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ORIGINAL PAPER

Environmentally relevant parameters affecting PCB degradation: carbon source- and growth phase-mitigated effects of the expression of the biphenyl pathway and associated genes in *Burkholderia xenovorans* LB400

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Abstract The principal means for microbial degradation of polychlorinated biphenyls (PCBs) is through the biphenyl pathway. Although molecular aspects of the regulation of the biphenyl pathway have been studied, information on environmental facets such as the effect of alternative carbon sources on (polychlorinated) biphenyl degradation is limited. Here we explore the effect of environmental conditions (e.g., carbon source and growth phase) on the variation in PCB degradation profiles of *Burkholderia xenovorans* LB400. Genome-wide expression patterns reveal 25 genes commonly up-regulated during PCB degradation and growth on biphenyl to be upregulated in the transition to stationary phase (relative to growth on succinate) including two putative detoxification pathways. Quantitative reverse transcription PCR (Q-RT-PCR) analysis of the upper biphenyl pathway (*bphA*, *bphD*, and *bphR1*), and detoxification genes in response to environmental conditions suggest associated regulation of the biphenyl pathway and chloroacetaldehyde dehydrogenase. The response of genes in the upper biphenyl pathway to carbon source competition and growth phase reveals inhibition of the biphenyl pathway by PCBs. Although PCBs are not degraded during growth on succinate with PCBs, expression data indicate that the biphenyl pathway is induced, suggesting that post-transcriptional regulation or active transport of biphenyl maybe limiting PCB degradation. Identification of the involvement of peripheral pathways in degradation of PCBs is crucial to understanding PCB degradation in an environmental context as bacteria capable of biodegradation experience a range of carbon sources and growth phases.

Keywords Polychlorinated biphenyl degradation · Cometabolism · Biphenyl degradation pathway · *Burkholderia xenovorans* LB400

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Introduction

Over the past three decades, microbial biodegradation of polychlorinated biphenyls (PCBs) has focused primarily on the molecular regulation and gene structure of the biphenyl pathway fortuitously

responsible for the co-metabolism of lower-chlorinated congeners (Ahmed and Focht 1973). In order for successful in situ microbial biodegradation of PCBs to occur; PCBs must be available to biodegrading microorganisms, and PCB-degrading pathways in aerobic bacteria need to be induced (reviewed in Ohtsubo et al. 2004). The biphenyl degradation pathway responsible for the degradation of PCBs has been analyzed and several studies have identified molecular components key to the regulation of the biphenyl pathway (see reference Pieper and Seeger 2008 for a review of PCB degradation). BphS is a GntR-type repressor (Mouz et al. 1999) shown in *Pseudomonas* sp. KKS102 to negatively affect the biphenyl pathway (Ohtsubo et al. 2000). ORF0 is another regulator belonging to the GntR family and was identified as a positive regulator for itself and the biphenyl pathway in *Pseudomonas pseudoalcaligenes* KF707 (Seeger et al. 1995) and as a regulator for *bphA1* in *Burkholderia xenovorans* LB400 (Beltrametti et al. 2001; Denef et al. 2004; Erickson and Mondello 1993).

Burkholderia xenovorans LB400 (LB400) (Chain et al. 2006; Goris et al. 2004) has become one model organism to study the aerobic biodegradation of PCBs because of the wide range of congeners oxidized as well as its ability to use some congeners as sources of carbon and energy (Bopp 1986; Gibson et al. 1993; Maltseva et al. 1999; Mondello et al. 1997). While LB400 has no homolog for BphS, regulation of the biphenyl pathway in LB400 involves BphR1 (formerly ORF0) (Beltrametti et al. 2001; Denef et al. 2004; Watanabe et al. 2003). BphR1 is a positive regulator of the biphenyl pathway in LB400 through a promoter localized upstream of *bphA* and has been identified as a biphenyl-inducible regulator of *bphA* (Beltrametti et al. 2001; Denef et al. 2004; Erickson and Mondello 1993), and possibly *bphD* (Denef et al. 2004).

Although molecular mechanisms involved in the regulation of the biphenyl pathway have been described, information on environmental factors affecting PCB degradation is limited. Previous results suggest that the degradation of PCBs does not occur during exponential-phase growth with simple carbon compounds such as glucose, succinate and benzoate (Billingsley et al. 1997; Furukawa et al. 1983; Parnell et al. 2006). However, following removal of simple carbon sources (either artificially, as in resting cell conditions, or by natural consumption), PCBs are capable of inducing the biphenyl pathway (Barriault et al. 1998; Bedard and Quensen 1995; Bedard et al. 1987, 1986; Gibson et al. 1993; Seeger et al. 1995). Although the effect of carbon source on PCB degradation has been studied (Billingsley et al. 1997), details have been limited to overall degradation profiles.

Most environmental settings where in situ bioremediation of PCBs by microorganisms would be most advantageous contain a wide range of naturally-occurring carbon sources at different concentrations and the growth phase of biodegrading bacteria varies. This investigation focuses on the expression of the biphenyl pathway and genes suggested to be co-regulated with the biphenyl pathway based on transcriptomic studies from an ecological context of competing carbon sources (succinate, biphenyl and PCBs) and different physiological states (growth phase).

Materials and methods

Media and growth conditions

Growth curve experiments allowed us to determine the effects of simple carbon sources and growth phase (carbon starvation) on the biphenyl pathway in *Burkholderia xenovorans* LB400. We grew triplicate cultures of LB400 in 25 ml mineral medium (K1) with combinations of biphenyl (3 g/l), succinate (1 g/l) and Aroclor 1242 (Monsanto Co., St. Louis, Mo.) (500 ppm) as described previously (Denef et al. 2004; Parnell et al. 2006). Cultures were grown in 125 ml Wheaton® flasks and incubated on an orbital shaker (200 rpm) at 30 ± 2°C. The growth rate of LB400 on different carbon sources was determined by measuring the maximum slope during logarithmic growth. Cell cultures were harvested at different growth phases—either mid-logarithmic [(ML) 0.3–0.4 OD_{600} nm] or transition to stationary-phase [(TP) 0.8–1.0 OD_{600} nm] growth (see 10)—for further analysis. For carbon source utilization, LB400 was grown on biphenyl until early logarithmic growth (OD 0.35) and amended with succinate. Following the addition of succinate, samples were harvested at 5, 30 min, and 1 h to determine the effect of simple

carbon compounds on regulation of the biphenyl pathway.

Growing cell assays

Starting cultures for each condition (succinate and biphenyl) were grown in K1 medium and transferred to 25 ml cultures of K1 medium with the same carbon source and 500 ppm Aroclor 1242. Due to the difference in the growth rates on each carbon source, the harvest time for LB400 (when mid-logarithmic phase was reached) varied from 12 to 24 h. Aroclor 1242 was added directly to the media at least 24 h prior to inoculation to allow stabilization. PCB degradation by LB400 was measured in triplicate by determining the disappearance of PCBs from cultures harvested at mid-log (ML), late log, and transition to stationary phase (TP) (0.4, 0.7 and 1.0 OD_{600}, respectively) under different carbon source growth conditions. Each PCB-containing treatment was extracted three times using an equal volume of 1:1 hexane:acetone solution by shaking for 30 min and analyzed by gas chromatography as described previously (Quensen et al. 1990). Controls were performed by extraction from uninoculated media. Percent degradation was calculated by determining the difference between PCB concentrations in experimental cultures and in controls as described earlier (Parnell et al. 2006).

Resting cell assays

PCB degradation profiles by LB400 were determined from cultures grown on succinate and biphenyl harvested from ML and from TP by centrifugation and rinsed to remove exogenous carbon. Cells were grown to an optical density of 1–1.5 at 600 nm and washed in sterile K1 medium twice before resuspending in the same medium at an optical density of 2.0. Reactions were carried out at 30 ± 2°C in 1-dram glass vials fitted with Teflon-lined lids containing 990 µl of cell culture and 10 µl of chlorobiphenyl solution. Chlorobiphenyl congeners (2-, 2,2′-, 2,4-, 2,3-, 4,4′-, 2,4,2′,4′- and 2,5,2′-chlorobiphenyl) used in resting cell assays were each at a final concentration of 2 ppm and a control for each assay was conducted in parallel consisting of heat-inactivated (121°C, 10 min) cells. PCB degradation assessment for each of the seven different PCB congeners was measured in triplicate using traditional methods described previously (Bedard et al. 1986), except for growth phase. PCBs were extracted after 0, 2, 4, 12, and 24 h.

cDNA preparation

RNAlater (Ambion) was added in a 1:1 ratio to the culture to protect RNA against degradation. Bacterial cells were harvested by centrifugation at 5,000× g for 10 min at 4°C. RNA was extracted using RNeasy RNA extraction kit (Qiagen), and remaining DNA was removed by 30 min incubation at room temperature with 1.5 U DnaseI/(g nucleic acid (Roche, 10 U/(l). Integrity of RNA and absence of DNA was verified by 1.2% agarose gel electrophoresis. 2 µg of total RNA was incubated overnight at 45°C using 6 µg random primers (Invitrogen), and Superscript II reverse transcriptase (Invitrogen). Purification was performed using the QiaQuick PCR purification kit (Qiagen). Total cDNA concentration was determined spectrophotometrically (Nanodrop® Wilmington, DE).

Q-RT-PCR analysis

Primers for *bphA*, *bphD*, *bphR1* and genes involved in putative detoxification pathways were designed using Primer Express software (Applied Biosystems, Foster City, CA). Triplicate Q-RT-PCR runs were performed for each condition and each targeted gene. One ng of cDNA from each condition was utilized in triplicate for 40 cycle, two step PCR in an ABI 7900HT (Applied Biosystems, Foster City, CA) using SYBR Green Master Mix (Applied Biosystems, Foster City, CA) and 125 nM of each primer (Table 1). Amplicon size (80–100 bps) and reaction specificity were confirmed by agarose gel electrophoresis and product dissociation curves. The number of target copies in each sample was interpolated from its detection threshold (CT) value using a purified PCR product standard curve. l6S rRNA expression was measured as internal control, and the measured internal control signal was used to normalize variations due to different reverse transcription efficiency (Denef et al. 2004). All conditions were normalized to succinate-grown cells harvested during ML growth.

Table 1 Primers used for Q-RT-PCR analysis in this study including biphenyl pathway elements and formaldehyde and chloroacetladehyde detoxification pathway genes

Primer	Sequence
bphA-f	3-gctacgtgggtacaaggc-5
bphA-r	3-tagccgacgttgccagg-5
bphD-f	3-ccgcactcaccgaaagttctac-5
bphD-r	3-ttacccgcctcgttgtagtg-5
bphR1-f	3-gtcagttcgtatcaccggc-5
bphR1-r	3-ccactgattgaacaagtgaacc-5
ClAc-f	3-atcgccaagattgcgtttac-5
ClAc-r	3-gtacacacctcgccctgatt-5
xoxF-f	3-aaggcaggtgaaaccaacac-5
xoxF-r	3-catacgtcgtcgtcttgtcg-5
Fae-f	3-aacaaggtcaccatcaaggg-5
Fae-r	3-ggaccgaacatctgcacc-5

Results

Effect of growth phase on PCB degradation

Growing-cell assays for PCB degradation of LB400 with succinate demonstrated a growth phase-dependent shift to PCB degradation (Fig. 1). Early growth (0.4 OD) indicated little degradation of PCBs; primarily degradation consisted of congeners with a chlorine-free ring. Although some degradation of higher chlorinated PCBs were evident in later logarithmic phase (0.7 OD), the greatest amount of PCB degradation occurred as the initial growth substrate was consumed during transition to stationary phase (1.0 OD) where cells were still growing, but likely in a state of carbon limitation.

Based on resting cell PCB degradation assays, the most significant change in degradation profile was revealed in LB400 harvested from TP biphenyl-grown cells. The increased degradation was generally observed for di-para substituted PCBs (Fig. 1). Ortho substituted congeners appear readily degraded on removal of exogenous carbon consistent with growing cell data while highly chlorinated para-substituted congeners show little indication of degradation except when harvested from biphenyl transition-phase growth.

Transcriptional profile comparison

Using genome-wide expression data from earlier studies (Denef et al. 2004; Parnell et al. 2006), a comparison of the gene expression patterns from biphenyl transition-phase and degradation of PCBs during growth on biphenyl indicate the induction of several common genes and pathways. Of the 114 genes up-regulated during PCB degradation (ML growth on biphenyl with PCBs) with reference to succinate ML, 49 (43%) were also induced during TP growth on biphenyl. Of the 49 genes commonly induced during PCB degradation and TP growth on biphenyl, 25 were unique relative to biphenyl ML growth (Table 2), including several genes involved in C_1 degradation and a putative chloroacetaldehyde dehydrogenase.

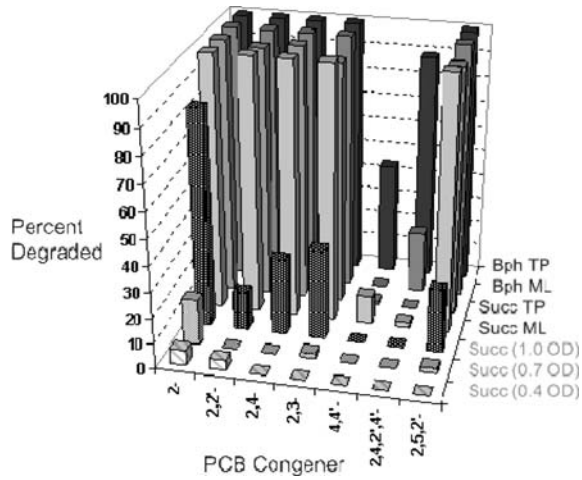


Fig. 1 Percent degradation of PCBs by *Burkholderia xenovorans* LB400. Degradation of selected PCB congeners analyzed by growing-cell and resting-cell assays associated with different growth substrates [succinate (Succ) and biphenyl (Bph)] and growth phases [Mid-Logarithmic (ML) and Transition to stationary-phase (TP)]. Resting cell assay data (Succ ML, TP and Bph ML, TP) rows are *black*, *gray rows* represent growing cell assays

Q-RT-PCR expression analysis

Samples harvested from various points along the growth curve give a representation of the induction of the biphenyl pathway according to carbon source competition as well as growth stage. During growth on succinate with PCBs, the *bphA* and *bphD* were down-regulated compared to growth on succinate alone (0.6 ± 0.2 and 0.3 ± 0.2, respectively). LB400 grown with succinate and biphenyl concurrently demonstrate an increased expression in the biphenyl pathway as compared to succinate alone during both

Table 2 Genes identified through genome-wide microarray analyses as commonly induced during growth on biphenyl transition to stationary-phase (TP) and exposure to PCBs during mid-log (ML PCBs) divergent from genes induced during growth on biphenyl alone (ML)

Gene ID	Annotation	Associated function	Expression ratio[a]		
			ML[c]	ML PCBs[b]	TP[c]
BxeC0962	Hemerythrin-like metal binding protein	Unknown	1.6	2.5	5.3
BxeC1188	(BphF) acetaldehyde dehydrogenase	Biphenyl pathway	1.8	2.2	6
BxeB1679	Putative transport-associated protein	Unknown	1.5	2.8	12.9
BxeB2425	**MxaJ-like solute binding protein**	**Formaldenyde detoxification**[d]	1.4	2.3	12
BxeB2426	**Cytochrome c-555 precursor**	**Formaldenyde detoxification**	1	4.8	42.3
BxeB2427	**(XoxF) Methanol dehydrogenase-like protein**	**Formaldenyde detoxification**	0.9	8	47.1
BxeB2436	**(Fae) Formaldehyde activating enzyme**	**Formaldenyde detoxification**	1.3	20.7	160.3
BxeB2437	**Conserved hypothetical protein**	**Formaldenyde detoxification**	1.1	5.8	66.8
BxeB2470	**Coenzyme PQQ synthesis protein E**	**Formaldenyde detoxification**	1.2	2.2	7
BxeB2473	**TonB-dependent receptor precursor**	**Formaldenyde detoxification**	0.9	2.8	14.8
BxeB2474	**Conserved hypothetical protein**	**Formaldenyde detoxification**	0.9	3	22
BxeB2469	**Coenzyme PQQ synthesis protein D**	**Formaldenyde detoxification**	1.1	2.6	9.7
BxeA0355	Conserved hypothetical protein	Unknown	1.1	2.1	5.9
BxeA1028	Putative exported protein	Unknown	2	2.7	4
BxeA1129	Chloromuconate cycloisomerase	Chlorobenzoate degradation	1	2.6	2.2
BxeA1130	Chlorocatechol 1,2-dioxygenase	Chlorobenzoate degradation	1.1	3.1	4.6
BxeA1629	Putative sugar ABC transport, substrate-binding protein	Unknown	1.8	6.7	2.3
BxeA1994	Putative Cyd operon protein YbgT	Unknown	1.3	2.2	2.3
BxeA2773	Regulatory protein, TetR	Unknown	1.6	2.6	4.3
BxeA2801	Putative peptidase A24A, prepilin type IV precursor	Unknown	1.7	2.1	3.8
BxeA2944	Conserved hypothetical protein	Unknown	1.4	2.6	2.9
BxeA3322	Putative membrane protein	Unknown	1.8	2.2	2.7
BxeA4441	**(Chloro)actetaldehyde dehydrogenase**	**Chloroacetaldehyde detoxification**[b]	1.8	8.1	34.3
BxeA4442	**Conserved hypothetical protein**	**Chloroacetaldehyde detoxification**	1	2.2	7.3
BxeA4478	Conserved hypothetical protein	Unknown	1.2	3.5	39.1

Highlighted genes indicate previously identified detoxification pathways that may be involved in PCB degradation

[a] Expression ratio is reported relative to LB400 grown on succinate and harvested during ML growth

[b] Data from Parnell et al. (2006) (36)

[c] Data from Denef et al. (2004) (13)

[d] Formaldehyde detoxification pathway characterized by Marx et al. (2004) (27)

ML- and TP-growth as *bphA* expression was increased 4.3 ± 1.8- and 9.7 ± 1.8-fold and *bphD* 2.8 ± 1.7- and 4.0 ± 2.1-fold (Table 3).

Regression analysis of the expression patterns gave an indication of the association of different genes with carbon source and growth phase. As expected, the biphenyl pathway elements (*bphA*, *bphD* and *bphR1*) are all statistically correlated. In addition, the putative chloroacetaldehyde dehydrogenase gene (ClAc) expression is strongly correlated with the biphenyl pathway (Table 4).

Carbon source competition

Growth rates of LB400 on a combination of carbon sources indicate primary substrate utilization. Maximum growth rates were calculated for growth on succinate, succinate and biphenyl, succinate and

Table 3 Expression values from quantitative RT-PCR analysis demonstrating the effect of different carbon sources [succinate (Succ), biphenyl (Bph), and polychlorinated biphenyls (PCB)] and different growth phases [Mid-Logarithmic (ML) and Transition to stationary-phase (TP)] on biphenyl pathway components, regulatory elements, and detoxification genes potentially involved in biphenyl degradation

	bphA	*bphD*	*bphR1*	Fae	*xoxF*	ClAc
Succ TP	1.3 ± 0.5	1.9 ± 1.4	0.8 ± 0.1	1.2 ± 0.3	1.2 ± 0.3	0.4 ± 0.1
Succ PCB ML	0.6 ± 0.2	0.3 ± 0.1	1.4 ± 0.1	0.8 ± 0.1	1.2 ± 0.3	1.3 ± 0.5
Succ PCB TP	2.1 ± 0.8	1.1 ± 0.6	1.0 ± 0.2	7.2 ± 2.3	2.0 ± 0.6	6.9 ± 2.4
Succ Bph ML	4.3 ± 1.8	2.8 ± 0.7	4.4 ± 1.7	1.1 ± 0.4	3.6 ± 1.4	10.8 ± 5.5
Succ Bph TP	9.7 ± 1.8	4.0 ± 2.1	6.6 ± 1.4	10.0 ± 3.3	6.0 ± 2.4	15.7 ± 6.2
Bph PCB ML	26.2 ± 4.9	27.0 ± 3.2	17.5 ± 2.1	9.5 ± 2.0	5.4 ± 0.5	356.2 ± 123.8
Bph PCB TP	14.4 ± 3.1	34.5 ± 7.2	8.0 ± 2.3	70.1 ± 21.2	9.1 ± 2.0	251.3 ± 75.8

Expression values are reported relative to succinate-grown *Burkholderia xenovorans* LB400 harvested from mid-logarithmic growth (Succ ML)

±SE

Table 4 Correlation matrix of r^2 values from regression analysis of the expression of components of the biphenyl pathway and detoxification pathway genes possibly associated with biphenyl degradation in *Burkholderia xenovorans* LB400

	bphA	*bphD*	*bphR1*	Fae	*xoxF*	ClAc
bphA	–	**0.84**	**0.84**	0.30	0.27	**0.85**
bphD		–	**0.77**	**0.31**	0.19	**0.82**
bphR1			–	0.23	0.26	**0.84**
Fae				–	**0.57**	**0.60**
xoxF					–	0.19

Quantitative RT-PCR expression data were converted to $\log_2$ values prior to regression analysis

Significant *P*-values (<0.05) are bold

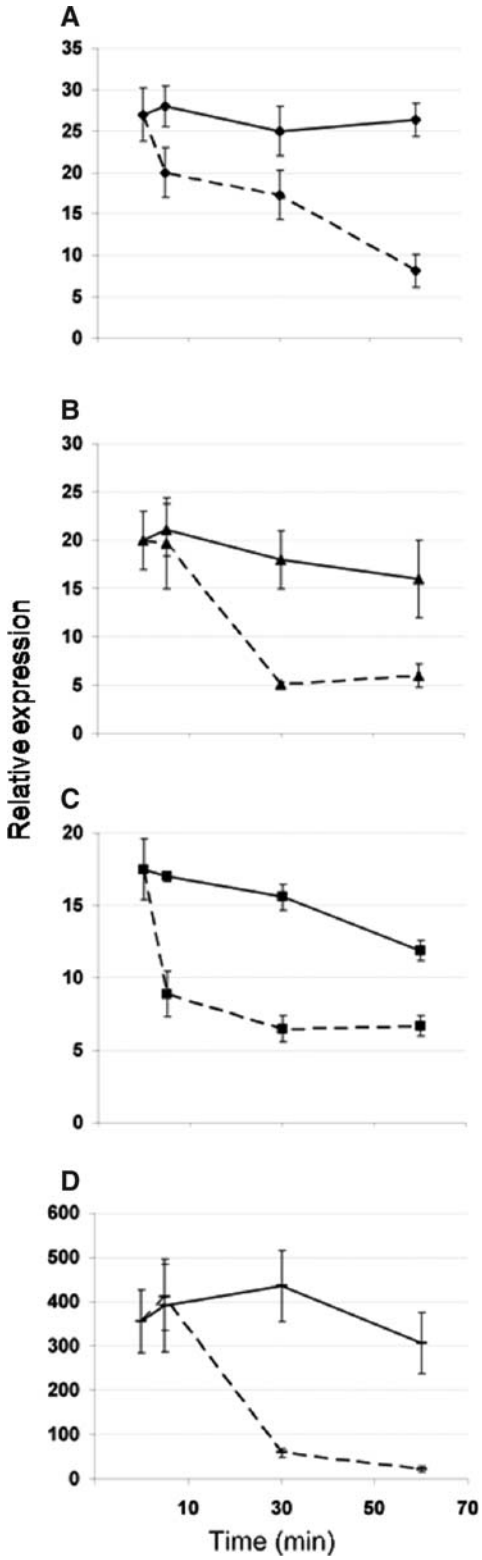
PCBs, biphenyl, and biphenyl with PCBs. The growth rate of LB400 on succinate alone was 0.42 ± 0.03 doubling/h (±SE), 0.41 ± 0.06 for succinate and biphenyl together and 0.42 ± 0.05 for LB400 grown with succinate and PCBs. The growth rate of LB400 on biphenyl alone was 0.21 ± 0.02 doubling/h and 0.20 ± 0.02 for biphenyl with PCBs. Once succinate was added to a biphenyl-grown culture, the growth rate changed from 0.22 ± 0.03 to 0.37 ± 0.02 within 30 min.

Within 5 min of addition of succinate to biphenyl-grown culture during ML phase, the expression of *bphD* decreased significantly (from 27.3 ± 3.2- to 20.0 ± 3.1-fold induction relative to succinate ML). Similarly, *bphR1* expression decreased significantly (from 17.5 ± 2.1- to 8.9 ± 1.6-fold induction) within 5 min of succinate addition. The expression level of *bphA* did not show significant decrease until between 5 and 30 min of succinate addition (20.0 ± 3.0- to 5.1 ± 0.2-fold induction) (Fig. 2).

Discussion

This study places (polychlorinated) biphenyl degradation in a context relevant to environmental scenarios by studying factors such as carbon source competition and growth phase effects on expression of the biphenyl pathway in LB400. We demonstrate the complexity of the regulation of the biphenyl pathway from an environmental perspective that has not been viewed before. Although inhibition of the biphenyl pathway by simple carbon sources has been demonstrated (Billingsley et al. 1997; Furukawa et al. 1983; Parnell et al. 2006), the detailed approach here provides insight to subtle, yet profound regulatory mechanisms. During growth of LB400 on succinate, PCB degradation was very limited, yet expression data indicate induction of the biphenyl pathway. This evidence suggests post-translational regulation or active transport as another layer of regulation for the biphenyl pathway.

Billingsley et al. (1997) reported that LB400 conditioned to biphenyl growth results in more efficient PCB degradation than LB400 grown on other sources (Billingsley et al. 1997). These results are somewhat expected as the physiological state of LB400 grown on biphenyl is more comparable to PCB-degrading conditions (same pathways are



◄ **Fig. 2** Q-RT-PCR expression following addition of succinate. Following growth of LB400 to mid-log phase, succinate was added (time 0) and data collected at 0, 5, 30 and 60 min following addition of succinate. Expression of *bphD* (**a**), *bphA* (**b**), the regulator *bphR1* (**c**), and chloroacetaldehyde dehydrogenase (**d**) relative to succinate mid-log growth of LB400. Unamended culture (*solid*) and amended with succinate (*dashed*)

induced). As co-metabolism of PCBs relies on removal of all exogenous carbon sources, energy becomes a limiting factor and dynamic changes in gene expression patterns (such as a switch from a dissimilar growth substrate like succinate) require energy that could otherwise be allocated to PCB degradation. The effect of energy availability on PCB degradation was demonstrated by Kohler et al. (1988) by comparing growing cell assays with resting cell assays (both grown on biphenyl). Not only did they find an increase in the magnitude of PCB degradation, but the range of congeners degraded also increased in growing cells (Kohler et al. 1988). They concluded that PCB degradation during growth on biphenyl affords more physiological stability leading to more active cells (Kohler et al. 1988). Similarly, resting cell assays presented here of cells harvested from different carbon sources (succinate and biphenyl) and growth phases (ML and TP) demonstrate a physiologically-mediated range in PCB degradation (Fig. 1). These findings in conjunction with recent genomic (Denef et al. 2004; Parnell et al. 2006) and proteomic analyses (Denef et al. 2005) suggest the activation of mechanisms beyond the biphenyl pathway that provide stability and improve PCB degradation for LB400.

Transcriptional analysis of active PCB degradation (during growth on biphenyl) and of TP biphenyl cells (condition harvested for resting cells that included greatest PCB degradation) identified 25 potential genes involved in efficient degradation of PCBs (Table 2). Two pathways induced during PCB degradation and biphenyl TP growth have potential for detoxification of damaging compounds. Marx et al. (2004) identified the C_1 pathway in LB400 as responsible for removal of formaldehyde (Marx et al. 2004). Additionally, chloroacetaldehyde dehydrogenase may eliminate toxic products of incomplete PCB degradation (Parnell et al. 2006). Induction

of these pathways prior to exposure to increased levels of formaldehyde- or chloroacetaldehyde-like compounds would impart an enormous advantage toward physiological stability of resting cell conditions and improve PCB degradation. Regression analysis of the expression patterns indicate that the expression chloroacetaldehyde dehydrogenase is significantly correlated to expression of the biphenyl pathway. Conversely, the expression of elements of the C_1 pathway (XoxF and Fae) was not strongly correlated to the induction of biphenyl pathway genes, although Fae and *bphD* expression is significant (Table 4).

Despite previous studies indicating that the biphenyl pathway (degradation of PCBs) is not induced (and possibly repressed) during growth on simple carbon compounds (Billingsley et al. 1997; Denef et al. 2004, 2005; Furukawa et al. 1983; Parnell et al. 2006), information on the effect of simple carbon sources on the induction of the biphenyl pathway is cursory. Carbon source utilization analysis indicates that despite an extended lag phase for adaptation, the eventual maximum growth rate for LB400 grown on both succinate and biphenyl was identical to growth on succinate alone. Addition of succinate to biphenyl-grown ML cultures changed the growth rate of LB400 from biphenyl- to succinate-like growth rates, suggesting a switch to succinate as carbon source and possible catabolite repression. In addition, compared to biphenyl treatments, the expression of *bphD* and *bphR1* in succinate amended treatments declined significantly (within 5 min). By 30 min, expression of *bphA* decreased significantly in succinate amended treatments (Fig. 2). By 1 h (Bph Succ ML + 1 h), the expression level of all three components of the biphenyl pathway examined diminished to levels similar to succinate with biphenyl (Succ Bph ES) (Table 3). This information indicates that succinate does play a role in repression or inactivation of the biphenyl pathway. Additional evidence presented in this study, in effect suggests that PCB degradation does not occur during growth on succinate until transition to stationary-phase growth where succinate becomes limited (Fig. 2).

On the surface, this evidence appears to contradict previous work by Master and Mohn (2001) indicating constitutive expression of the biphenyl pathway in LB400 during growth on simple carbon sources (Master and Mohn 2001). However, this study clarifies this paradox by evaluating the expression of the biphenyl pathway during growth on multiple carbon compounds and during different physiological states. Q-RT-PCR analyses indicate that genes in the biphenyl pathway (*bphA*, *bphD* and *bphR1*) were induced during growth on succinate with biphenyl (Table 3) as compared to succinate only. In addition, during growth on succinate with PCBs, the biphenyl pathway was down-regulated indicating some level of expression of the biphenyl pathway during growth on succinate and repression of the biphenyl pathway by PCBs. Although the biphenyl pathway was expressed during growth on simpler carbon compounds such as succinate, little degradation occurred. These data suggest additional elements possibly involved post-transcriptional regulation or that the active transport of biphenyl in LB400 as reported by Master et al. (2005) play a more crucial role in biphenyl degradation than previously thought (Master et al. 2005).

This work shows a repression of the biphenyl pathway in LB400 by PCBs during growth with a simple carbon source such as succinate. As many environmental sites contaminated with PCBs contain a range of carbon sources, this becomes an important concern for in situ PCB degradation. Furthermore, although biphenyl appears not to be utilized during growth on succinate with biphenyl, the biphenyl pathway is expressed, suggesting post-transcriptional regulation or active transport involved in biphenyl degradation (Master et al. 2005). Additionally, the expression of the chloroacetaldehyde dehydrogenase correlates to expression of the biphenyl pathway in LB400 suggesting involvement of the chloroacetaldehyde dehydrogenase in biphenyl (and PCB) degradation. It is interesting to note that the chloroacetaldehyde dehydrogenase that is induced is not the *bphF* acetaldehyde dehydrogenase gene. This work identifies genes associated with the biphenyl pathway in imparting stability to LB400 during PCB degradation and fills a gap in the knowledge of the activation of the biphenyl pathway under competing carbon sources and the effect PCBs may have on the biphenyl pathway in environmental scenarios.

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