Characterization of novel PAH dioxygenases from the bacterial metagenomic DNA of a contaminated soil

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Table S2 : Sequences potentially coding for RHD components found among the 600 longest contigs

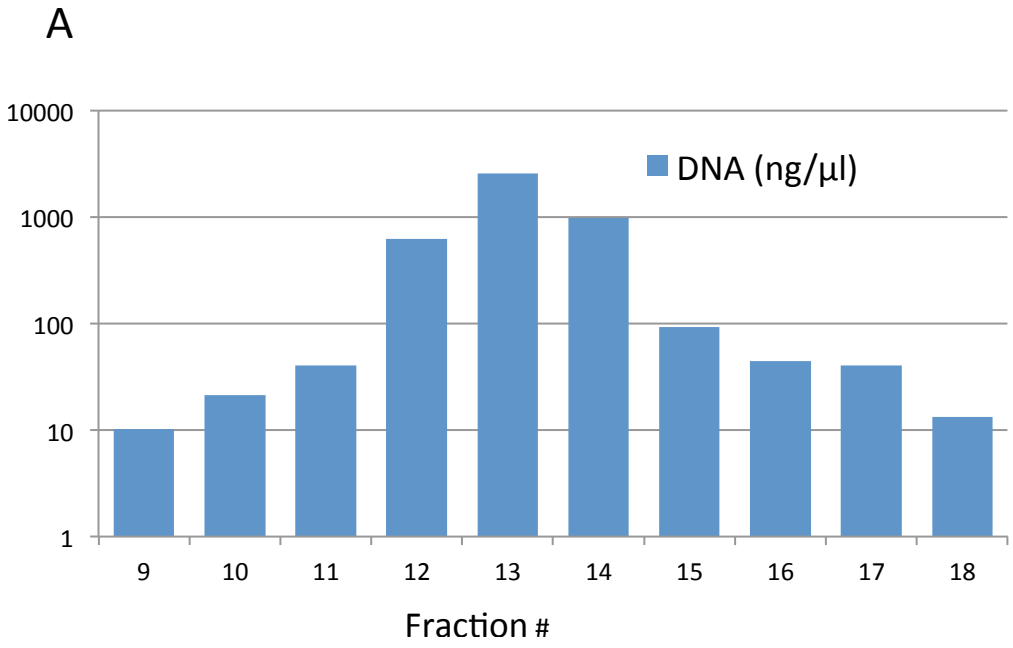
Contig	Length (bp)	Coding sequence [a] (length in bp)	Best match in the UniProtKB database					Comment
			Gene product	Accession	% identity [b]	RHD subunit	Microorganism	
95	3041	Orf95-2 (1026)	PahAa	AFH77976.1	82	Reductase	Uncultured [c]	See contig map in Fig. 1
113	2818	Orf113-2 (324)	PahAb	AFH77975.1	95	Ferredoxin	Uncultured	See contig map in Fig.1
204	2274	Orf204-2 (570)	Orf	G7UVF1	54	Beta	*P. spadix* BD-a59	
		Orf204-3 (1062)	PhnA1b	Q65AS5	60	Alpha	*Sphingomonas* CHY-1	Partial sequence
332	1884	Orf332-2 (507)	PahAd4	AFH77970.1	82	Beta	Uncultured	
		Orf332-3 (561)	PahAc4	AFH77962.1	87	Alpha		Missing N-ter (cf.contig 763)
341	1862	*Orf341-1 (945)*	PahAc2	AFH77960.1	96	Alpha	Uncultured	Missing N-ter
		Orf341-2 (507)	PahAd2	AFH77968.1	85	Beta		(cf. contig 55951)
427	1675	*Orf427-1 (1068)*	PahAc8	AFH77966.1	92	Alpha	Uncultured	Mising N-ter
		Orf427-2 (531)	PahAd8	AFH77974.1	87	Beta		(cf. contig 5241)
451	1644	Orf451-1 (1320)	PahAc5	AFH77963.1	92	Alpha	Uncultured	Missing β subunit (cf. contig 3411)

550	1501	*Orf550-1 (849)*	Orf	EGD05954.1	76	Alpha	*Burkholderia* TJI49	Missing N-ter
569	1471	Orf569-1 (1332)	PahAc3	AFH77961.1	94	alpha	Uncultured	Missing β subunit (cf. contig 2271)
763	1312	*Orf763-2 (798)*	PahAc4	AFH77962.1	87	alpha	Uncultured	Partial sequence
1214	1085	*Orf1214-2 (873)*	PahAc6	AFH77964.1	93	Alpha	Uncultured	Partial sequence
2271	862	*Orf2271-1 (639)*	PahAd3	AFH77969.1	94	Beta	Uncultured	Partial sequence
3411	746	*Orf3411-1 (180)*	PahAd5	AFH77971.1	79	beta	Uncultured	Partial sequence

[a] Orfs in italics represent partial gene sequences

[b] Percent amino acid identity between gene product and best match

[c] Uncultured bacteria found in a pyrene-degrading consortium (see main text)



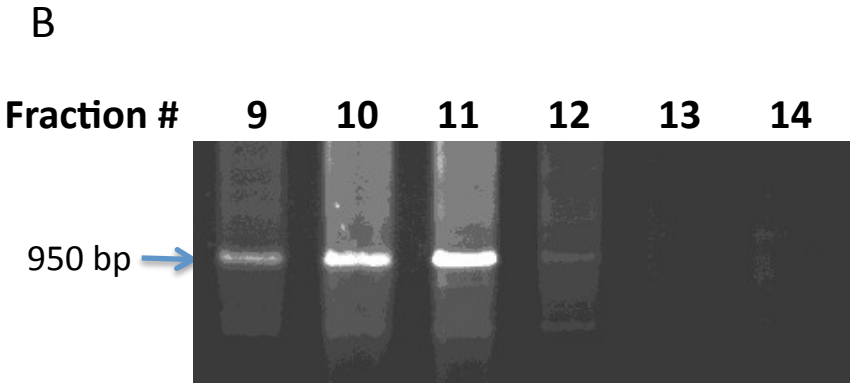


Figure S1 : Selection of heavy fractions containing labeled DNA after isopycnic centrifugation

A: Typical concentration profile of DNA-containing fractions after CsCl gradient centrifugation of soil DNA from SIP experiments. The gradient was fractionated into twenty 160-µl aliquots, from which DNA was precipitated, then taken up in 20 µl of Tris buffer as described in Methods. Concentrations were determined with a Nanodrop apparatus and plotted in log scale. Fractions are numbered from the bottom to the top of the centrifuge tube.

B: PCR analysis of central fractions of the gradient. Primers used afforded specific amplification of a 950-bp fragment internal to the RHD alpha subunit genes found in Betaproteobacteria (see Methods for details). Fractions 9-11 were pooled with other selected fractions from similar runs for subsequent DNA sequencing.

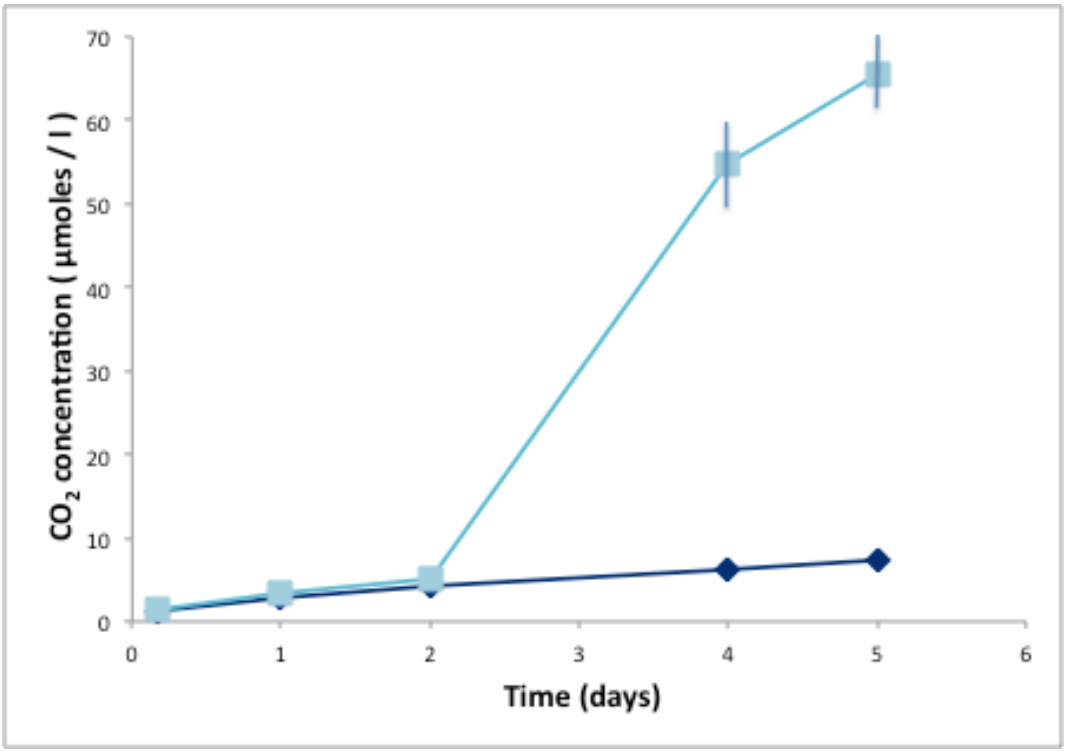


Figure S2 : Time course of ^{13}C-phenanthrene mineralization in soil microcosms

$^{13}CO_2$ produced in the headspace of microcosms was estimated by GC/MS. Concentrations are means of determinations made in 5 microcosms supplemented with labeled phenanthrene (squares) or in 2 unamended microcosms (diamonds). Standard deviations are indicated as error bars when greater than symbol size.

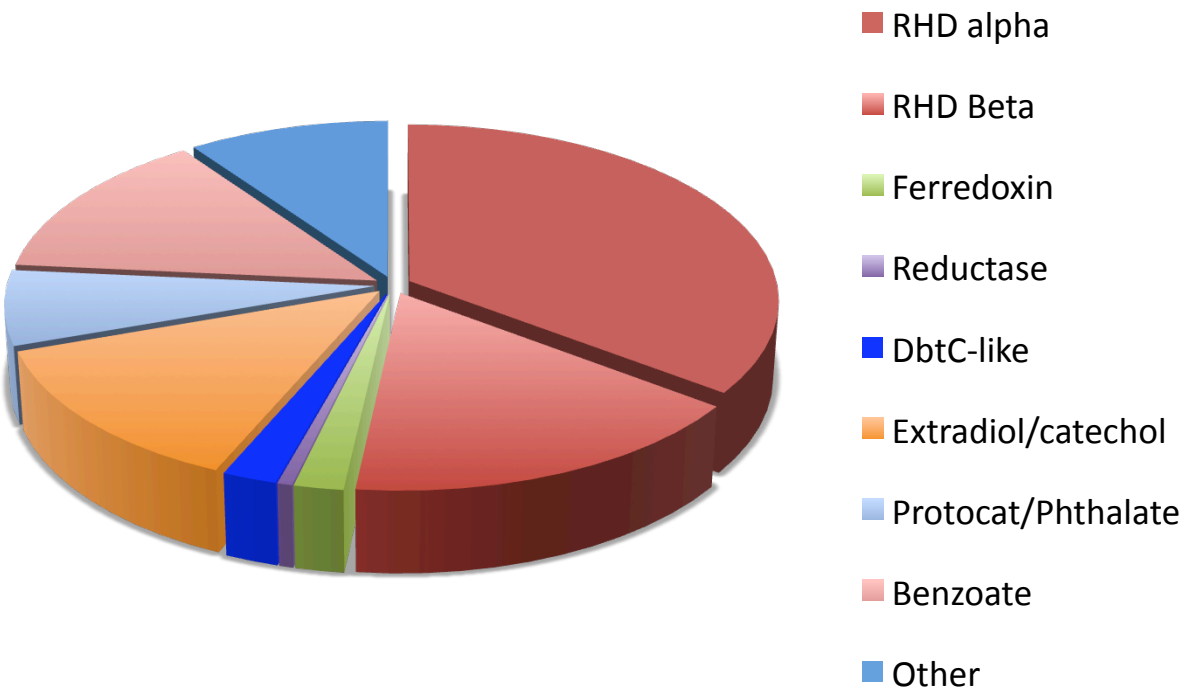


Figure S3 : Predicted dioxygenase-related sequences possibly involved in aromatic hydrocarbon metabolism

From the metagenomic data, a total 510 sequences were found to be related to RHD alpha or beta subunits, benzoate dioxygenases, as well as enzymes likely involved in later steps of the aromatic hydrocarbon degradation pathway, such as protocatechuate and phthalate dioxygenases. Ring-cleavage dioxygenases including DbtC-like enzymes thought to attack polycyclic substrates were also identified.