total of 28 genes involved in the TCA cycle. As shown in Table 2 and Fig. 4, we reconstructed a complete steps for the conversion of citrate to oxaloacetate, some of which were assigned with multiple copies of genes. All of the genes, with the sole exception of the succinate dehydrogenase flavoprotein subunit-encoding gene (Mvan_0484), were distributed across the entire genome.

In order to demonstrate that the TCA cycle is operative in *M. vanbaalenii* PYR-1, these sequence similarity-based predictions were related to existing proteome information. We identified that 23 out of 28 genes were expressed as proteins (Table 2 and Fig. 4) (Kim et al. 2007; Kweon et al. 2007). Previously, the expression levels of many enzymes involved in PAH degradation were shown to be induced in strain PYR-1 grown with PAHs (Kim et al. 2007; Kweon et al. 2007). Interestingly however, most of the TCA cycle genes did not appear to be changed in abundance (Table 2). They exhibited similar expression profiles showing quite stable quantities of protein across all three tested growth conditions. Although there were some differences, these were considered to be marginal, or no more than 2-fold. This indicates that these TCA cycle genes in the PAH induced PYR-1 do not seem to be differently affected as compared to the sorbitol-grown PYR-1. The only exception is the gene encoding succinate dehydrogenase flavoprotein subunit (Mvan_0484), which was 15-fold more abundant in the pyrene-induced PYR-1. Because this gene is encoded in region A near genes that are significantly induced in the presence of pyrene, such as NidB2 (Mvan_0483), NidD (0486), and NidA (0488), the succinate dehydrogenase is likely coordinately upregulated with them.

Conclusion

In this study, we undertook genomic analyses to determine the molecular factors responsible for the degradation of HMW PAHs by *M. vanbaalenii* PYR-1. Overall, the *M. vanbaalenii* PYR-1 genome had 194 and 28 genes involved in the degradation of PAHs and the TCA cycle, respectively. A total of 90 gene predictions were additionally verified at the protein level. Based on the results, we proposed a pathway in which HMW PAHs are degraded into the *β*-ketoadipate pathway through protocatechuate and then mineralized to carbon dioxide via the TCA cycle. The PAH catabolic genes are mostly located in genomic regions A and B with others scattered all over the chromosome. The sequences and arrangement of aromatic catabolic genes of strain PYR-1 appear to have diverged considerably from those of other bacterial species. A considerable multiplicity of the genes with a high range of diversity involved in PAH catabolism was revealed. This complex suite of enzymes with different catalytic potential appears to contribute to the exceptional ability of strain PYR-1 to degrade HMW PAHs. From the standpoint of aromatic hydrocarbon degradation, the genome of *M. vanbaalenii* PYR-1, as featured in this analysis, appears to be geared toward the degradation of HMW PAHs. Although this study addressed some important questions about molecular background and allowed us to assemble a whole picture for the degradation of HMW PAHs by strain PYR-1, understanding the

Table 2 Identified genes coding for the enzymes related to the citric acid cycle in *M. vanbaalenii* PYR-1

Enzyme (EC number)	Gene	Mvan number[a]	Normalized peptide counts[b]		
			Control	Pyrene	Fluoranthene
Citrate synthase (2.3.3.1)	*gltA*	*5022*	25.5	39.2	27.5
		5025	3.0	5.4	1.9
Citrate lyase (4.1.3.6)					
β Chain	*citE*	2378	2.5	1.4	2.8
		2654	–	–	–
		4087	–	–	–
		4487	1.5	2.7	–
Aconitate hydratase (4.2.1.3)	*acnA*	2745	29.5	35.2	37.0
Isocitrate dehydrogenase, $NADP^+$ (1.1.1.42)	*icd*	*3212*	52.5	73.1	48.4
2-Oxoglutarate dehydrogenase (1.2.4.2)	*sucA*	4477	117.5	110.9	144.2
Dihydrolipoamide succinyltransferase (2.3.1.61)	*sucB*	3579	27.0	13.5	36.1
Dihydrolipoamide dehydrogenase (1.8.1.4)	*pdhD*	0794	20.5	36.5	26.6
2-Oxoglutarate synthase (1.2.7.3)					
α Subunit	*korA*	3965	16.0	21.6	17.1
β Subunit	*korB*	3964	3.0	5.4	4.7
Succinyl-CoA synthetase (6.2.1.5)					
α Subunit	*sucD*	4869	6.0	6.8	8.5
β Subunit	*sucC*	*4870*	9.5	6.8	5.7
Succinate dehydrogenase/fumarate reductase (1.3.99.1,1.3.5.1)					
Flavoprotein subunit	*sdhA*	*0284*	18.5	24.4	23.7
		0484	2.0	31.1	–
		1587	10.5	20.3	13.3
		2799	–	–	–
Iron sulfur subunit	*sdhB*	0283	8.0	5.4	6.6
		1586	3.0	4.1	2.8
Cytochrome b subunit	*sdhC*	1589	0.5	1.4	–
Hydrophobic membrane anchor protein	*sdhD*	1588	–	–	–
Fumarate hydratase class II (4.2.1.2)	*fumC*	4649	17.5	21.6	13.3
Malate dehydrogenase (1.1.1.37)	*mdh*	*3067*	1.0	–	1.9
Malate synthase G (2.3.3.9)	*aceB*	*3097*	20.0	25.7	19.0
Isocitrate lyase (4.1.3.1)	*Icl*	0801	–	–	–
	aceAa	2977	75.5	85.2	78.8

[a] Mvan indicates the locus tag number assigned to each ORF in the *M. vanbaalenii* PYR-1 genome sequence. Mvan numbers shown in italics indicate ORFs that were proposed by KEGG to be involved in the citric acid cycle

[b] See Table 1 footnote c

overall physiology of the cell towards PAH metabolism may still be far from complete. Experimentation to further verify annotated functional roles of catabolic genes is necessary as well as the expansion of genome analysis to the entire PYR-1 genome. In concert with this work, additional functional genomic studies, to obtain more information about genes and their interactions, are being conducted, which will lead to a new and more holistic view on the regulatory mechanisms as well as gene functions in this bacterium with respect to PAH metabolism.

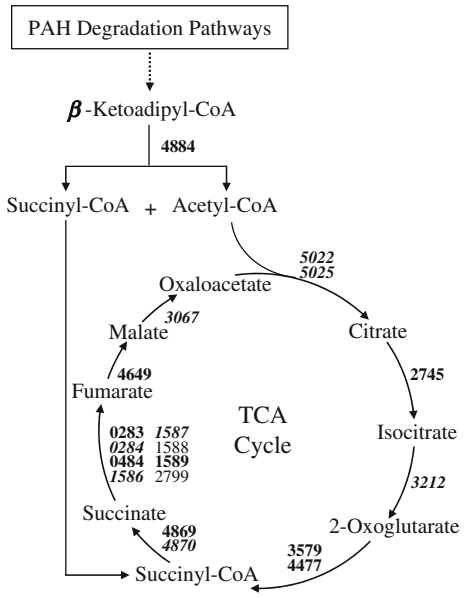


Fig. 4 TCA cycle of *M. vanbaalenii* PYR-1 as predicted from genomic and proteomic information. Enzymes identified to be responsible for the respective reaction steps are represented by Mvan numbers. Numbers shown in bold and italic indicate ORFs that were previously identified in the proteome and that were proposed by KEGG to be involved in TCA cycle, respectively

Acknowledgments We thank Robin L. Stingley and Ashraf A. Khan for critical review of the manuscript and Thomas D. Yun for graphical assistance. The authors acknowledge Charles D. Miller and Ronald C. Sims at the Utah State University and the staff of the Joint Genome Institute for their efforts with the genome sequencing of *M. vanbaalenii* PYR-1. This work was supported by an appointment to the Postgraduate Research Program at the National Center for Toxicological Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U. S. Department of Energy and the U. S. Food and Drug Administration.

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Barbe V, Vallenet D, Fonknechten N, Kreimeyer A, Oztas S, Labarre L, Cruveiller S, Robert C, Duprat S, Wincker P, Ornston LN, Weissenbach J, Marliere P, Cohen GN, Medigue C (2004) Unique features revealed by the genome sequence of *Acinetobacter* sp. ADP1, a versatile and naturally transformation competent bacterium. Nucleic Acids Res 32:5766–5779

Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141–147

Boldrin B, Tiehm A, Fritzsche C (1993) Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by a *Mycobacterium* sp. Appl Environ Microbiol 59:1927–1930

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000) Role of quinones in toxicology. Chem Res Toxicol 13:135–160

Brezna B, Khan AA, Cerniglia CE (2003) Molecular characterization of dioxygenases from polycyclic aromatic hydrocarbon-degrading *Mycobacterium* spp. FEMS Microbiol Lett 223:177–183

Brezna B, Kweon O, Stingley RL, Freeman JP, Khan AA, Polek B, Jones RC, Cerniglia CE (2006) Molecular characterization of cytochrome P450 genes in the polycyclic aromatic hydrocarbon degrading *Mycobacterium vanbaalenii* PYR-1. Appl Microbiol Biotechnol 71:522–532

Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J (2005) ACT: the Artemis Comparison Tool. Bioinformatics 21:3422–3423

Chain PS, Denef VJ, Konstantinidis KT, Vergez LM, Agullo L, Reyes VL, Hauser L, Cordova M, Gomez L, Gonzalez M, Land M, Lao V, Larimer F, LiPuma JJ, Mahenthiralingam E, Malfatti SA, Marx CJ, Parnell JJ, Ramette A, Richardson P, Seeger M, Smith D, Spilker T, Sul WJ, Tsoi TV, Ulrich LE, Zhulin IB, Tiedje JM (2006) *Burkholderia xenovorans* LB400 harbors a multi-replicon, 9.73-Mbp genome shaped for versatility. Proc Natl Acad Sci USA 103:15280–15287

Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393:537–544

Dos Santos VA, Heim S, Moore ER, Stratz M, Timmis KN (2004) Insights into the genomic basis of niche specificity of *Pseudomonas putida* KT2440. Environ Microbiol 6:1264–1286

Eulberg D, Lakner S, Golovleva LA, Schlömann M (1998) Characterization of a protocatechuate catabolic gene cluster from *Rhodococcus opacus* 1CP: evidence for a merged enzyme with 4-carboxymuconolactone-decarboxylating and 3-oxoadipate enol-lactone-hydrolyzing activity. J Bacteriol 180:1072–1081

Fiorenza S, Ward CH (1997) Microbial adaptation to hydrogen peroxide and biodegradation of aromatic hydrocarbons. J Ind Microbiol Biotechnol 18:140–151

Gescher J, Ismail W, Olgeschlager E, Eisenreich W, Worth J, Fuchs G (2006) Aerobic benzoyl-coenzyme A (CoA) catabolic pathway in *Azoarcus evansii*: conversion of ring cleavage product by 3, 4-dehydroadipyl-CoA semialdehyde dehydrogenase. J Bacteriol 188:2919–2927

Gibson DT, Parales RE (2000) Aromatic hydrocarbon dioxygenases in environmental biotechnology. Curr Opin Biotechnol 11:236–243

Gilmartin N, Ryan D, Sherlock O, Dowling D (2003) BphK shows dechlorination activity against 4-chlorobenzoate, an end product of *bph*-promoted degradation of PCBs. FEMS Microbiol Lett 222:251–255

Gonçalves ER, Hara H, Miyazawa D, Davies JE, Eltis LD, Mohn WW (2006) Transcriptomic assessment of isozymes in the biphenyl pathway of *Rhodococcus* sp. strain RHA1. Appl Environ Microbiol 72:6183–6193

Grosser RJ, Warshawsky D, Vestal JR (1991) Indigenous and enhanced mineralization of pyrene, benzo[*a*]pyrene, and carbazole in soils. Appl Environ Microbiol 57:3462–3469

Guengerich FP, Wu ZL, Bartleson CJ (2005) Function of human cytochrome P450 s: characterization of the orphans. Biochem Biophys Res Commun 338:465–469

Habe H, Chung JS, Ishida A, Kasuga K, Ide K, Takemura T, Nojiri H, Yamane H, Omori T (2005) The fluorene catabolic linear plasmid in *Terrabacter* sp. strain DBF63 carries the ß-ketoadipate pathway genes, *pcaRHGBDC-FIJ*, also found in proteobacteria. Microbiology 151: 3713–3722

Habe H, Omori T (2003) Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Biosci Biotechnol Biochem 67:225–243

Haggblom MM, Nohynek LJ, Salkinoja-Salonen MS (1988) Degradation and *O*-methylation of chlorinated phenolic compounds by *Rhodococcus* and *Mycobacterium* strains. Appl Environ Microbiol 54:3043–3052

Hall K, Miller CD, Sorensen DL, Anderson AJ, Sims RC (2005) Development of a catabolically significant genetic probe for polycyclic aromatic hydrocarbon-degrading mycobacteria in soil. Biodegradation 16:475–484

Hamann C, Hegemann J, Hildebrandt A (1999) Detection of polycyclic aromatic hydrocarbon degradation genes in different soil bacteria by polymerase chain reaction and DNA hybridization. FEMS Microbiol Lett 173:255–263

Harayama S, Rekik M, Wasserfallen A, Bairoch A (1987) Evolutionary relationships between catabolic pathways for aromatics: conservation of gene order and nucleotide sequences of catechol oxidation genes of pWW0 and NAH7 plasmids. Mol Gen Genet 210:241–247

Harwood CS, Parales RE (1996) The ß-ketoadipate pathway and the biology of self-identity. Annu Rev Microbiol 50:553–590

Hearn EM, Dennis JJ, Gray MR, Foght JM (2003) Identification and characterization of the *emhABC* efflux system for polycyclic aromatic hydrocarbons in *Pseudomonas fluorescens* cLP6a. J Bacteriol 185:6233–6240

Heitkamp MA, Cerniglia CE (1988) Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. Appl Environ Microbiol 54:1612–1614

Heitkamp MA, Cerniglia CE (1989) Polycyclic aromatic hydrocarbon degradation by a *Mycobacterium* sp. in microcosms containing sediment and water from a pristine ecosystem. Appl Environ Microbiol 55:1968–1973

Heitkamp MA, Freeman JP, Miller DW, Cerniglia CE (1988) Pyrene degradation by a *Mycobacterium* sp.; identification of ring oxidation and ring fission products. Appl Environ Microbiol 54:2556–2565

IARC (1983) Monograph on the evaluation of carcinogenic risk of chemicals to man: polynuclear aromatic compounds. Part 1: chemical, environmental and experimental data, vol. 32, World Health Organization. Lyon, France

Jimenez JI, Minambres B, Garcia JL, Diaz E (2002) Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. Environ Microbiol 4:824–841

Jouanneau Y, Meyer C, Jakoncic J, Stojanoff V, Gaillard J (2006) Characterization of a naphthalene dioxygenase endowed with an exceptionally broad substrate specificity toward polycyclic aromatic hydrocarbons. Biochemistry 45:12380–12391

Kanaly RA, Harayama S (2000) Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J Bacteriol 182:2059–2067

Kanehisa M, Goto S, Hattori M, Aoki-Kinoshita KF, Itoh M, Kawashima S, Katayama T, Araki M, Hirakawa M (2006) From genomics to chemical genomics: new developments in KEGG. Nucleic Acids Res 34:D354–D357

Karlson U, Dwyer DF, Hooper SW, Moore ER, Timmis KN, Eltis LD (1993) Two independently regulated cytochromes P-450 in a *Rhodococcus rhodochrous* strain that degrades 2-ethoxyphenol and 4-methoxybenzoate. J Bacteriol 175:1467–1474

Kelley I, Cerniglia CE (1995) Degradation of a mixture of high-molecular-weight polycyclic aromatic hydrocarbons by a *Mycobacterium* strain, PYR-1. J Soil Contam 4:77–91

Kelley I, Freeman JP, Cerniglia CE (1990) Identification of metabolites from degradation of naphthalene by a *Mycobacterium* sp. Biodegradation 1:283–290

Kelley I, Freeman JP, Evans FE, Cerniglia CE (1991) Identification of a carboxylic acid metabolite from the catabolism of fluoranthene by a *Mycobacterium* sp. Appl Environ Microbiol 57:636–641

Kelley I, Freeman JP, Evans FE, Cerniglia CE (1993) Identification of metabolites from the degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1. Appl Environ Microbiol 59:800–806

Khan AA, Wang RF, Cao WW, Doerge DR, Wennerstrom D, Cerniglia CE (2001) Molecular cloning, nucleotide sequence, and expression of genes encoding a polycyclic aromatic ring dioxygenase from *Mycobacterium* sp. strain PYR-1. Appl Environ Microbiol 67:3577–3585

Khan AA, Kim SJ, Paine DD, Cerniglia CE (2002) Classification of a polycyclic aromatic hydrocarbon-metabolizing bacterium, *Mycobacterium* sp. strain PYR-1, as *Mycobacterium vanbaalenii* sp. nov. Int J Syst Evol Microbiol 52:1997–2002

Kim YH, Engesser KH, Cerniglia CE (2003) Two polycyclic aromatic hydrocarbon *o*-quinone reductases from a pyrene-degrading *Mycobacterium*. Arch Biochem Biophys 416:209–217

Kim SJ, Jones RC, Cha CJ, Kweon O, Edmondson RD, Cerniglia CE (2004a) Identification of proteins induced by polycyclic aromatic hydrocarbon in *Mycobacterium*

vanbaalenii PYR-1 using two-dimensional polyacrylamide gel electrophoresis and de novo sequencing methods. Proteomics 4:3899–3908

Kim YH, Moody JD, Freeman JP, Engesser KH, Cerniglia CE (2004b) Evidence for the existence of PAH-quinone reductase and catechol-*O*-methyltransferase in *Mycobacterium vanbaalenii* PYR-1. J Ind Microbiol Biotechnol 31:507–516

Kim YH, Engesser KH, Cerniglia CE (2005a) Numerical and genetic analysis of polycyclic aromatic hydrocarbon-degrading mycobacteria. Microbial Ecol 50:110–119

Kim YH, Freeman JP, Moody JD, Engesser KH, Cerniglia CE (2005b) Effects of pH on the degradation of phenanthrene and pyrene by *Mycobacterium vanbaalenii* PYR-1. Appl Microbiol Biotechnol 67:275–285

Kim SJ, Kweon O, Freeman JP, Jones RC, Adjei MD, Jhoo JW, Edmondson RD, Cerniglia CE (2006) Molecular cloning and expression of genes encoding a novel dioxygenase involved in low- and high-molecular-weight polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1. Appl Environ Microbiol 72:1045–1054

Kim SJ, Kweon O, Jones RC, Freeman JP, Edmondson RD, Cerniglia CE (2007) Complete and integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on systems biology. J Bacteriol 189:464–472

Kurbatov L, Albrecht D, Herrmann H, Petruschka L (2006) Analysis of the proteome of *Pseudomonas putida* KT2440 grown on different sources of carbon and energy. Environ Microbiol 8:466–478

Kweon O, Kim SJ, Jones RC, Freeman JP, Adjei MD, Edmondson RD, Cerniglia CE (2007) A polyomic approach to elucidate the fluoranthene degradative pathway in *Mycobacterium vanbaalenii* PYR-1. J Bacteriol 189: 4635–4647

Lamb DC, Ikeda H, Nelson DR, Ishikawa J, Skaug T, Jackson C, Omura S, Waterman MR, Kelly SL (2003) Cytochrome P450 complement (CYPome) of the avermectin-producer *Streptomyces avermitilis* and comparison to that of *Streptomyces coelicolor* A3(2). Biochem Biophys Res Commun 307:610–619

Larkin MJ, Allen CC, Kulakov LA, Lipscomb DA (1999) Purification and characterization of a novel naphthalene dioxygenase from *Rhodococcus* sp. strain NCIMB12038. J Bacteriol 181:6200–6204

Lloyd-Jones G, Lau PC (1997) Glutathione *S*-transferase-encoding gene as a potential probe for environmental bacterial isolates capable of degrading polycyclic aromatic hydrocarbons. Appl Environ Microbiol 63:3286–3290

López Z, Vila J, Grifoll M (2005) Metabolism of fluoranthene by mycobacterial strains isolated by their ability to grow in fluoranthene or pyrene. J Ind Microbiol Biotechnol 32:455–464

MacLeod CT, Daugulis AJ (2003) Biodegradation of polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor in the presence of a bioavailable solvent. Appl Microbiol Biotechnol 62:291–296

Mason JR, Cammack R (1992) The electron-transport proteins of hydroxylating bacterial dioxygenases. Annu Rev Microbiol 46:277–305

McLellan SL, Warshawsky D, Shann JR (2002) The effect of polycyclic aromatic hydrocarbons on the degradation of benzo[*a*]pyrene by *Mycobacterium* sp. strain RJGII-135. Environ Toxicol Chem 21:253–259

McLeod MP, Warren RL, Hsiao WW, Araki N, Myhre M, Fernandes C, Miyazawa D, Wong W, Lillquist AL, Wang D, Dosanjh M, Hara H, Petrescu A, Morin RD, Yang G, Stott JM, Schein JE, Shin H, Smailus D, Siddiqui AS, Marra MA, Jones SJ, Holt R, Brinkman FS, Miyauchi K, Fukuda M, Davies JE, Mohn WW, Eltis LD (2006) The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. Proc Natl Acad Sci USA 103:15582–15587

Moody JD, Freeman JP, Doerge DR, Cerniglia CE (2001) Degradation of phenanthrene and anthracene by cell suspensions of *Mycobacterium* sp. strain PYR-1. Appl Environ Microbiol 67:1476–1483

Moody JD, Fu PP, Freeman JP, Cerniglia CE (2003) Regio- and stereoselective metabolism of 7, 12-dimethylbenz[a]anthracene by *Mycobacterium vanbaalenii* PYR-1. Appl Environ Microbiol 69:3924–3931

Moody JD, Freeman JP, Fu PP, Cerniglia CE (2004) Degradation of benzo[*a*]pyrene by *Mycobacterium vanbaalenii* PYR-1. Appl Environ Microbiol 70:340–345

Moody JD, Freeman JP, Cerniglia CE (2005) Degradation of benz[*a*]anthracene by *Mycobacterium vanbaalenii* strain PYR-1. Biodegradation 16:513–526

Mukerjee-Dhar G, Shimura M, Miyazawa D, Kimbara K, Hatta T (2005) *bph* Genes of the thermophilic PCB degrader, *Bacillus* sp. JF8: characterization of the divergent ring-hydroxylating dioxygenase and hydrolase genes upstream of the Mn-dependent BphC. Microbiology 151:4139–4151

Page RD (1996) TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Patrauchan MA, Florizone C, Dosanjh M, Mohn WW, Davies J, Eltis LD (2005) Catabolism of benzoate and phthalate in *Rhodococcus* sp. strain RHA1: redundancies and convergence. J Bacteriol 187:4050–4063

Penning TM, Burczynski ME, Hung CF, McCoull KD, Palackal NT, Tsuruda LS (1999) Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active *o*-quinones. Chem Res Toxicol 12:1–18

Pinyakong O, Habe H, Yoshida T, Nojiri H, Omori T (2003) Identification of three novel salicylate 1-hydroxylases involved in the phenanthrene degradation of *Sphingobium* sp. strain P2. Biochem Biophys Res Commun 301:350–357

Ramirez N, Cutright T, Ju LK (2001) Pyrene biodegradation in aqueous solutions and soil slurries by *Mycobacterium* PYR-1 and enriched consortium. Chemosphere 44:1079–1086

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sakai M, Masai E, Asami H, Sugiyama K, Kimbara K, Fukuda M (2002) Diversity of 2, 3-dihydroxybiphenyl dioxygenase genes in a strong PCB degrader, *Rhodococcus* sp. strain RHA1. J Biosci Bioeng 93:421–427

Schneider J, Grosser R, Jayasimhulu K, Xue W, Warshawsky D (1996) Degradation of pyrene, benz[*a*]anthracene, and benzo[*a*]pyrene by *Mycobacterium* sp. strain RJGII-135, isolated from a former coal gasification site. Appl Environ Microbiol 62:13–19

Seah SY, Labbe G, Nerdinger S, Johnson MR, Snieckus V, Eltis LD (2000) Identification of a serine hydrolase as a key determinant in the microbial degradation of polychlorinated biphenyls. J Biol Chem 275:15701–15708

Stingley RL, Khan AA, Cerniglia CE (2004) Molecular characterization of a phenanthrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1. Biochem Biophys Res Commun 322:133–146

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Vila J, Lopez Z, Sabate J, Minguillon C, Solanas AM, Grifoll M (2001) Identification of a novel metabolite in the degradation of pyrene by *Mycobacterium* sp. strain AP1: actions of the isolate on two- and three-ring polycyclic aromatic hydrocarbons. Appl Environ Microbiol 67:5497–5505

Vuilleumier S, Pagni M (2002) The elusive roles of bacterial glutathione *S*-transferases: new lessons from genomes. Appl Microbiol Biotechnol 58:138–146

Wang RF, Wennerstrom D, Cao WW, Khan AA, Cerniglia CE (2000) Cloning, expression, and characterization of the *katG* gene, encoding catalase-peroxidase, from the polycyclic aromatic hydrocarbon-degrading bacterium *Mycobacterium* sp. strain PYR-1. Appl Environ Microbiol 66:4300–4304

Zhou HW, Guo CL, Wong YS, Tam NF (2006) Genetic diversity of dioxygenase genes in polycyclic aromatic hydrocarbon-degrading bacteria isolated from mangrove sediments. FEMS Microbiol Lett 262:148–157

The views presented in this article do not necessarily reflect those of the Food and Drug Administration.