

Original Research

# PCB Biodegradation and *bphA1* Gene Expression Induced by Salicylic Acid and Biphenyl with *Pseudomonas fluorescence* P2W and *Ralstonia eutropha* H850

Chaohua Hu\*, Youchi Zhang, Xiaoda Tang, Wensui Luo\*\*

Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

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## Abstract

Organic carbon substrate amendments are promising bioremediation strategies to induce polychlorinated biphenyls (PCB) aerobic degradation. However, their selective induction on PCB degraders has not been well studied. In this study, the substrate interaction effects of salicylic acid and biphenyl on PCB biodegradation were investigated with pure cultured isolates, including a newly isolated *Pseudomonas fluorescence* (P2W) and the veteran PCB degrader *Ralstonia eutropha* (H850). A significant biodegradation of lower-chlorinated PCB in H850 was induced by both salicylic acid and biphenyl amendments, while the biodegradation in P2W was induced only by salicylic acid. The binary substrates of salicylic acid and biphenyl resulted in a significantly inhibited effect on PCB removal in both strains. The expression of the functional gene *bphA1* in the upper biphenyl degradation pathway was further investigated by quantitative reverse transcription PCR. Compared to H850, P2W had higher expression in the *bphA1* gene induced mainly by salicylic acid rather than biphenyl. Particularly, the binary substrate induction led to an excessive expression of *bphA1* gene in both strains, which was in good agreement with their biomass growth. These results suggested that the special induction of PCB biodegradation depends on the selection of organic carbon substrates and the acclimation of degrader strains.

**Keywords:** polychlorinated biphenyl, aerobic biodegradation, *bphA1*, selective induction, salicylic acid

## Introduction

Among the representatives of persistent organic contaminants, polychlorinated biphenyls (PCB) have been found to be widely present in waters, sediments, and soils. Because of lipophilic properties of PCB, trace concentration of environmental PCB could gradually accumulate along the food chain to concentration levels that pose seri-

ous threats to human and animal health [1-3]. Microbial aerobic degradation has been commonly regarded as a promising approach for *in situ* remediation of the PCB-contaminated environment [4, 5]. Many indigenous bacteria from soils or sediments, such as *R. eutropha* H850, *Burkholderia xenovorans* LB400, and *Rhodococcus jostii*. RHA1, have been isolated and demonstrated to be capable of degrading a wide range of PCB congeners in laboratory and field studies in the past two decades [6-10]. However, indigenous bacteria from diverse habitats show significant difference in their capability of degrading PCB [11].

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\*e-mail: chhu@iue.ac.cn

\*\* e-mail: wsluo@iue.ac.cn

The aerobic degradation of PCB by microbe is necessary to be regulated by using specific inducers to open a degradation pathway. Some organic carbons such as biphenyl, salicylic acid, and plant secondary metabolites (e.g., coumarin, carvone, naringin, etc.) have been shown to influence aerobic PCB removal and bacterial community structure in different types of environments [12, 13]. Especially salicylic acid, which is a kind of plant-derived compound, has great potential to enhance biodegradation of PCBs by inducing the cometabolic pathway [14]. The biphenyl pathway of PCB degradation has been thought to be the principal means for aerobic PCB degradation in a wide range of microorganisms [15]. The upper biphenyl pathway is composed of four enzymes involving biphenyl 2,3-dioxygenase (BphA), 2,3-dihydroxybiphenyl-2,3-dehydrogenase (BphB), 2,3-dihydroxybiphenyl-1,2-dioxygenase (BphC), and 2-hydroxy-6-oxo-6-phenyl-hexa-2,4-dienoic acid hydrolase (BphD) [16, 17]. The first degrading enzyme BphA, which consists of large and small subunits of terminal dioxygenase (BphA1 and BphA2, respectively), ferredoxin (BphA3), and ferredoxin reductase (BphA4), is responsible of the initial dioxygenation step by catalyzing the introduction of two oxygen atoms into a benzene ring [8, 18]. Among them, BphA1 plays a crucial role in determination of substrate specificity and biodegradation efficiency for PCB degraders [7]. Particularly, the *bphA1* gene encoding the large subunit of iron-sulfur protein of BphA with a substrate-binding site may be used as a molecular mark to evaluate substrate specificity and degradation capability for PCB degraders [19]. However, to the best of our knowledge the response of PCB degrader from diverse habitats under influence of co-metabolic substrates on the expressions of biphenyl degradation pathway and functional genes have not been thoroughly investigated so far.

Two PCB-degrading bacteria were chosen for investigation in this study, one was a new isolate (*Pseudomonas fluorescence* P2W) from a top red earth soil in southern China and the other (*Ralstonia eutropha* H850) was from a bacterial collection and reportedly isolated from PCB-contaminated sediment environment. The veteran degrader H850, which exhibits high bioactivity in PCB degradation and is most likely to be further induced by exogenous organic substrates [20], was deemed to have a model induction pathway for PCB biodegradation relative to the new isolate P2W. At the same time, the two tested degraders, H850 and P2W, were found to have similar morphology. The growth and PCB degradation capability of two tested bacteria were mediated by amending different organic inducers, including salicylic acid, biphenyl, and salicylic acid plus biphenyl. The distinguished expression of the functional gene *bphA1* between the two degraders was further investigated by quantitative reverse transcription PCR (Q-RT-PCR). The results from this study will provide valuable alternatives for utilizing various organic co-substrate amendments to improve the degradation capabilities of indigenous microorganisms at abruptly PCB-contaminated sites, where the natural biodegradation processes might be extremely slow.

## Materials and Methods

### Isolation and Identification

The wetland soil used to isolate indigenous strains which could be quickly induced for PCB biodegradation was collected from Xiamen of China. The physical-chemical properties of the collected soil were reported in our previous work [21]. The collected soil was first co-incubated with 1.94 mmol·kg<sup>-1</sup> biphenyl at 25°C for 35 d. Then, the domesticated strains from the co-incubated soil were plated on a mineral salts agar with biphenyl crystals (MSAB) placed on the inverted lid, according the method by Park et al. [20]. After incubation at 30°C for 7 d, the dominant populations on the plates were selected and tested for their PCB degradation level in mineral medium amended with PCB. According to the PCB degradation capacity, one of the bacterial strains was purified by transferring two times on MSAB. To identify the classification of the isolated strain, the 16S rRNA gene sequences were amplified using the common primer. The phylogenetic tree was constructed by comparing the known 16S rRNA gene sequences from the nucleotide database, according to previous reports [22, 23].

### Growing Cell Assays

*Ralstonia eutropha* strain H850 was obtained from the German Resource Center for Biological Material (DSMZ). The growth and degradation profiles of the two bacterial strains were determined by growing in the mineral-salts medium [20], which was amended with different organic substrates and 10 mg·kg<sup>-1</sup> of the commercial PCB mixtures (Aroclor 1254) obtained from AccuStandard Inc. (New Haven, USA). The PCB mixtures dissolved in hexane were added into an 8 mL brown bottle at least 2 h prior to amendment of culture medium to allow for solvent volatilization. The PCB stayed while hexane evaporated because of the significant difference in evaporation pressure (EP) between solvent (EP<sub>hexane</sub> = 20.2 kPa at 25°C) and solute (EP<sub>PCB</sub> < 6.7×10<sup>-5</sup> kPa at 25°C) [12]. Afterward, the PCB degraders were inoculated in those bottles with a 3 mL mineral salt medium amended with 5 mM of different organic substrates, including salicylic acid, biphenyl, biphenyl plus salicylic acid. After the bacterial cultures were incubated at 30°C for 72 hrs, the two PCB-degrading strains were both observed to reach stationary-phase growth, and their biomass growth was monitored by measuring the change in optical density at 600 nm using the culture supernatants as blank controls [24].

Simultaneously, the PCBs in the cultures were extracted with an equal volume of 1:1 hexane:acetone solution using a shaker at 150 rpm for 3 h, according to our previous study [13]. The extracted PCBs were diluted with hexane and analyzed by an Agilent gas chromatograph (Agilent 7890A) equipped with a 30 m HP-5 column (0.25 mm film thickness and 0.32 mm i.d.) and an electron capture detector. Oven temperature was started at 120°C, and then increased at 20°C min<sup>-1</sup> to 200°C for 3 min, and increased to 240°C at 1.5°C min<sup>-1</sup>, and finally increased to 300°C and

held for 2 min. To accomplish the determination of specific congeners in the Aroclor 1254 mixture, the commercial PCB mixtures dissolved in hexane were analyzed by GC-MS (Agilent 5975C inert XL E1/C1 MSD) with a standard 28 composition of PCB congeners (AccuStandard Inc.). Among these PCB congeners we selected 18 chlorinated biphenyl (CB) congeners, including 2,4,4'-CB; 2,2',5,5'-CB; 2,2',3,5'-CB; 2,3',4,4'-CB; 2,2',4,5,5'-CB; 3,4,4',5-CB; 3,3',4,4'-CB; 2,3,4,4',5-CB; 2,3,3',4,4'-CB; 2,2',3,4,4',5-CB; 3,3',4,4',5-CB; 2,2',3,4',5,5',6-CB; 2,2',3,3',4,4'-CB; 2,3,3',4,4',5-CB; 2,3,3',4,4',5'-CB; 2,2',3,4,4',5,5'-CB; 3,3',4,4',5,5'-CB; and 2,2',3,3',4,4',5-CB.

### cDNA Synthesis and Q-RT-PCR Analysis

The total RNA extractions for quantitative reverse transcription PCR (Q-RT-PCR) were performed according to the method described by Parnell et al. [9] with a minor modification. In brief, 1.45 ml of cell cultures at the same growing stage was collected by centrifugation at 12,000 g for 2 min, and the total RNA was extracted with RNAPrep pure Bacteria RNA Purification Kit from Tiangen Biotech (Beijing) Co., Ltd, and the remaining DNA was degraded by DNase digestion with 27 U DNaseI at 30°C for 15 min. After purification with removal protein solution, the total RNA concentration was measured by a micro-volume UV spectrophotometer (ND-100, USA). Then the cDNA was synthesized by reverse transcription reaction using PrimeScript RT reagent Kit (TaKaRa). The bacterial *bphA1* gene encoding a large subunit of biphenyl dioxygenase was analyzed by Q-RT-PCR [9, 25], which was performed with the gene-specific primers (forward primer: 5'-TTCATTCCTGCCACCTTC-3'; reverse primer: 5'-AGCCGACGTTGCCAGGAAAAT-3'). The synthesized cDNA was used as a template for PCR amplification. The amplifi-

cation was carried out on an ABI Prism 7500 Fast Real-Time PCR System using SYBR Premix Ex Taq (TaKaRa) with the following temperature program: initial activation/predenaturation at 95°C for 10 min; followed by 40 cycles, with 1 cycle consisting of 15 s of denaturation at 95°C, 35 s of annealing at 58°C, and 1 min of extension at 87°C. At the end of the real-time PCR, a dissociation protocol was performed by a final step of measuring the SYBR Green I signal intensities from 60 to 95°C.

### Statistical Analysis

To evaluate the significance of different substrate effects on the cell growth of degraders and their PCB degradation efficiency, analysis of variance (ANOVA) was performed with SPSS 15.0. Before ANOVA analysis, the data of percentage removal of different PCBs was arcsine square root-transformed. Each data represents the mean of three replications with one standard deviation. In the case where the p-value was less than 0.05, the significance of different substrate effects on cell growth or PCB removal were evaluated by the least significant difference (LSD) test, with different letters (a, b, or c) denoting significant treatment differences.

## Results

### Isolation and Growth Profiles of PCB Degraders

A bacterial strain P2W with capability of PCB degradation was isolated from a PCB-uncontaminated soil amended with biphenyl crystals. The isolate P2W was determined as a Gram-negative bacterium by Gram staining, and further identified according to the classification by 16S rRNA

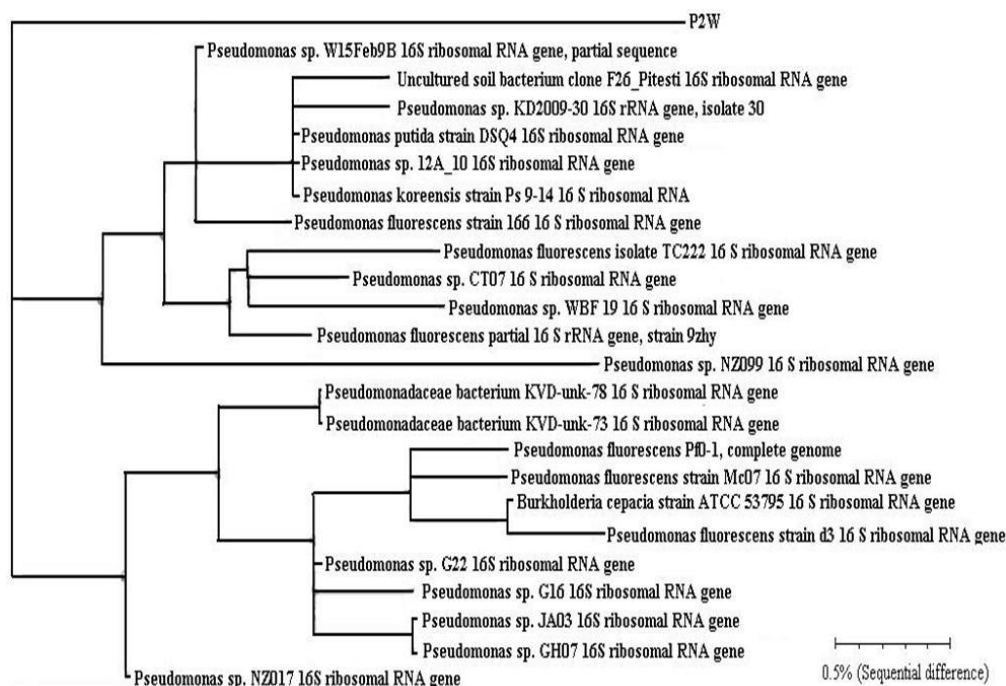


Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequence data of the isolate P2W and other relative species.

Table 1. Percent removal (%) of the select PCB congeners in *Ralstonia eutropha* H850 and *Pseudomonas fluorescens* P2W responding to different substrate amendments.

	Neg.	Bip.	Sal.	Bip.+ Sal.	P-values
H850					
2,4,4'	16.6±17.1c	100.0±0.0a	100.0±0.0a	64.6±33.5b	0.01
2,2',5,5'	34.8±20.0b	77.5±6.5a	74.8±6.1a	71.2±8.6a	0.01
2,2',3,5'	37.2±18.1b	76.8±6.5a	76.8±5.4a	70.3±5.6a	<0.01
2,3',4,4'	10.9±3.4c	52.3±3.8a	51.9±3.9a	32.6±14.5b	<0.01
2,2',4,5,5'	15.8±2.5c	53.4±2.5ab	62.3±5.9a	44.7±9.1b	<0.01
3,4,4',5	8.5±1.5c	45.3±1.7a	50.3±3.8a	25.2±15.4b	<0.01
3,3',4,4'	1.2±2.6c	30.1±2.1a	28.5±3.7ab	13.4±11.1b	0.02
2,3,4,4',5	-0.4±3.6	6.7±2.4	7.7±2.1	7.0±8.0	0.84
2,3,3',4,4'	0.9±4.7b	20.4±1.6a	24.2±1.8a	14.1±7.8a	<0.01
2,2',3,4,4',5'	-2.0±1.9	2.8±3.1	4.7±2.3	5.6±7.8	0.79
3,3',4,4',5	1.8±6.8	15.5±2.2	15.6±3.4	9.7±8.5	0.53
2,2',3,4',5,5',6	0.4±3.6	3.5±2.7	5.2±2.0	6.7±7.9	0.72
2,2',3,3',4,4'	-2.1±4.0	4.7±2.3	7.3±1.2	9.1±7.7	0.18
2,3,3',4,4',5	2.8±7.0	13.3±3.5	9.7±2.6	11.2±7.4	0.41
2,3,3',4,4',5'	-0.8±3.0	0.3±2.7	3.2±1.9	6.4±7.5	0.46
2,2',3,4,4',5,5'	0.5±7.9	2.1±2.6	3.5±2.1	8.9±7.1	0.41
3,3',4,4',5,5'	0.0±6.4	3.8±3.3	6.1±2.0	11.0±8.1	0.64
2,2',3,3',4,4',5	-1.1±5.6	5.8±3.7	8.7±2.0	11.3±8.6	0.55
P2W					
2,4,4'	13.8±2.3b	5.9±3.1c	26.8±6.8a	7.7±9.0bc	<0.01
2,2',5,5'	26.1±1.7b	5.7±2.9c	45.7±24.5a	2.6±6.5c	0.02
2,2',3,5'	11.7±1.7b	5.9±2.9b	21.8±10.4a	2.1±5.9b	0.05
2,3',4,4'	7.8±1.9	5.6±3.1	13.4±5.1	0.8±6.2	0.15
2,2',4,5,5'	9.6±1.7	5.5±3.0	19.3±9.7	2.2±5.9	0.11
3,4,4',5	7.5±1.5	7.2±2.9	13.4±4.6	3.4±5.1	0.19
3,3',4,4'	8.2±1.8	7.6±3.2	12.7±4.5	3.6±4.8	0.11
2,3,4,4',5	11.5±3.7	8.4±4.7	12.2±4.4	5.7±3.2	0.24
2,3,3',4,4'	25.5±16.0	16.6±15.2	11.5±4.6	2.4±1.7	0.10
2,2',3,4,4',5'	9.3±1.3	9.6±3.8	15.9±4.0	10.0±1.7	0.08
3,3',4,4',5	6.0±1.6	2.4±4.6	7.6±4.8	-0.9±3.2	0.68
2,2',3,4',5,5',6	7.8±1.6	7.1±3.7	12.8±4.4	5.5±1.8	0.11
2,2',3,3',4,4'	6.8±1.3	7.4±2.7	11.5±4.3	-1.4±2.1	0.04
2,3,3',4,4',5	7.0±1.8	2.4±5.2	10.8±6.4	1.7±2.5	0.26
2,3,3',4,4',5'	9.4±2.1a	9.5±3.8a	15.4±5.4a	3.7±0.9b	0.01
2,2',3,4,4',5,5'	8.7±3.3	2.9±4.2	17.3±13.8	2.3±1.3	0.13
3,3',4,4',5,5'	8.3±0.7ab	7.1±4.6b	13.6±3.6a	3.2±1.1c	0.02
2,2',3,3',4,4',5	8.9±0.3ab	5.5±4.8bc	16.6±7.5a	1.6±1.8c	0.01

Different letters (a, b, or c) denote the statistical significance ( $p < 0.05$ ) among different substrate-amended treatments.

gene sequences (GeneBank accession No. JN029804). As shown in Fig. 1, the isolated strain was classified to *Pseudomonas fluorescens* based on the high 16S rRNA gene sequence similarities (~99%) obtained by blasting the known 16S rRNA gene sequences of other species in the National Center for Biotechnology Information (NCBI). When observed by SEM, the exterior morphology of the newly isolated P2W showed short rods with approximately 1-1.5  $\mu\text{m}$  in length and 0.5-1  $\mu\text{m}$  in width, which was similar to the morphology of the well-known PCB degrader *Ralstonia eutropha* H850.

The growth rates of the new isolate P2W and the veteran PCB degrader H850 were determined to compare their propagation abilities under different organic substrates (Fig. 2). The two tested PCB degraders displayed different utilization efficiency in response to different organic substrates. When treated in salicylic acid or biphenyl alone, H850 had faster growth rate than P2W under experimental conditions. In comparison with biphenyl, the salicylic acid amendment appeared to further enhance the growth of P2W, but the difference was not significant. Notably, when compared with the sole salicylic acid or biphenyl amendment, the binary organic substrates (salicylic acid plus biphenyl) resulted in a significantly higher growth rate for both tested strains ( $p$ -value < 0.05).

#### Salicylic Acid and Biphenyl Effects on PCB Biodegradation

To evaluate the reliability of PCB extraction and analysis protocol, the average recovery efficiency was determined and shown as  $78.7 \pm 6.6\%$  and  $88.6 \pm 3.0\%$  for lower chlorinated (tri- and tetra-chlorinated) and higher chlorinated (penta-, hexa-, and hepta-chlorinated) PCB congeners, respectively. As shown in Table 1, the growing cell assay in the two tested strains induced by salicylic acid, biphenyl, and salicylic acid plus biphenyl showed a distinguishing pattern in the biodegradation of lower chlorinated congeners. The percentage of PCB removal depended on the

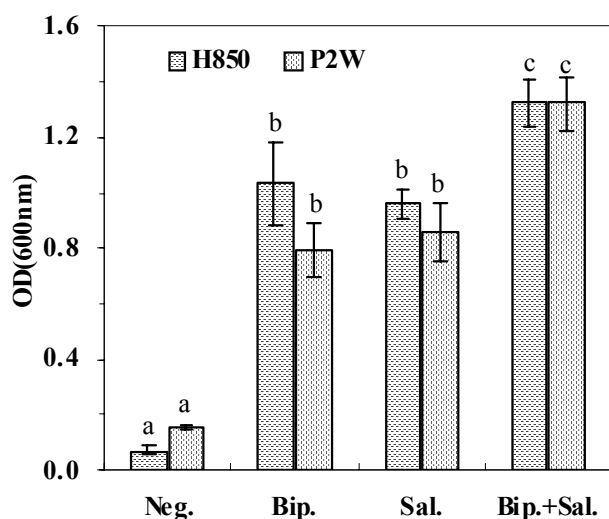


Fig. 2. Salicylic acid and biphenyl effects on the growth of *Ralstonia eutropha* H850 and *Pseudomonas fluorescens* P2W.

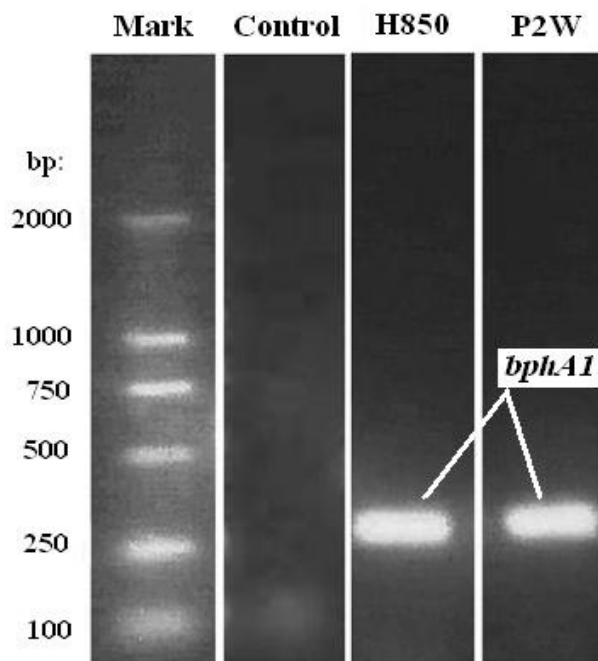


Fig. 3. Agarose gel electrophoretic proof for the presence of *bphA1* gene in the two investigated degraders H850 and P2W. Negative control represents that the *bphA1* gene was not detected from a non-PCB-degrading strain.

type of co-metabolic substrates, degrading strains, and chlorinated extent. Compared with the negative controls, the treatments of salicylic acid and biphenyl in H850 led to a significant removal ( $p$ -value < 0.05) for lower chlorinated congeners such as 2,4,4'-trichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, 2,2',3,5'-tetrachlorobiphenyl, 2,3,4,4'-tetrachlorobiphenyl, and 2,2',4,5,5'-tetrachlorobiphenyl. While only the salicylic acid treatment boosted P2W to remove lower chlorinated PCBs such as 2,4,4'-trichlorobiphenyl, 2,2',3,5'-tetrachlorobiphenyl, and 2,2',5,5'-tetrachlorobiphenyl. The biodegradation of *para*-chlorinated biphenyls (e.g., 3,3',4,4'-CB) by both strains was inhibited relative to most ortho-chlorinated biphenyls under the treatments of salicylic acid or biphenyl. In particular, the salicylic acid plus biphenyl treatment resulted in a significantly inhibited effect in the aerobic biodegradation of lower chlorinated congeners, especially for the tetrachlorobiphenyls ( $p$ -value < 0.05). In the presence of salicylic acid and biphenyl, the removal of 3,3',4,4'-CB was only  $13.4 \pm 11.1\%$  and  $3.6 \pm 4.8\%$  in H850 and P2W, respectively.

#### Expression Analysis of the *bphA1* Gene

To investigate salicylic acid and biphenyl effects on the regulation of biphenyl pathway for the biodegradation of PCB congeners, the expression of the *bphA1* gene was analyzed by quantitative RT-PCR. A small partial sequence (302 bp) from the conserved region of the *bphA1* gene was verified both in the genomic DNA of H850 and P2W (Fig. 3). The relative expression levels of the *bphA1* gene in different substrate-amendment treatments were then acquired by reference to the amplification levels recorded in una-

mended controls. As shown in Fig. 4, the relative expression value of the *bphA1* gene induced by biphenyl appeared to be up-regulated in H850, while it showed down-regulation in the presence of salicylic acid. By contrast, the *bphA1* gene expression in P2W exhibited the contrary trend, which up-regulated by salicylic acid rather than biphenyl. Notably, salicylic acid plus biphenyl treatments produced much higher up-regulation in the expression levels of the *bphA1* gene than the sole salicylic acid or biphenyl amendment for both P2W and H850. In comparison with the unamended control, the complex substrates produced a  $2.7 \pm 0.7$  fold enhancement in the *bphA1* gene expression of H850, and enhanced  $374.0 \pm 48.3$ -fold in that of P2W.

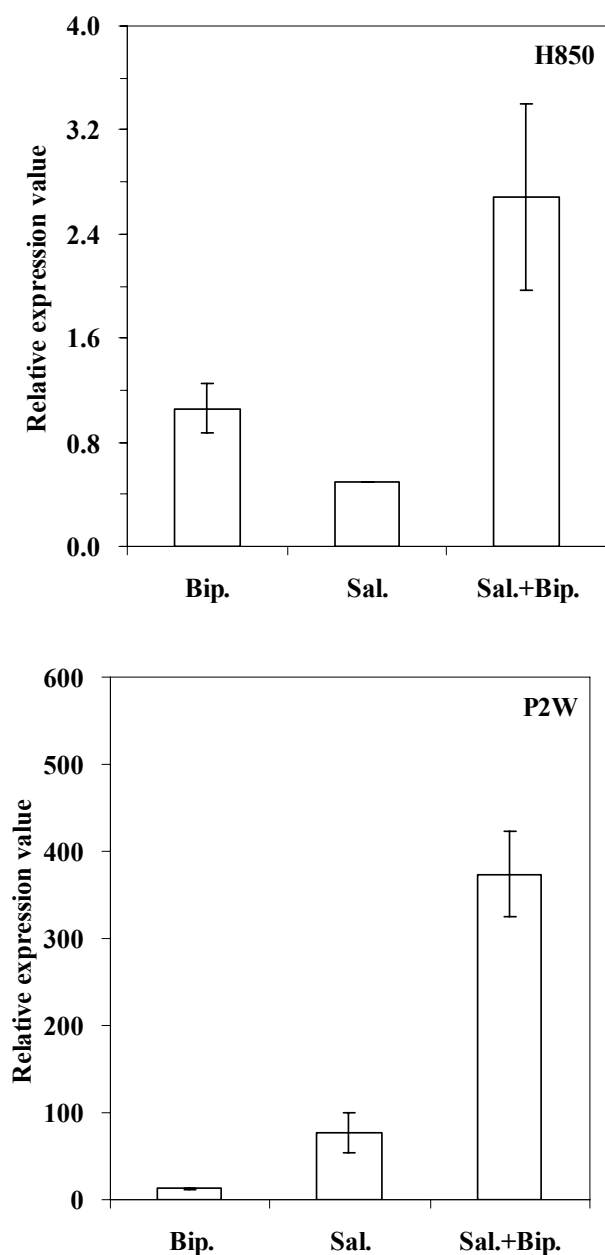


Fig. 4. The relative expression values of the *bphA1* gene from quantitative RT-PCR analysis in the PCB biodegradation process by *Ralstonia eutropha* H850 and *Pseudomonas fluorescens* P2W responding to different substrate amendments relative to the unamended control. Error bars denote the standard deviation of mean data from three independent assays.

## Discussion

As is well-known, PCB biodegradation is commonly influenced by the chlorinated amounts and positions of the congeners. In the present study, the lower chlorinated congeners such as trichlorobiphenyl (2, 4, 4'-CB) and tetrachlorobiphenyls (e.g., 2,2',3,5'-CB, 2,2,5',5-CB, and 3, 4, 4',5-CB) were degraded mostly during growth of H850 in the presence of salicylic acid or biphenyl. However, the *para* chlorinated substituent like 3,3',4,4'-tetrachlorobiphenyl was hardly degradable. This is in accordance with the earlier report of Kim et al. [1] indicating that the metabolism of PCB by *Alcaligenes* sp. JB1 was restricted to the congeners with up to four chlorine substituents and the congeners containing *para* chlorine substituent were rarely degraded. A similar removal mode for different PCB congeners was also reported by Luo et al. [12, 13]. However, they suggested that PCB removal by indigenous bacterial strains could be induced and depended on the type of inducible substrates such as biphenyl and certain plant secondary metabolites.

In this study the two investigated bacteria possessed a distinguished mode on PCB removal by the induction with biphenyl or salicylic acid, although there is no significant difference between the growths of both strains. The veteran PCB-degrading strain H850, which was isolated from PCB-contaminated dredge sediment of the Hudson River, displayed an effective removal (> 50%) for most of lower chlorinated congeners when induced by biphenyl or salicylic acid. Different from H850, P2W was newly isolated from wetland soil and domesticated in a laboratory with biphenyl-enriched culture only for one month, and appeared to be capable of degrading PCB by salicylic acid, but not by biphenyl. This indicated that P2W had a more strict selectivity to inducible substrates than H850. Compared to the commercial biphenyl, which is artificial and impacted on people's health and environmental security, salicylic acid could be a better alternative to induce PCB aerobic biodegradation. Because salicylic acid is one of the naturally occurring substrates from plant metabolites, it has more accessibility to be utilized as carbon and energy sources than biphenyl. In our previous study [24], salicylic acid was proved to support the growth and PCB degradation of two indigenous bacteria strains strongly related (> 99%) to *Ralstonia eutropha* and *Burkholderia cepacia*, respectively. Therefore, salicylic acid could be an important inducer and carbon source for PCB-degrading strains.

On the other hand, when treated with salicylic acid, the relative expression value of the functional gene *bphA1*, which indicated the expression of biphenyl degradation pathway, was significantly up-regulated in P2W. The up-regulation might be attributed to P2W being likely easy to be induced by salicylic acid to open the biphenyl degradation pathway through increasing the expression of *bphA1* [20]. In contrast, the expression of *bphA1* gene in H850 was inhibited in response to the induction of salicylic acid. However, the salicylic acid amendment in H850 resulted in a comparable removal for lower chlorinated congeners of PCBs relative to the biphenyl treatment. It was deduced that H850 could have developed various degradation pathways

besides the biphenyl pathway. Parnell et al. [26] demonstrated the peripheral pathways in PCB degradation during the growth of *Burkholderia xenovorans* LB400 on succinate and/or biphenyl, and suggested the detoxification pathway regulated by the chloroacetaldehyde dehydrogenase, which may eliminate toxic products of incomplete PCB degradation.

Additionally, the interaction of salicylic acid with the other organic substrate (e.g., biphenyl) was observed to inhibit PCB biodegradation. When amended with salicylic acid plus biphenyl, PCB removal by H850 and P2W was both found to be markedly weakened or not even detected. This is probably attributed to the carbon source competition among the co-metabolic substrates of salicylic acid and biphenyl, as well as PCB congeners. It might also be related to the substrate selectivity of PCB degraders, which could be regulated by the expression of *bphA1* gene [8, 18]. As suggested by Q-RT-PCR, salicylic acid plus the biphenyl amendment resulted in an enhanced expression of *bphA1* gene, and thus the enhanced expression could cause the post-translational regulation of biphenyl pathway or the shift of active site for PCB biodegradation [9]. However, under the experimental condition of this study, the enhanced expression of *bphA1* gene could mainly be associated with the fact that the binary organic substrates boosted significantly the biomass growth of H850 and P2W, in comparison with the sole substrate of salicylic acid or biphenyl (p-value < 0.05).

### Conclusion

In this study, PCB biodegradation and *bphA1* gene expression were investigated with isolates *R. eutropha* H850 and *P. fluorescens* P2W. H850 and P2W bacteria can be induced to degrade certain lower chlorinated PCB congeners by the amendment of salicylic acid. However, the binary substrate induction of salicylic acid and biphenyl resulted in an inhibited effect on PCB removal in both strains. The relative expression level of the functional gene *bphA1* indicated that the newly isolated strain P2W is more accessible to being induced by salicylic acid than the veteran strain H850. In particular, the binary substrates of salicylic acid and biphenyl induced an excessive expression of gene *bphA1* in both strains, well conforming with the significant increase of their biomass growth. The issue of the interaction mechanism between inducible substrates is still open and needs to be further studied to uncover the factual influence of substrate interactions on the synthesis and activity of PCB-degrading enzymes.

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