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Transcriptional Response of *Rhodococcus aetherivorans* I24 to Polychlorinated Biphenyl-Contaminated Sediments

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Abstract We used a microarray targeting 3,524 genes to assess the transcriptional response of the actinomycete *Rhodococcus aetherivorans* I24 in minimal medium supplemented with various substrates (e.g., PCBs) and in both PCB-contaminated and non-contaminated sediment slurries. Relative to the reference condition (minimal medium supplemented with glucose), 408 genes were upregulated in the various treatments. In medium and in sediment, PCBs elicited the upregulation of a common set of 100 genes, including gene-encoding chaperones (*groEL*), a superoxide dismutase (*sodA*), alkyl hydroperoxide reductase protein C (*ahpC*), and a catalase/peroxidase (*katG*). Analysis of the *R. aetherivorans* I24 genome sequence identified orthologs of many of the genes in the canonical biphenyl pathway, but very few of these genes were upregulated in response to PCBs or biphenyl. This study is one of the first to use microarrays to assess the transcriptional response of a soil bacterium to a pollutant under conditions that more closely resemble the natural environment. Our results indicate that the transcriptional response of *R. aetherivorans* I24 to PCBs, in both medium and sediment, is primarily directed towards reducing oxidative stress, rather than catabolism.

Abbreviations

PCBs	Polychlorinated biphenyls
GLU	Glucose control
BP	Biphenyl
C	Contaminated sediment
NC	Non-contaminated sediment
LB	Luria–Bertani Broth
MM	Minimal medium
PBS	Phosphate-buffered saline
COG	Cluster of orthologous groups of proteins

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Introduction

Polychlorinated biphenyls (PCBs) are a group of molecules which were widely used in a variety of industrial applications prior to their total ban in 1977 [32]. PCBs are chemically and thermally stable and usually recalcitrant to biodegradation. Thus, they tend to accumulate in soils and aquatic sediments, where their toxicity and persistence as pollutants makes them a threat to the ecosystem and to human health [22].

Bioremediation is thought to be a promising approach for restoring PCB-contaminated sites [20]. Anaerobic microbial communities found in sediments can reductively dechlorinate PCBs, such that fewer chlorinated *ortho*- and *ortho*- plus *para*-chlorinated congeners accumulate. Subse-

quently, aerobic organisms can attack the major congeners resulting from anaerobic dechlorination [13]. With the exception of monochlorinated molecules, chlorinated biphenyls are not known to be growth substrates for bacteria; rather, co-metabolism induced by biphenyl, or other compounds, is required to achieve growth [26]. Indeed, the enzymatic pathways involved in bacterial metabolism of PCBs are also involved in biphenyl metabolism.

Much of our knowledge of biphenyl metabolism in bacteria is derived from studies of *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400. Both of these strains were isolated from sites contaminated with chlorinated hydrocarbons, and the enzymatic pathways underlying biphenyl metabolism in each organism are similar [5, 22, 30]. Briefly, biphenyl is first metabolized to benzoate and 2-hydroxypenta-2,4-dienoate by the sequential action of a 4-component dioxygenase (e.g., BphAa, Ab, Ac, Ad), and the enzymes BphB, BphC, and BphD. Benzoate is then degraded by enzymes encoded by the *ben* and *cat* (catechol) genes while 2-hydroxypenta-2,4-dienoate is further metabolized to pyruvate and acetyl-CoA by BphE, BphF, and BphG (reviewed in [22]). Interestingly, in *R. jostii* RHA1, multiple isozymes exist for each step in this bifurcated pathway; sequence analysis, in some cases supported by experimental data, identified three complete (or partial) 4-component dioxygenases and numerous putative homologs of *bphC* (13), *bphD* (eight), *bphE*, (eight) *bphF* (seven), and *bphG* (seven). It has been suggested that this extensive complement of isozymes may contribute to this organism's aggressive catabolism of complex PCB mixtures [12].

Using microarrays, Goncalves et al. [12] compared transcript abundance in *R. jostii* RHA1 cells grown in media supplemented with either biphenyl or pyruvate as a sole carbon source. During growth on biphenyl, the *bph*, *ben*, and *cat* genes were significantly upregulated, in some cases by as much as 10,000-fold. However, while some of the predicted isozymes in the biphenyl pathway were upregulated, the majority were not. Notably, some of these apparently unresponsive genes appear to be expressed at constitutively high levels. Similar transcriptomic and proteomic studies of *B. xenovorans* LB400 have also detected the coordinated upregulation of *bph* genes in response to biphenyl. However, in addition to the induction of catabolic genes, PCBs and biphenyl were also observed to elicit a stress response, upregulating the chaperones DnaK and GroEL as well as the AhpC [1, 9, 10].

Apart from *R. jostii* RHA1, other bacteria from the genus *Rhodococcus* have been found to metabolize a wide variety of environmental pollutants including alkanes, aromatic compounds, and halogenated hydrocarbons [3, 33]. *Rhodococcus aetherivorans* I24 was isolated from a toluene-contaminated aquifer by Chartrain et al. [7]. It is able to metabolize naphthalene and toluene as sole carbon-energy sources and to convert indene to a variety of indandiols, some of which can be used as precursors of the HIV protease inhibitor indinavir sulfate [23], but it has not been tested yet for its response to PCBs.

A common feature of the studies cited above is that they focus on measuring changes in gene expression brought about by specific substrate molecule (e.g., biphenyl) under laboratory conditions (i.e., in culture media). To date, high throughput techniques have not been applied to assess the effect of PCBs on bacteria in complex environments, such as polluted soils or sediments. Several challenges associated with the presence of humic acids, organic contaminants, and metals, which may interfere with the hybridization [36], and with the difficulty of identifying which factors caused the expression of specific genes, must be addressed; in this context, studies employing pure cultures, polluted and unpolluted soil, or sediment matrices can be very useful. Similarly, there have been few (if any) investigations of how "naïve" organisms such as *R. aetherivorans* I24—those which have not been extensively exposed to PCBs—respond to these pollutants.

This study employs microarrays to assess gene expression in *R. aetherivorans* I24 during growth in a variety of sediment slurries and culture media. The primary objective of this work is to explore gene expression in contaminated sediments and contrast this with what is observed in defined medium supplemented with PCB mixtures. In addition, as *R. aetherivorans* I24 was not isolated from a site contaminated with polychlorinated hydrocarbons, its response to PCB/biphenyl exposure may offer insight into the evolution of xenobiotic degrading bacteria.

Methods

Experimental Conditions

R. aetherivorans I24 was grown overnight in 10 ml LB before inoculating 1 L of minimal medium (MM) [19] supplemented with glucose (3 gL^{-1}) and biphenyl (0.69 mg L^{-1}, i.e., 4.5 μM). Cells were incubated overnight at 30°C on a rotary shaker before repeating biphenyl supplementation and incubating for a further 3 h. Prior to treatment, the cells were washed twice in 1 L PBS and then resuspended in MM containing 3 gL^{-1} glucose (OD600=0.5). For treatments, 60 ml aliquots of this culture were then dispensed into 500-ml flasks. The treatments were as follows:

1. glucose only (GLU)
2. 0.69 mg L^{-1} of BP
3. 5 mg L^{-1} of the PCB mixture Aroclor 1254 (PCB)
4. a contaminated sediment (C)
5. a non-contaminated sediment (NC)

All samples were incubated for 48 h at 30°C on a rotary shaker (200 rpm). RNA was then isolated from 20 ml volumes which were harvested by centrifugation. For all conditions, three biological replicates were performed, each of which was hybridized in duplicate. Since the variation among these hybridization replicates was minimal (data not shown), the average of the two was taken for all data analyses.

For the sediment treatments, 9 g of contaminated or non-contaminated sediment was added to each culture flask. These sediment samples were collected in 2000 from the Wijnhaven area of Rotterdam harbor [11]. This site has 30 years history of contamination by a variety of pollutants, PCBs included. The amounts of PCBs, heavy metals, polycyclic aromatic hydrocarbons, and pesticides in both sediment samples are presented in Table 1. In a previous work, it was shown that in the contaminated sediment, the average bioavailability of PCBs congeners was 42% of the total content, with a maximum of 67% for PCB 52 and a minimum of 23% for PCB138 [24]. All sediment samples were autoclaved for 30 min at 120°C prior to the experiments.

Aroclor 1254 (ULTRA Scientific, Inc) is the commercially available PCB mixture which most closely resembles the congener pattern found in the contaminated sediment. MM was spiked with PCBs to a concentration of 5 mg L^{-1} using acetone as a carrier solvent. The acetone was allowed to evaporate completely prior to beginning the experiment.

Table 1 Total content of PCBs, PAHs, DDT, and its breakdown products DDD and DDE in the non-contaminated and contaminated sediments

	NC	C
PCB 28 (μg kg^{-1})	15	132
PCB 52 (μg kg^{-1})	7	241
PCB 101 (μg kg^{-1})	11	64
PCB 114 (μg kg^{-1})	8	82
PCB 138 (μg kg^{-1})	9	114
PCB 153 (μg kg^{-1})	11	226
PCB 180 (μg kg^{-1})	6	247
Zn (mg kg^{-1})	480	2,100
Cu (mg kg^{-1})	76	270
Cr (mg kg^{-1})	86	340
Pb (mg kg^{-1})	100	500
Cd (mg kg^{-1})	2.2	15
Phenanthrene (mg kg^{-1})	0.6	3.2
Napthalene (mg kg^{-1})	nd	0.9
Pyrene (mg kg^{-1})	0.8	4.2
Crysene (mg kg^{-1})	0.8	2.7
DDE (mg kg^{-1})	nd	0.02
DDD (mg kg^{-1})	nd	0.02
DDT (mg kg^{-1})	nd	nd

Adapted from [11]

nd not detected

Preliminary experiments (data not shown) indicated that the conditions described and the presence of glucose in the minimal medium allowed in all treatments the growth of the strain and sufficient RNA yields for microarray analyses.

RNA Isolation

RNA was extracted from sediment treatments with the QBIOgen FastRNA Pro Soil Direct Kit as per the manufacturer's recommendations (Morgan Irvine, CA). For nonsediment treatments, the QBIOgen Pro Blue Kit was used. Following isolation, RNA samples were subjected to a double DNAse treatment with Qiagen's RNase-free DNase and RNeasy mini kit (Valencia, CA). All RNA samples were stored at −80°C prior to microarray analyses, and their quality was confirmed by Bioanalyzer (Agilent, Santa Clara, CA).

DNA Microarrays

Array manufacturing, cDNA labeling, hybridization, and scanning were carried out by Roche NimbleGen, Inc (Madison, WI). A total of 3,524 genes were represented on the arrays; of these, 41 and 176 are found on the *R. aetherivorans* I24 plasmids pRA2 and pRA3, respectively. Based on the current annotation of the *R. aetherivorans* I24 genome, this accounts for 64% of the chromosomal genes, 55% from pRA2, and 54% from pRA3 (Cahill et al. in preparation). On average, there were ~3 probes per gene with a range of 1–43.

Raw probe-level intensities were initially processed using Nimblescan (Roche NimbleGen, Inc, Madison, WI). Data were subjected to quantile normalization and gene expression summary values were then calculated using the robust multiarray average algorithm [4, 15]. Statistical analysis of gene expression data was carried out with Gene Spring v.10 (Agilent, Santa Clara CA). Data were log-transformed and percentile filtered with a lower cutoff of 20 (3,366 of 3,524 genes passed filtering). Significant differences in gene expression between each treatment and the glucose-only reference were identified using volcano plot comparisons (*T* test unpaired, Benjamin–Hochberg multiple testing correction, *p* value cutoff 0.05, fold change cutoff 2.0). Fold changes were expressed as the ratio (treatment/reference) of averaged normalized expression values. All array data generated in this study have been deposited in the NCBI's Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE17033.

Sequence Analysis

To assign genes to cluster of orthologous groups of proteins (COG) functional categories, BLASTP was used to identify the reciprocal best hit for each *R. aetherivorans* I24 gene

among the sequences in eggNOG (database downloaded Mar. 25, 2009) [16]. *R. aetherivorans* I24 genes which could be assigned to COGs by this method were then mapped to functional categories using the legend provided by the COG database (http://www.ncbi.nlm.nih.gov/COG/grace/uni.html) [31].

Similarly, reciprocal best hits were used to identify putative *R. aetherivorans* I24 orthologs of isozymes from the biphenyl pathway of *R. jostii* RHA1 (as identified by Goncalves et al. [12]). The *R. jostii* RHA1 protein sequences were downloaded from http://www.rhodococcus.ca and are accessible in the NCBI under the accession numbers NC_008268.1, NC_008269.1, NC_008270.1, and NC_008271.1.

Results

Patterns in Gene Expression

Comparison of each of the four experimental treatments to the glucose-only condition identified a total of 626 genes which were significantly up- or downregulated (A full list of upregulated genes is included as part of the Supplementary material; data are summarized in Table 2). The greatest number of differentially expressed genes was found in the contaminated sediment treatment (383 total) whereas expression in non-contaminated sediment was most similar to that of the glucose-only reference (111 total). Among the sediment treatments, there was considerable overlap of the genes which were upregulated. Specifically, 37 of the 44 genes upregulated in the non-contaminated sediment were also upregulated in the contaminated sediment. Similarly, PCBs, regardless of whether in medium or contaminated sediments, elicited the upregulation of 100 genes. These common genes constitute a large proportion of the total number of genes upregulated in each PCB-containing condition (129 and 207 genes, respectively). In contrast, there were very few upregulated genes in common between the biphenyl and PCB treatments in media (12 of 184 and 129, respectively).

An examination of upregulated genes in the context of their membership in orthologous groups (i.e., COGs) did not reveal any obvious biases (Table 3). Under all conditions, similar percentages of upregulated genes fell into the categories of "Cellular processes and signaling," "Metabolism," or are "Poorly characterized." The only apparent trend was that in both sediment and media among the genes upregulated by PCBs, a greater proportion was involved in "Information storage and processing" than in the other categories. More specifically, PCBs elicited the upregulation of a large number of genes involved in "Translation, and ribosomal structure and biogenesis."

Sediment Gene Expression

Depicting the upregulated genes in a Venn diagram consisting of four sets allowed us to refine our pairwise comparisons (Fig. 1). While a common set of 37 genes were upregulated in both sediment conditions, only eight of these were found exclusively in these conditions. In fact, the majority of the 37 common genes, 23 in total, were also upregulated by PCBs in medium (Table 4). This latter set included two paralogs of the chaperone *groEL* and a universal stress protein. In addition, a thioredoxin as well as the alkyl hydroperoxide reductase protein C (*ahpC*) were upregulated in both sediment conditions and the PCB-containing medium. Both of these proteins are known to be involved in the response to oxidative stress [25, 29].

PCB Gene Expression

The 100 upregulated genes which were common to the PCB-containing medium and contaminated sediment conditions comprise two major groups. The minority was the 23 genes which were also upregulated in non-contaminated sediment, as discussed in the preceding section. A second set of 67 upregulated genes was specific to PCBs (Table 4). This group included genes encoding additional proteins related to oxidative stress response, namely a superoxide dismutase (*sodA*), a catalase/peroxidase (*katG*), and a

Table 2 Summary of up- and downregulated genes in each condition, relative to the glucose-only treatment

Treatment	Upregulated	Downregulated	Total	Common upregulated genes			
				PCB	C	NC	
BP	184	59	243	12	14	8	BP
PCB	129	87	216		100	29	PCB
C	207	176	383			37	C
NC	44	67	111				

Common upregulated genes are not necessarily *specific* to the pair of conditions being compared (see Fig. 1)

BP biphenyl in medium, *PCB* PCBs in medium, *C* contaminated sediment, *NC* non-contaminated sediment

Table 3 Functional classification of up-regulated genes based on COG membership

Category	Description	BP	PCB	C	NC
J,K,L	Information storage and processing	10.3 (19)	13.2 (17)	19.8 (41)	9.1 (4)
J	Translation, ribosomal structure and biogenesis	4.3 (8)	9.3 (12)	13 (27)	4.5 (2)
K	Transcription	4.9 (9)	2.3 (3)	5.3 (11)	2.3 (1)
L	Replication, recombination, and repair	4.3 (8)	1.6 (2)	1.4 (3)	2.3 (1)
D,M,O,T,U,V	Cellular processes and signaling	12.5 (23)	11.6 (15)	9.2 (19)	15.9 (7)
D	Cell cycle control, cell division, chromosome partitioning	1.1 (2)	0 (0)	0 (0)	0 (0)
M	Cell wall/membrane/envelope biogenesis	4.9 (9)	3.1 (4)	1.9 (4)	0 (0)
O	Posttranslational modification, protein turnover, chaperones	2.2 (4)	3.9 (5)	3.4 (7)	6.8 (3)
T	Signal transduction mechanisms	3.3 (6)	2.3 (3)	2.4 (5)	6.8 (3)
U	Intracellular trafficking, secretion, and vesicular transport	0 (0)	0.8 (1)	0.5 (1)	0 (0)
V	Defense mechanisms	1.1 (2)	1.6 (2)	1 (2)	2.3 (1)
C,E,F,G,H,I,P,Q	Metabolism	50 (92)	48.8 (63)	52.2 (108)	54.5 (24)
C	Energy production and conversion	4.9 (9)	14 (18)	9.7 (20)	15.9 (7)
E	Amino acid transport and metabolism	8.2 (15)	8.5 (11)	9.7 (20)	6.8 (3)
F	Nucleotide transport and metabolism	3.3 (6)	1.6 (2)	1.9 (4)	4.5 (2)
G	Carbohydrate transport and metabolism	4.9 (9)	6.2 (8)	5.8 (12)	6.8 (3)
H	Coenzyme transport and metabolism	3.8 (7)	2.3 (3)	3.4 (7)	2.3 (1)
I	Lipid transport and metabolism	9.2 (17)	4.7 (6)	12.1 (25)	2.3 (1)
P	Inorganic ion transport and metabolism	10.3 (19)	6.2 (8)	4.3 (9)	9.1 (4)
Q	Secondary metabolites biosynthesis, transport and catabolism	5.4 (10)	5.4 (7)	5.3 (11)	6.8 (3)
R,S	Poorly characterised	18.5 (34)	17.1 (22)	16.9 (35)	20.5 (9)
R	General function prediction only	13 (24)	12.4 (16)	12.6 (26)	15.9 (7)
S	Function unknown	5.4 (10)	4.7 (6)	4.3 (9)	4.5 (2)
	Unassigned	29.9 (55)	21.7 (28)	17.9 (37)	22.7 (10)

The value in parentheses is the total number of upregulated genes from the treatment which were assigned to the category. Percentages were calculated based on the total number of genes upregulated in each condition. As some genes belong to more than one category, these values do not sum to 100

BP biphenyl in medium, *PCB* PCBs in medium, *C* contaminated sediment, *NC* non-contaminated sediment

thioredoxin reductase. This PCB-specific set also included several ribosomal proteins (six total) and a ribosomal recycling factor.

A relatively small proportion of the genes upregulated in PCB-containing medium were specific to that condition (26 of 129), and the predicted functions of these genes are diverse. In contrast, almost one half of the genes upregulated in contaminated sediment were specific to this treatment (95 of 207). Among this set were numerous additional ribosomal proteins (12 total), alkyl hydroperoxide reductase protein F (*ahpF*), and several catabolic genes (e.g., Raet_200057—a putative catechol 2,3-dioxygenase).

Biphenyl Gene Expression

R. aetherivorans I24 can use biphenyl as its sole carbon and energy source. To account for this, the complete genome sequence of *R. aetherivorans* I24 was examined to identify orthologs of enzymes in the biphenyl pathway of the closely related bacterium *R. jostii* RHA1 (Table 5). *R. aetherivorans*

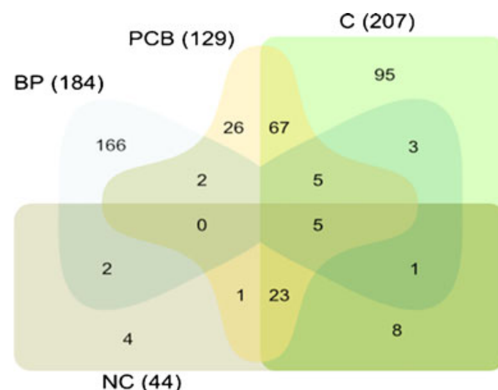

Figure 1 A Venn diagram depicting the relationships (i.e., overlaps) between the sets of genes upregulated in each treatment, relative to the glucose-only reference. (*BP* biphenyl in medium; *PCB* PCBs in medium; *C* contaminated sediment, *NC* non-contaminated sediment). Total numbers of upregulated genes per treatment are reported *in parenthesis*

Table 4 Fold changes of selected genes

Locus ID	Gene Name	Product description	C	NC	PCB
Up-regulated in sediments and PCB-containing medium (specific)					
Raet_100123		L-carnitine dehydratase/bile acid-inducible protein F	3.4	2.8	3.2
Raet_100408		Aldehyde dehydrogenase	52.9	51.1	45.1
Raet_100409		Copper amine oxidase precursor	21.7	19.5	19.5
Raet_100909		Proline/betaine transporter, MFS family	2.0	2.4	2.3
Raet_100925		Hypothetical protein	4.7	4.9	5.7
Raet_100963	ahpC	Alkyl hydroperoxide reductase protein C	3.6	2.8	2.6
Raet_101119		Universal stress protein family	2.4	2.7	2.4
Raet_101409		Possible transcriptional regulator, TetR family	2.9	3.2	2.9
Raet_101494	glpX	Fructose-1,6-bisphosphatase, GlpX type	5.1	3.1	3.2
Raet_101735		Probable ketoreductase	2.2	2.0	2.1
Raet_101877		Hydroxymethylpyrimidine ABC transporter, substrate-binding component	5.5	2.7	7.9
Raet_102058		Thioredoxin	6.1	2.7	3.4
Raet_102153	trmD	tRNA (Guanine37-N1) -methyltransferase	2.3	2.7	2.4
Raet_102170	tsf	Translation elongation factor Ts	2.9	2.3	3.0
Raet_103078	ilvC	Ketol-acid reductoisomerase	5.0	3.5	4.1
Raet_103384	groEL	Heat shock protein 60 family chaperone GroEL	10.5	6.7	8.1
Raet_103523		Alcohol dehydrogenase	15.8	18.3	98.2
Raet_103757	groEL	Heat shock protein 60 family chaperone GroEL	3.6	2.8	2.8
Raet_103861		Hypothetical protein	5.4	3.4	4.4
Raet_104170		Amide hydrolase	4.3	4.1	4.7
Raet_104512		Arsenate reductase	3.1	3.1	2.2
Raet_104985		FMNH2-utilizing oxygenase	2.3	2.2	2.8
Raet_105079		Regulatory protein	9.1	7.6	8.2
Up-regulated in PCB-containing medium and contaminated sediment (specific):					
Raet_100072		Immune-responsive protein 1	2.9	–	2.5
Raet_100181	sodA	Superoxide dismutase [Mn]	13.8	–	11.2
Raet_100302		Long-chain-fatty-acid–CoA ligase	2.9	–	2.8
Raet_100336		Putative transcriptional regulator	3.7	–	3.7
Raet_100411		Polysaccharide deacetylase	11.6	–	10.2
Raet_100427		3-ketoacyl-CoA thiolase [isoleucine degradation]	4.5	–	2.4
Raet_100656		Short chain dehydrogenase	2.2	–	2.3
Raet_101147		Isoprenylcysteine carboxyl methyltransferase	2.6	–	2.6
Raet_101177		Possible resuscitation-promoting factor	5.3	–	4.2
Raet_101192	serC	Phosphoserine aminotransferase	2.5	–	2.6
Raet_101215		Acetyl-coenzyme A synthetase	2.3	–	2.5
Raet_101259		Sodium:solute symporter protein	2.1	–	2.2
Raet_101262		Putative conserved integral membrane protein	2.4	–	2.1
Raet_101307	sucC	Succinyl-CoA ligase [ADP-forming] beta chain	2.4	–	2.1
Raet_101308	sucD	Succinyl-CoA ligase [ADP-forming] alpha chain	3.1	–	2.2
Raet_101317		Hypothetical protein	2.7	–	2.4
Raet_101451	eno	Enolase	4.3	–	3.7
Raet_101480		Serine hydroxymethyltransferase	2.6	–	2.0
Raet_101485		Putative esterase	2.0	–	2.2
Raet_101490		Metabolite transporter, MFS superfamily	2.2	–	2.4
Raet_101661		Long-chain-fatty-acid–CoA ligase	2.0	–	2.2
Raet_101738		Flavin-dependent oxidoreductase	2.7	–	3.1
Raet_101812		Hypothetical protein	2.0	–	2.2
Raet_101977		Ribonuclease PH	3.5	–	3.6

Table 4 (continued)

Locus ID	Gene Name	Product description	C	NC	PCB
Raet_102035		Hypothetical protein	3.0	–	2.9
Raet_102107		Diaminobutyrate-pyruvate aminotransferase	2.3	–	2.9
Raet_102172		Ribosome recycling factor	2.6	–	2.9
Raet_102225	dapB	Dihydrodipicolinate reductase	3.6	–	3.9
Raet_102314		Hypothetical protein	2.1	–	2.2
Raet_102472	gapA	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	3.5	–	3.5
Raet_102754		ABC transporter, substrate-binding component	2.3	–	2.3
Raet_102882		DNA polymerase IV	2.3	–	2.7
Raet_102935		Cytochrome c oxidase polypeptide II	3.0	–	4.5
Raet_102946		Dihydrolipoamide succinyltransferase component (E2) of 2-oxoglutarate dehydrogenase complex	3.6	–	3.2
Raet_102973		Phosphoglycolate phosphatase	3.4	–	2.5
Raet_103048	rpsT	SSU ribosomal protein S20p	2.8	–	2.5
Raet_103072	gltX	Glutamyl-tRNA synthetase	2.0	–	2.1
Raet_103079	ilvH	Acetolactate synthase small subunit	2.3	–	2.0
Raet_103086		Aspartyl-tRNA(Asn) amidotransferase subunit B	2.0	–	2.1
Raet_103104		Electron transfer flavoprotein, beta subunit	2.9	–	2.5
Raet_103310		Uracil phosphoribosyltransferase	2.1	–	2.3
Raet_103425	rpsE	SSU ribosomal protein S5p (S2e)	4.0	–	2.9
Raet_103435	rplP	LSU ribosomal protein L16p (L10e)	5.6	–	3.2
Raet_103436	rpsC	SSU ribosomal protein S3p (S3e)	2.6	–	2.4
Raet_103437	rplV	LSU ribosomal protein L22p (L17e)	7.9	–	5.8
Raet_103441	rplD	LSU ribosomal protein L4p (L1e)	3.8	–	2.8
Raet_103828		Ferredoxin–NADP(+) reductase, actinobacterial (eukaryote-like) type	2.4	–	4.3
Raet_103972		Transposase, IS4	2.4	–	2.7
Raet_104027		Possible transcriptional regulator	2.5	–	2.5
Raet_104035		Transposase	2.3	–	2.5
Raet_104214		Hypothetical protein	2.1	–	2.4
Raet_104250		Probable ATP-binding component of ABC transporter	2.2	–	2.0
Raet_104597		Putative type I restriction-modification system subunit R	4.2	–	4.6
Raet_104662		3-ketoacyl-CoA thiolase	4.9	–	3.5
Raet_104714		NADH dehydrogenase	6.9	–	6.4
Raet_104777		NADH:flavin oxidoreductase	2.1	–	2.2
Raet_104817		probable amino-acid transmembrane abc transporter protein	2.2	–	2.8
Raet_104861		Hypothetical protein	2.1	–	2.4
Raet_104924		Putative sugar kinase	2.7	–	4.5
Raet_105100		Possible Linoleolyl-CoA Desaturase	2.3	–	2.7
Raet_105138	katG	Catalase/ Peroxidase	3.9	–	2.3
Raet_105150		Thioredoxin reductase	2.9	–	3.7
Raet_105158		OxaI/YidC membrane insertion protein	2.1	–	2.4
Raet_300097	thiC	Thiamin biosynthesis protein ThiC	3.0	–	6.3
Raet_300153		Large subunit of N,N-dimethylformamidase	3.0	–	2.1
Raet_300154		3-oxoacyl-[acyl-carrier protein] reductase	3.2	–	3.3
Raet_300250		Conserved hypothetical protein, Fic family	2.9	–	3.0

A full list is available in the supplement

Table 5 Isozymes from the biphenyl pathway of *R. jostii* RHA1 from Goncalves et al. [12] and their putative orthologs in *R. aetherivorans* I24.

bphAaAbAcAd	bphB	bphC	bphD	bphE	bphF	bphG
Ro08060;08059;08058;08057 [1] Raet_300032;300031;300030;300029	Ro08054 (*bphB1*) Raet_300026 [2]	Ro08055 (*bphC1*) Raet_300027				
		Ro04541 (*hsaC*) Raet_100698	Ro04540 (*hsaD*) Raet_100697	Ro04533 (*hsaE*) Raet_100681	Ro04535 (*hsaF*) Raet_100683 [2]	Ro04534 (*hsaG*) Raet_100682
		Ro05803 Raet_104238 [2]		Ro05799 Raet_104242 [2]		Ro05800 Raet_104241
		Ro03865 Raet_101559			Ro03867	Ro03866 Raet_101558
		Ro10315 Raet_105110				
		Ro03975 Raet_104705				
			Ro08044 (*etbD1*) Raet_300012			
			Ro00519 (*ohpC*) Raet_101733	Ro00517	Ro00515	Ro00516
				Ro08085 (*bphE3*) Raet_300169	Ro08083 (*bphF3*) Raet_300167	Ro08084 (*bphG3*) Raet_300168 [2]
				Ro03881 Raet_101545		
Ro10133;10134 (*etbAa1Ab1*)		Ro10135 (*etbC*)	Ro10136 (*bphD1*)	Ro10137 (*bphE2*)	Ro10138 (*bphF2*)	
Ro10143;10144;10145 (*etbAa2Ab2Ac*)			Ro10146 (*etbD2*)			
Ro10125 (*etbAd*)	Ro10126 (*bphB2*)					
		Ro00477				
		Ro00536				
		Ro02488				
		Ro04905				
		Ro08079				
		Ro09005				
			Ro05797			
			Ro08081			
			Ro09014			
				Ro09021 (*bphE1*)	Ro09019 (*bphF1*)	Ro09018 (*bphG1*)
				Ro10117 (*bphE4*)	Ro10112 (*bphF4*)	Ro10116 (*bphG4*)

Column headers (in bold) refer to the enzymes which catalyze each step of the pathway. The locus ID of the *R. aetherivorans* I24 ortholog appears underneath the corresponding gene in *R. jostii* RHA1. Physically co-located genes are boxed (i.e., Ro08083, Ro08084, and Ro08085 are found adjacent to one another on the plasmid pRHL1). Genes for which no *R. aetherivorans* I24 orthologs could be found are shaded gray

[1] *bphAaAbAcAd*

[2] These genes were not represented on the arrays

I24 was found to possess all of the enzymes required for a complete biphenyl pathway, as well as a complete collection of *ben* genes. Specifically, the linear plasmid pRA3 encodes orthologs of *bphAaAbAcAd*, *bphB1*, and *bphC1* in the same arrangement as the corresponding genes in *R. jostii* RHA1. In addition, orthologs of *etbD1* and *bphE3*, *bphG3*, and *bphF3* are also present on pRA3; the arrangement of the latter three is syntenic with their orthologs in *R. jostii* RHA1. While *R. aetherivorans* I24 does possess a core set of enzymes required for biphenyl metabolism, it lacks orthologs of many of the isozymes identified by Goncalves et al. [12].

Of each of the treatments in this study, biphenyl elicited the most distinctive response. Of 184 genes upregulated in the biphenyl treatment, 166 were specifically induced by biphenyl. However, none of the orthologs of key enzymes in the biphenyl pathway, for example *bphAaAbAcAd*, were featured in this set, nor were they upregulated under any of the other conditions. In fact, only two orthologs of isoyzmes noted by Goncalves et al. [12] were upregulated by the biphenyl treatment (Raet_101733 and Raet_100698). In addition, biphenyl upregulated components of numerous transporters as well as genes with putative functions that suggest a role in aromatic catabolism. These include a ring hydroxylating dioxygenase (Raet_101087), a carveol dehydrogenase (Raet_101089), and a phenol hydrolase (Raet_101551).

Discussion

To the best of our knowledge, this is the first study in which microarrays have been applied to assess the global gene expression of a bacterial strain in PCB-contaminated sediments. To distinguish sediment-induced effects from those specific to PCBs, we included two additional treatments: non-contaminated sediment and defined medium supplemented with PCBs. Our aim was to assess how a bacterial strain of ecological importance such as *R. aetherivorans* I24 reacts to pollutants in the sediment matrix and to identify genes of potential ecological interest, e.g., for the development of biosensors. After some preliminary experiments (data not shown), we decided to pre-induce the cultures to be used in the experiments with biphenyl (0.69 mg L^{-1}), and resuspend them in MM added with glucose (3 gL^{-1}). The choice of biphenyl was based on the fact that co-

metabolism or pre-induction with biphenyl is a necessary condition for PCBs metabolism in other degrading strains such as *R. jostii* RHA1 *B. xenovorans* LB400 [8, 10]. On the other hand, the addition of glucose in all treatments ensured an active growth of cells and at the same time a sufficient RNA yield for microarray analyses. We think this choice is also representative of the ecology of soils and sediments, where easily available carbon sources such as glucose are usually present [27].

As part of this study, we also assessed gene expression in defined medium supplemented with biphenyl. This simplified treatment was intended to aid in the interpretation of the more complex treatments involving sediments and/or PCB mixtures. As well, we hoped to gain insight into the catabolism of this substrate by a bacterium which had not been extensively exposed to polychlorinated hydrocarbons. Our analysis of the complete genome sequence of *R. aetherivorans* I24 showed that while this species does possess the core enzymes required for biphenyl metabolism, it lacks orthologs of the majority of the isozymes in the biphenyl pathway of *R. jostii* RHA1 (Table 5). Given that multiple isozymes are thought to be advantageous for the catabolism of mixtures of related compounds (e.g., PCBs) [2], it may be that the extensive collection of isozymes in this latter species reflects a selective pressure imposed by the environment from which it was first isolated.

Even though *R. aetherivorans* I24 possesses all of the core enzymes of the biphenyl pathway, most of which were represented on the arrays (19 of 24), very few of these genes were upregulated in any of the treatments in this study. Of the *R. aetherivorans* I24 genes which are likely to be orthologs of the isozymes identified by Goncalves et al. [12], only isozymes of *bph*C and *bph*D were upregulated in the biphenyl treatment. In addition to these, we also observed upregulation of several genes which, while not obvious orthologs of *bph* genes, are predicted to be involved in aromatic catabolism. Thus, while some of the genes upregulated in biphenyl imply that the cells are responding to this substrate, we did not observe the coordinated upregulation of all of the core enzymes in the pathway, along with numerous isozymes, as seen under comparable conditions in *R. jostii* RHA1. One explanation for this may be the presence of glucose in all of the treatments used here. Taken together, the observations of Goncalves et al. [12] and Sakai et al. [28] suggest that in *R. jostii* RHA1, the expression of the *bphE1F1G1* operon is repressed in rich medium. If the same were true for all of the *bph* genes in *R. aetherivorans* I24, then we might not expect significant upregulation of these genes in any of the treatments. An alternative possibility is that the response observed in *R. jostii* RHA1 is, like its extensive complement of isozymes, a product of selective pressures operating in the environment from which it was isolated. This scenario implies that in a common ancestor of the rhodococci, enzymes in the *bph* pathway were not primarily involved in biphenyl metabolism; rather, selective pressures specific to the *R. jostii* RHA1 lineage led to the recruitment of these enzymes for the metabolism of polychlorinated hydrocarbons.

Sequence analysis, taken together with our microarray observations, thus revealed that although *R. aetherivorans* I24 encodes all of the necessary genes for biphenyl/PCB metabolism, these genes do not appear to be subject to the same coordinated regulation that is characteristic of bacteria which have been extensively exposed to polychlorinated hydrocarbons.

Our measurements of gene expression in PCB-containing medium broadly agree with observations of the proteome of *B. xenovorans* LB400 under similar conditions. In common with Agullo et al. [1], we note that exposure to PCBs upregulates chaperones (e.g., *groEL*) and enolase. However, while we observed that PCBs induced numerous genes involved in the response to oxidative stress, Agullo et al. [1] only identified a single gene (*ahpC*), and it was induced in response to biphenyl, rather to PCBs. In contrast, our observations have little in common with what was found in a microarray-based investigation of PCB-induced changes in gene expression in *B. xenovorans* LB400 [21].

One mechanism by which PCBs are toxic to bacteria is through the production of reactive oxygen species [8]. Accordingly, PCBs, in both sediment and medium, increased the expression of *sodA*, *katG*, *ahpC*, the chaperone *groEL*, thioredoxin, and thioredoxin reductase. All of these genes are known to be induced in response to oxidative stress and/or PCBs [1, 8, 14, 34, 35]. These findings suggest that the response of *R. aetherivorans* I24 to PCBs, regardless of whether in sediment or medium, is directed at ameliorating the effect of reactive oxygen species, rather than catabolism. Furthermore, the fact that PCBs induced a very similar transcriptional response of the strain in both sediment and medium suggest that the interference of sediment and soil components such as humic acids on the gene expression patterns and hybridization process [35] is minimal. This needs to be confirmed but sounds promising for future studies aimed at comparing the transcriptional response of bacterial strains in media of different complexity.

Exposure of cells to contaminated sediments or PCBs in pure cultures induced a significantly higher ribosome synthesis. No previous findings on this induction in bacteria by PCB are available; only one study found a higher induction of these genes by a microarray study of gene expression in mammalian cells exposed to PCBs [6].

Of the 408 genes which were upregulated among all treatments, only eight were restricted to the sediment treat-

ments. Among the 37 genes common to C and NC treatments, 23 are also found in PCB-containing medium. This common set includes chaperones and a universal stress protein. Thus, it may be that even in non-contaminated sediment, most of the upregulated genes are part of a general stress response that is also activated by PCBs, regardless of whether they are in sediment or medium.

The genes upregulated in contaminated sediment include those common to the non-contaminated sediment and PCB-containing medium (discussed above), as well as those specific to the conditions which included PCBs. Among this set are numerous genes that may play a role in the cell's response to the oxidative stress caused by the PCBs. There were also a considerable number of genes (95) which were upregulated only in the contaminated sediment. The sediment samples used in this study are known to be contaminated with other pollutants (Table 1) in addition to PCBs. These condition-specific genes may reflect the cell's response to these additional substrates.

Our assessment of global gene expression in *R. aetherivorans* I24 revealed that response in pure culture can be to a certain extent a predictor of the ecological behavior of the strain in presence of contaminated sediments. The high induction of stress genes and, on the other hand, the lower or absent induction of genes related to biphenyl metabolism in cells exposed to PCBs in pure culture or in contaminated sediment may indicate that the strain, even if pre-induced with biphenyl and containing PCBs degradation genes, is actually more involved in counterbalancing the stress caused by the presence of pollutants rather than degrading them, at least in the 48 h incubation used here and even in the presence of glucose. This can hold important consequences for biosensor-based risk assessment and for the bacterial-aided bioremediation of contaminated sites. Monitoring of the induction of stress genes in contaminated samples may in fact represent a way to identify potential constraints to soil bioremediation practices, while the in situ application of biosensors specific for pollutants such as PCBs in soils and sediments may be limited by a low or absent induction of genes involved in the degradation if those genes are used as promoters [17, 18]. On the other hand, some of the genes identified here could be used as indicators not of the effects of a single class of specific molecules such as PCBs but rather to abiotic stresses caused by chemical pollution of different origins. Finally, specific experiments aimed at understanding the optimal exposure time and conditions for the expression of catabolic genes would be very useful for bioremediation purposes.

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