Biphenyl-Metabolizing Microbial Community and a Functional Operon Revealed in E-Waste-Contaminated Soil

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S *Supporting Information*

ABSTRACT: Primitive electronic waste (e-waste) recycling activities release massive amounts of persistent organic pollutants (POPs) and heavy metals into surrounding soils, posing a major threat to the ecosystem and human health. Microbes capable of metabolizing POPs play important roles in POPs remediation in soils, but their phylotypes and functions remain unclear. Polychlorinated biphenyls (PCBs), one of the main pollutants in e-waste contaminated soils, have drawn increasing attention due to their high persistence, toxicity, and bioaccumulation. In the present study, we employed the culture-independent method of DNA stable-isotope probing to identify active biphenyl and PCB degraders in e-waste-contaminated soil. A total of 19 rare operational taxonomic units and three dominant bacterial genera (*Ralstonia*, *Cupriavidus*, and uncultured bacterium DA101) were enriched in the ^{13}C heavy DNA fraction, confirming their functions in PCBs metabolism. Additionally, a 13.8 kb *bph* operon was amplified, containing a *bphA* gene labeled by ^{13}C that was concentrated in the heavy DNA fraction. The tetranucleotide signature characteristics of the *bph* operon suggest that it originated from *Ralstonia.* The *bph* operon may be shared by horizontal gene transfer because it contains a transposon gene and is found in various bacterial species. This study gives us a deeper understanding of PCB-degrading mechanisms and provides a potential resource for the bioremediation of PCBs-contaminated soils.
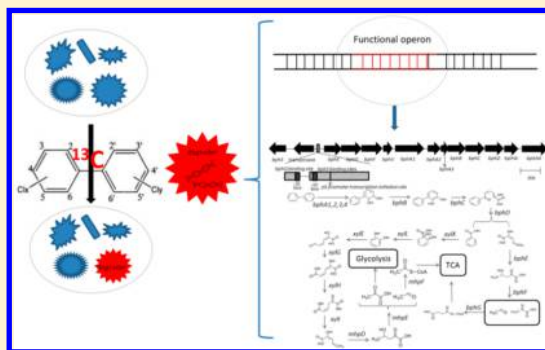
INTRODUCTION

The increasing demand for electronic products and their frequent replacement has led to an accumulation of outdated electronic devices and associated waste. Electronic waste (e-waste)-recycling activities can recover considerable quantities of valuable and reusable materials from such waste.[1] However, due to the crude and low-tech methods used in e-waste recycling, these activities release high concentrations of pollutants into the environment, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins dibenzofurans (PCDD/Fs), lead, cadmium, and copper, which has become a global environmental problem.[2−4] These pollutants are mobile and ubiquitous in the sediment, air, water, and soil around e-waste-recycling sites, threatening ecological functions and human health.[5−7]

PCBs, one of the main pollutants present in e-waste-contaminated soils, have drawn increasing attention due to their high persistence, toxicity, and bioaccumulation.[8,9] As an eco-friendly and cost-effective remediation option, microbial metabolism of waste, known as bioremediation, has become a hot topic in recent decades.[10,11] To date, various bacteria have been isolated using PCBs as the sole source of carbon and energy, including *Paenibacillus*, *Stenotrophomonas*, and *Pseudomonas*.[12,13] Bioaugmentation treatment with these degraders appears to be a promising technology that can enhance the

removal efficiency of PCBs from e-waste-contaminated soils. However, the survival and metabolic function of these degraders are highly dependent on environmental conditions, limiting their application.[14] Additionally, heavy metals in e-waste-contaminated soils can inhibit biodegradation and bioremediation of organic pollutants due to their toxicity toward microorganisms.[15] Autochthonous bioaugmentation of e-waste-contaminated soils has been suggested as a potential practical alternative method of bioremediation.[15] However, autochthonous bioaugmentation suffers from the fact that soil PCBs degraders are scarcely cultivable. Hence, some studies in the past few years have been conducted to identify functional PCBs-metabolizing microbes via culture-independent methods in situ. Song et al. found that three dominant strains of dissimilatory iron-reducing bacteria are closely associated with PCBs dehalogenation in e-waste soils from Qingyuan, China.[16] Tang et al. investigated the bacterial community in soils around an e-waste-recycling workshop and identified several microbes that might catabolize PCBs.[17] However, the challenge of revealing functional PCBs-degraders with a high throughput

and reliable approach remains, and meeting this challenge will benefit future bioremediation efforts. To develop appropriate strategies to identify the active PCBs-metabolizing microbes and apply them effectively in autochthonous bioaugmentation, understanding the phylotypes and ecological behaviors of these functional degraders is a critical matter of great urgency.

PCBs can be metabolized both anaerobically and aerobically by microbes. Anaerobic degradation involves the initial reductive dehalogenation rather than the ring-opening of biphenyl skeleton, particularly for highly chlorinated PCBs.[18,19] In this step, atomic carbon does not incorporate into microbial components and the represent anaerobic degraders cannot be identified via SIP. Additionally, metabolism of lower chlorinated PCBs and biphenyl share the same enzymes as downstream anaerobic degradation of highly chlorinated PCBs,[13,20] encoded by *bphABCDEFG*.[13] Therefore, biphenyl is often used as a model compound for the study of the biodegradation of PCBs, especially for SIP.[21−23] Accordingly, in the present study, we employed DNA stable-isotope probing (DNA-SIP), a culture-independent method directly linking function to phylogeny of microbes in complex environments, to explore the in situ PCBs degrader community in e-waste-contaminated soils.

In the aerobic mineralization process of PCBs, biphenyl dioxygenase, which belongs to the large family of Rieske nonheme iron oxygenases and is composed of a large α subunit and small β subunit (a ferredoxin and a ferredoxin reductase, respectively), is considered as the key enzyme responsible for initiating PCB degradation.[11] The abundance of the *bphA* gene encoding biphenyl dioxygenase has been used to evaluate PCB mineralization capability.[24] In addition, its diversity and phylogenetic information has been studied to reveal functional gene resources and infer the possible transformation mechanism.[23,25] However, amplification of the *bphA* gene with conserved primers provides limited information, and the lack of a full map of the PCBs-degrading gene is a barrier to a deeper understanding of the PCB degradation pathway. In most cases, degradation genes are located in a operon, which is regulated or activated by the same stimulants.[26] Thus, further characterization of the complete operon containing the *bphA* gene and the regulatory mechanism of PCBs metabolism, which is frequently mediated by BPHQ and BPHS regulators, is necessary.[26,27] The entire operon has only been identified in cultivable microorganisms, but its occurrence within the in situ microbial community remains unknown. The complete operon could be a potential gene resource for bioremediation via transferring into genetically modified microbes or plants in the future.[28,29] In addition, the detailed sequence and structure of the PCBs degradation operon might hint at its evolutionary origin and indicate links between functional genes and PCBs-metabolizing bacteria based on G + C (guanine and cytosine) content and tetranucleotide frequencies.[30,31]

In the present study, we used ^{13}C-biphenyl as a labeled compound and combined DNA-SIP with high-throughput sequencing, which offered high-resolution identification of the functional microbial communities and *bphA* genes, to explore the active biphenyl-utilizing bacteria in e-waste-contaminated soil. In addition, we successfully amplified one functional *bphA* gene involved in biphenyl degradation, characterized the PCB degradation operon, and revealed the relationship between the active bacteria and operon responsible for in situ PCB metabolism. This study gives us a deeper insight into the mediator of aerobic PCB degradation as well as functional genes and their probable regulatory mechanisms in heavy-metal- and PCB-co-contaminated environments.

■ MATERIALS AND METHODS

SIP Microcosms. The soil sample was collected from Qingyuan, Guangdong Province, China, near an e-waste-recycling plant (23°21′0.72″ N, 113°11′29.04″ E). The concentration of soil $\sum$PCBs was 1220 ng/g, and the contents of other pollutants (polycyclic aromatic hydrocarbons [PAHs], polybrominated diphenyl ethers [PBDEs], Cu, Pb, and Zn) are listed in Table S1. The soil contained 45% sand, 21% silt, and 34% clay. After sampling, the soils were transported to the laboratory with an ice pack to maintain low temperatures.

Microcosms containing soil supplemented with either ^{13}C-biphenyl (99 atom % [13]C; Sigma) or unlabeled biphenyl were cultivated for 9 days and sampled on days 3, 6, and 9. A total of 4 treatments were conducted, each in triplicate: (1) microcosm amended with ^{13}C-biphenyl; (2) microcosm amended with unlabeled biphenyl; (3) soils amended with unlabeled biphenyl for chemical analysis; and (4) sterile soils with unlabeled biphenyl as a negative control. The low concentration of $\sum$PCBs in soils challenged the sensitivity of incorporating labeled elements into active microbial DNA, and we therefore used 10 mg/L biphenyl in SIP microcosms to achieve satisfactory enrichment of the heavier DNA after ultra-centrifugation and fractionation because a relative higher dosage of stable isotope-labeled substrates benefits the enrichment and separation of the heavier DNA of active microorganisms.[32] Briefly, 50 μL of biphenyl (dissolved in acetone) was transferred into a sterile 100 mL dark brown serum bottle and dried for 1−2 h to allow the acetone to evaporate completely. After the addition of 5 g of soil and 5 mL of sterile distilled water, the bottles were sealed with sealing film, which allowed air to exchange freely. The microcosms were cultivated at 25 °C with shaking at 120 rpm. After sampling, the soils were immediately frozen at −80 °C until further analysis.

Biphenyl Analysis. At each time point, biphenyl was extracted from the two treatments containing unlabeled biphenyl (sterile and nonsterile soils) and analyzed using gas chromatography mass spectrometry (GC-MS). The soils were first freeze-dried, homogenized, and pulverized. Biphenyl was extracted with dichloromethane (DCM) in a Soxhlet apparatus for 48 h, spiking with ^{13}C-biphenyl as the recovery standard and using active copper to remove sulfur. The extracts were then concentrated to approximately 0.5 mL following solvent exchange to hexane and finally purified in a multilayer column that contained neutral alumina (3% deactivated), neutral silica gel (3% deactivated), and anhydrous Na_2SO_4 (from bottom to top) and eluted with 20 mL of hexane/DCM (1:1, v/v). Prior to analysis, extracts were concentrated to ∼100 μL using a gentle stream of N_2 and amended with 1000 ng of hexamethylbenzene.

Biphenyl was detected using GC-MS (Agilent 7890) equipped with a capillary column (DB-5MS, 30 m, 0.25 mm, 0.25 μm). The temperatures of the injector and transfer lines were 290 and 300 °C, respectively. The initial oven temperature was 60 °C for 1 min and rose to 290 °C at a rate of 3 °C/min, where it was held for 20 min. The flow rate of the carrier gas (high-purity helium) was 1.83 mL/min.

A standard curve (R^2 = 0.9993) was constructed using a range of stock solutions prepared with biphenyl concentration of 0, 20, 40, 80, 200, 500, 2500 and 10 000 μg/L (Figure S1).

The recovery rates for all the samples were between 87% and 109%, within the acceptable range of 80%−120%. All the concentrations of biphenyl in different treatments were calibrated according to the recovery rate.

DNA Extraction and Ultracentrifugation. DNA was extracted from soils using the PowerSoil DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's instructions. The soil DNA samples from the same treatment at the same time point were combined. The concentration and quality of DNA were measured using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies). After ultra-centrifugation, which was performed as previously described,[33] 15 fractions from each tube were separated, and their buoyant densities were measured using an AR200 digital refractometer according to the previous study.[33] Finally, these fractions were purified using the MicroElute DNA-Pure Kit (Omega Bio-Tek) to remove CsCl.

Amplification and Bar-Coded High-throughput Sequencing of Bacterial 16S rRNA Genes. For bacterial 16S rRNA, amplification and high-throughput sequencing were performed on each fraction. Polymerase chain reaction (PCR) was carried out using the universal primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′). The 806R primer was labeled with a unique 12 bp barcode to distinguish among amplification products. PCR was performed to amplify 1 μL of template DNA in a 25 μL reaction system containing 12.5 μL of rTaq premix buffer (TaKaRa) and 100 nM (0.5 μL) of each primer. Amplification was performed in triplicate as follows: 94 °C for 5 min; 28 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. The triplicate amplification products for each sample were pooled and purified using the MicroElute Cycle-Pure Kit (Omega Bio-Tek). Their length, concentration, and quality were verified using agarose gel electrophoresis and a Qubit fluorometer (Thermo Fisher Scientific). Sequencing was performed at Beijing Genomics Institute using the Miseq PE300 sequencer after combining PCR products from different samples in approximately equimolar amounts.

Identification and Quantification of the bphA Gene. We targeted the *bphA* gene encoding the α subunit of biphenyl dioxygenase using the primer pair of bphA463f (5′-CGCGTSGMVACCTACAARG-3′) and bphA674r (5′-GGTACATGTCRCTGCAGAAYTGC-3′) as a representative functional gene for PCBs metabolism.[24] The PCR reaction mixture contained 1 μL of template DNA (50−100 ng), 12.5 μL of rTaq premixed buffer (TaKaRa), 0.5 μL (100 nM) of each primer, and 10.5 μL of deionized water. Amplification was performed according to the following program: 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. After purification and confirmation the products via 1.5% agarose gel electro-phoresis, the fragments were ligated into pMD 19T (TaKaRa) and transformed into *Escherichia coli* DH5α. Positive clones with the correct inserts were selectively grown on Luria−Bertani agar plates supplemented with 50 mg/L of ampicillin for 12 h at 37 °C. The plasmids were then extracted and submitted for sequencing.

The abundance of *bphA* in each fraction was determined using real-time quantitative PCR (qPCR) with the same primer pair of bphA463f and bphA674r. The qPCR reactions were performed in 20 μL of mixture containing 10 μL of SYBR green PCR Premix Ex *Taq*II (TaKaRa), 0.2 μL of each primer (10 μM), and 1 μL of DNA template. The recombinant sequence containing the *bphA* gene was extracted from *E. coli* and serially diluted (from 10^8 to 10 copies) as a standard curve. The reactions were conducted on an ABI 7500 real-time PCR system (Applied Biosciences) as follows: denaturation for 2 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 30 s at 55 °C, and 20 s at 72 °C. The SYBR green signal was obtained after the 20 s step at 72 °C in each cycle. Melting curve analysis was performed by increasing the temperature from 55 to 95 °C and analyzing the signal after 40 cycles of the PCR reaction.

Identification and Analysis of the Biphenyl Degradation Operon. The biphenyl degradation (*bph*) operon was amplified by extending the flanking sequences of the *bphA* gene according to Liu's method.[34] The primers used are listed in Table S2. A 13.8 kb DNA fragment was successfully amplified and sequenced. The relationships between the *bph* operon and six bacterial types (*Acidovorax*, *Comamonas*, *Ralstonia*, *Cupriavidus*, *Methylobacillus*, and *Escherichia*) were investigated using Pearson's correlation analysis based on tetranucleotide-derived z-scores.[35] *Acidovorax* and *Comamonas* contain a *bph* operon that was previously isolated and studied,[36] whereas *Ralstonia* and *Cupriavidus* were identified as the two dominant PCBs degraders in the present study. *Methylobacillus* belongs to the same order as *Acidovorax*, *Comamonas*, *Cupriavidus*, and *Ralstonia*, while *Escherichia* is affiliated with the same class as *Acidovorax*, *Comamonas*, *Cupriavidus*, *Ralstonia*, and *Methylobacillus*. Z-scores were calculated using eqs 1−3. Briefly, the observed frequencies of all 256 possible tetranucleotides $N(n1n2n3n4)$ were calculated using a custom Perl script (https://github.com/jlf8858/Tetranucleotide). Next, the expected frequency $E(n1n2n3n4)$ was calculated with the use of a maximal-order Markov model, calculated using eq(1:

$$E(n1n2n3n4) = \frac{N(n1n2n3)N(n2n3n4)}{N(n2n3)} \quad (1)$$

Then, the z-score $Z(n1n2n3n4)$ was estimated using eq 2 and the variance (var) $(N(n1n2n3n4))$ was approximated via eq 3:

$$Z(n1n2n3n4) = \frac{N(n1n2n3n4) - E(n1n2n3n4)}{\sqrt{\mathrm{var}(N(n1n2n3n4))}} \quad (2)$$

$$\mathrm{var}(N(n1n2n3n4)) = E(n1n2n3n4)$$
$$\times \frac{[N(n2n3) - N(n1n2n3)][N(n2n3) - N(n2n3n4)]}{N(n2n3)^2}$$
$$(3)$$

Bioinformatics Analysis. The data have been submitted to NCBI (SRP134258). Raw sequence data were processed and analyzed with Mothur[37] and Quantitative Insights Into Microbial Ecology (QIIME).[38] Briefly, reads with a quality score of <25 or a length of <250 bp were discarded. The sequences were assembled and assigned to samples based on their barcodes. To reduce the error rate, singletons were removed. Representative sequences were chosen at a similarity level of 97% of operational taxonomic units (OTUs). Next, chimeric sequences identified by the UCHIME algorithm[39] were discarded. The taxonomic identification of representative sequences was determined based on the Greengenes 13.5 database using QIIME with its default settings. The relative abundance of each taxon and OTU was calculated by comparing its number of sequences with the number of total sequences. We defined the relative enrichment factor, REF, to

identify the dominant active degraders, which was calculated according to eq 4:

$$\text{REF} = \frac{A_{13}}{A_{12}} \tag{4}$$

Here, A_{13} represents the ratio of the relative abundance of each OTU in the heavy DNA fraction to that in the light DNA fraction in the ^{13}C-biphenyl treatments, and A_{12} is the same ratio in the ^{12}C-biphenyl treatments. For the OTU representing microbes capable of dissimilating biphenyl, their genomic DNA is labeled with ^{13}C isotopes and therefore enriched in the heavy DNA fraction in the ^{13}C-biphenyl treatments. Accordingly, microbes represented by OTUs with REF > 1.0 are considered active biphenyl degraders. To predict the biphenyl degradation pathway, the gene contents of each sample were identified using the phylogenetic investigation of communities by reconstructing unobserved states (PICRUSt) method.[40]

RESULTS

Biphenyl Degradation by Soil Microbes. The biphenyl degradation efficiency was as illustrated in Figure 1. The
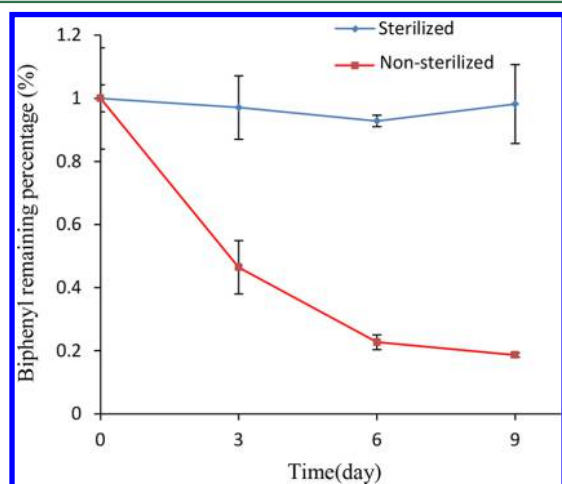

Figure 1. Percentage of biphenyl remaining.

biphenyl remaining in the nonsterile treatment after a 9 day aerobic incubation was only 18.7% of the original dosage, whereas 98.2% of the biphenyl remained in the sterile microcosms. Of the 79.5% of biphenyl degradation that is biotic degradation, over 50% occurred within the first 3 days, and degradation dramatically declined from day 6 to day 9, when only 4.1% of the biphenyl was metabolized.

Microbes Responsible for Biphenyl Biodegradation. A total of three bacteria types were identified as the dominant functional biphenyl degraders at different time points based on their enrichment in the ^{13}C-biphenyl heavy DNA fraction (Figure 2). A microbe affiliated with *Ralstonia* was highly active in biphenyl metabolism in the first 3 days and had a high REF value (17.8) (Figure 3). On day 6, the REF of *Ralstonia* decreased dramatically to 4.8, while the REF of *Cupriavidus* increased to 3.4 (Figure 3), indicating that *Cupriavidus* took over the dominant role in biphenyl degradation. On day 9, *Ralstonia* was no longer labeled with ^{13}C, but *Cupriavidus* was still active. Although only 4.1% of biphenyl was degraded from day 6 to day 9 (Figure 1), *Cupriavidus* had an extremely low abundance in the soil microbial community (0.33% on day 9), and the dissimilation of 4.1% biphenyl could sufficiently lead to

the label of genomic DNA of *Cupriavidus*. In addition, a candidate genus, DA101 (Greengenes database), began to participate in biphenyl metabolism, with a REF value of 1.3 (Figure 3). We also studied the abundances of the three bacteria identified as active degraders within the microbial community at various time points (Figure 2F). *Ralstonia* had the greatest abundance (25.2%) on day 3, which dramatically decreased on day 6 (2.5%), and was only 0.41% on day 9. Although the REF of *Cupriavidus* increased during the time series, its abundance decreased. DA101 was the rarest among the three degraders; its abundance changed little, making up 0.19%, 0.19%, and 0.22% of the community on days 3, 6, and 9, respectively.

Aside from these three dominant bacteria, some rare OTUs were affiliated with microbes involved in biphenyl degradation. In total, 19 OTUs were responsible for ^{13}C-biphenyl metabolism at various time points (Table S3). On days 3, 6, and 9, the number of enriched OTUs was 4, 6, and 9, respectively. However, their abundances were all below 0.1% throughout the biphenyl degradation process. Among these OTUs, three were affiliated with *Burkholderia*, and six were assigned to the order Burkholderiales.

Occurrence and Quantification of bphA Genes. Only one *bph*A gene was detected in the clone library among 20 positive clones. Their sequences were identical, sharing high similarity (98%) with *bph*A genes from *Comamonas* (LN879547) and *Acidovorax* (CP003872) (Figure S2). A significant enrichment of *bph*A in the heavy DNA fractions was detected in ^{13}C-biphenyl-amended soil samples (Figure 4). In contrast, *bph*A gene was only enriched in the light DNA fraction of the ^{12}C-biphenyl microcosms, indicating that the *bph*A gene was involved in biphenyl degradation and was labeled with ^{13}C in the ^{13}C-biphenyl microcosms (Figure 4). The abundance of the *bph*A gene was 4 578 204 copies in the heavy DNA fraction, which decreased to 501 828 and 94 839 on days 6 and 9, respectively (Figure S3), implying a significantly positive correlation with biphenyl degradation efficiency ($p = 0.034$).

bph Operon Identification and Biphenyl Degradation-Pathway Prediction. A single *bph* operon was obtained via amplification of the sequences flanking the identified *bph*A gene in the heavy DNA fraction of ^{13}C treatment. The sequence contained 14 genes with high similarity (99%) to the *bph* operons of *Comamonas* (LN879547.1 and AB706355.1) and *Acidovorax* (AB546270.1 and CP003872.1): *bphS*, transposase, *bphE*, *bphG*, *bphF*, *bphV*, *bphA1*, *bphA2*, *bphA3*, *bphB*, *bphC*, *bphD*, *bphW*, and *bphA4* (Figure 5A).

We combined the results of *bph* operon identification and PICRUSt gene content in the heavy DNA fraction of the ^{13}C-biphenyl microcosm to predict the biphenyl degradation pathway using the KEGG database[40] (Figure 5B). The aromatic ring of biphenyl was transferred to cis-2,3-dihydro-2,3-dihydroxybiphenyl via proteins encoded by *bphA1,2,3,4*. Then, *cis*-2,3-dihydro-2,3-dihydroxybiphenyl was dehydrogenized by the *bphB*-encoded protein. Following this, the ring of 2,3-dihydroxybiphenyl was cleaved by the activity of the *bphC*-encoded protein, and transformed into 2-hydroxy-2,4-pentadienoate and benzoic acid with the help of the protein encoded by *bphD*. Next, 2-hydroxy-2,4-pentadienoate was transformed to succinyl coenzyme A by the protein product of *bphEFG*, which is involved in the tricarboxylic acid (TCA) cycle. Benzoic acid could be transformed into acetyl coenzyme A and pyruvic acid via an enzyme-catalyzed reaction predicted by PICRUSt.
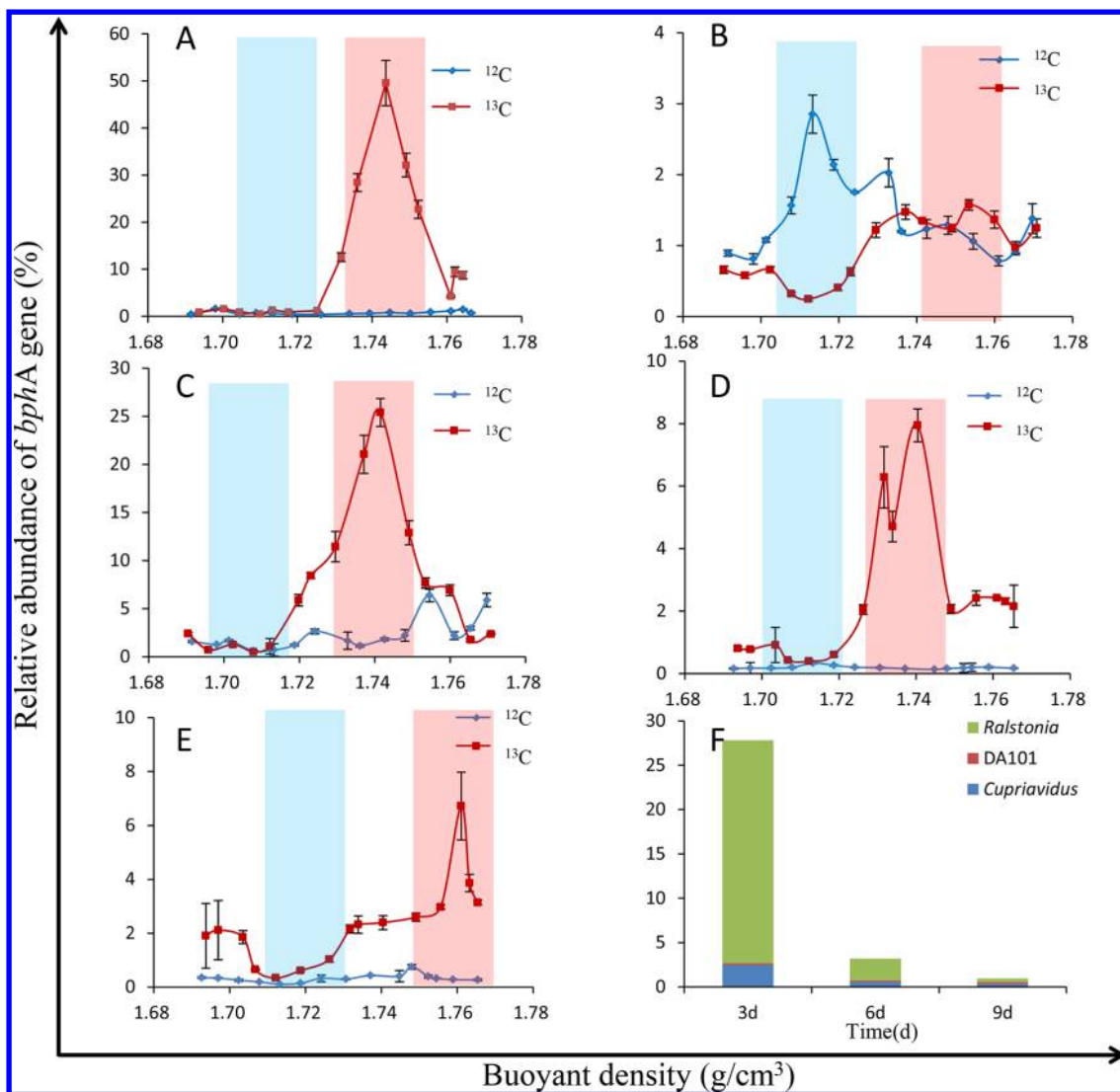
Figure 2. Relative abundances of dominant degraders in each DNA fraction and sample during incubation. (A) Relative abundance of *Ralstonia* in each DNA fraction on day 3; (B) relative abundance of *Ralstonia* in each DNA fraction on day 6; (C) relative abundance of *Cupriavidus* in each DNA fraction on day 6; (D) relative abundance of *Cupriavidus* in each DNA fraction on day 9; (E) relative abundance of DA101 in each DNA fraction on day 9; (F) relative abundances of *Ralstonia*, *Cupriavidus*, and DA101 in samples on days 3, 6, and 9, respectively. Blue and red shadows represent the light and heavy DNA fraction in each treatment, respectively.
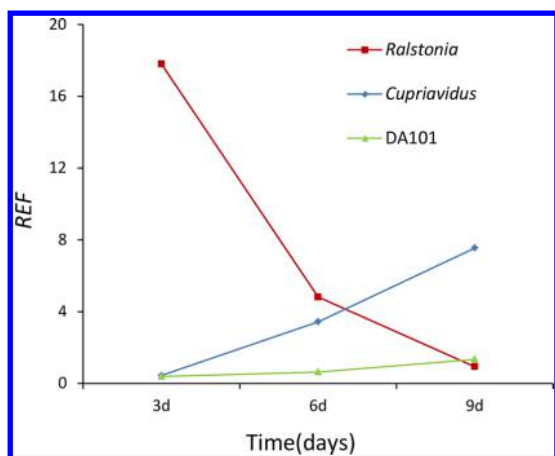

Figure 3. ^{13}C-labeling indicator REF change with time in the dominant functional degraders.

Acetyl coenzyme A and pyruvic acid are involved in the TCA cycle and glycolytic pathway, respectively. Finally, biphenyl is decomposed into carbon dioxide and water.

Relationships between the bph Operon and Biphenyl Degraders. The *bph* operon discovered in the present study exhibits high similarity with the operons identified in *Acidovorax* and *Comamonas*. In Teeling's research, 98.2% of fragments within a species have tetranucleotide-derived z-score correlations of 0.6 or greater.[35] As expected, the tetranucleotide signature of the *bph* operon was closely related to those of *Acidovorax* and *Comamonas*, both of which had indicator values of 0.73 (Figure 6). To validate this method, we investigated the tetranucleotide signature relationship of the *bph* operon with *Methylobacillus* ($R = 0.45$) and *Escherichia* ($R = 0.20$) (Figure S4), suggesting that prediction based on tetranucleotide-derived z-scores is credible. The tetranucleotide signatures of biphenyl-degrading microbes in the present study, *Cupriavidus* and *Ralstonia*, were similar to that of the *bph* operon. Pearson's R values of *Cupriavidus* and *Ralstonia* were 0.71 and 0.77,
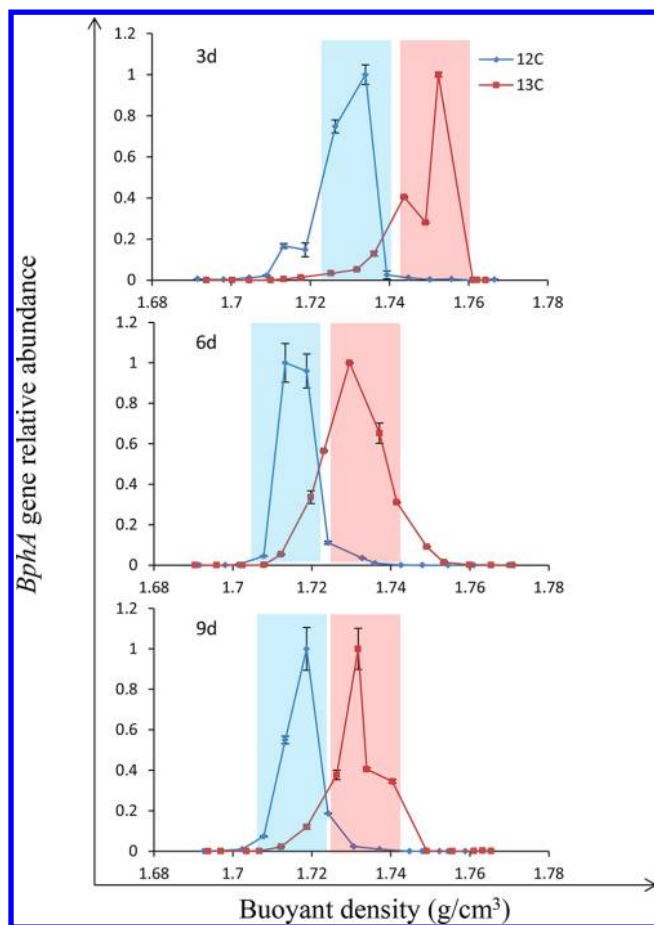
Figure 4. Relative abundance of *bphA* gene in ultracentrifugation fractions from ^{13}C-biphenyl and ^{12}C-biphenyl amended microcosms, as determined using real time quantitative PCR. The relative abundance was normalized to the maximum value in each treatment. Blue and red shadows represent the light and heavy DNA fraction in each treatment.

respectively (Figure 6), indicating that the *bph* operon we amplified likely originated from *Ralstonia*.

DISCUSSION

In the present study, 54.6% of the biphenyl was degraded within the first 3 days, which is remarkably higher than that of previous studies,[23,41] possibly due to the high abundance of functional biphenyl degrading microbes. The three major degraders were identified as *Ralstonia*, *Cupriavidus*, and DA101 based on the results of DNA-SIP. The PCB degrading capability of *Ralstonia* was identified decades ago, including the active enzymes involved in this process.[42] For example, 2,2′,5,5′-tetrachlorobiphenyl can be degraded by *Ralstonia eutropha* H850 via a 3,4-dioxygenase attack.[43] In addition, *Ralstonia* is a widely studied bacterium with a powerful ability to degrade organic pollutants. It can metabolize 2,4,6-trichlorophenol, nitroaromatic compounds, phenol, trichloroethene, biaryl compounds, and PAHs.[44−48] *Cupriavidus* is a genus of Gram-negative bacteria that belongs to the family Burkholderiaceae. Similar to *Ralstonia*, *Cupriavidus* can metabolize various pollutants, including PAHs, benzylpenicillin, 2-and 4-nitrobenzoates, diphenyl ethers, and PCBs.[49−53] Although both *Ralstonia* and *Cupriavidus* are powerful degraders of organic pollutants based on traditional cultiva-

tion-based methods, their degradation capacity in the field is often questioned,[54] challenging their application in bioaugmentation efforts. Hence, cultivation-independent approaches are necessary to identify functional microbes and confirm their functions in situ, but such studies are limited and have provided no direct evidence proving the PCB-degrading abilities of *Ralstonia* and *Cupriavidus* in soils. To the best of our knowledge, the present study is the first to definitively show *Ralstonia* and *Cupriavidus* metabolizing biphenyl (or PCBs) as a carbon source in situ. DA101 is a bacterium of uncertain genus belonging to the phylum Verrucomicrobia, and this study represents the first time it is identified as a biphenyl degrader. It is noteworthy that the increasing uptake of ^{13}C label might not lead to the increasing abundance of active degraders. For instance, *Cupriavidus* was identified as biphenyl degraders but its relative abundance decreased. These results suggested that rare bacterial species responsible for PCBs degradation might not adapt to the environment and did not dominate the complex microbial community. Accordingly, increasing the abundance of rare active degraders, such as bioaugmentation, might be an appropriate practical strategy to improve the remediation efficiency of PCBs contaminated soils.

In e-waste-contaminated soils, aside from PCBs, high concentrations of PAHs, PBDEs, Cu, Pb, and Zn were detected. Among the major degraders, *Ralstonia* and *Cupriavidus* might have an advantage over other microbes in such a heavily contaminated environment. *Ralstonia* and *Cupriavidus* have been found to degrade PAHs[44,49] and can tolerate high metal concentrations.[55,56] Together with the findings revealed by the SIP results in the present study, we speculated that *Ralstonia* and *Cupriavidus* might metabolize various organic pollutants and survive in heavy metal and POPs co-contaminated environments. In addition, the ability to use multiple carbon sources and heavy-metal resistance help these bacteria occupy a broader ecological niche in e-waste-contaminated soils, giving them great potential for application in field bioaugmentation.

Only one *bphA* gene was detected in our study, which was similar to the well-known genes of *Comamonas* and *Acidovorax*. When labeled with ^{13}C, this *bphA* gene had high copy numbers in the heavy DNA fraction, and thus, this gene was confirmed to be involved in the metabolism of biphenyl. The diversity of the *bphA* gene was quite low in the present soil community. Limitation by the primers used might result in only a few types of *bphA* genes being amplified, while genes with different conserved zones could be missed. The *bph* operon amplified using the flanking sequences of the *bph*A gene has almost the same sequence and structure as those from *Comamonas testosteroni* P19 and *Acidovorax* sp. KKS102. Although the sequence of *bph* operon from cultivable *Comamonas testosteroni* P19 is recorded in NCBI GenBank (accession no. LN879547.1), there is a lack of experimental evidence, and its ecological function is putative. First isolated in 1988, *Acidovorax* sp. KKS102 could only grow in biphenyl- or PCB-supplemented minimal medium when co-cultivated with the nonbiphenyl-degrading bacterium *Pseudomonas fluorescens* KKL101.[57] Both genetic and phenotypic evidence has supported its role in transforming biphenyl into succinyl-coenzyme A by following two distinct mechanisms regulated by *bphS* (repressor) or *bphQ* (activator).[26] The *pE* promoter transcription initiation site located 318 bp upstream of *bphE* was accompanied by four *bphS* binding sites named BS I, BS II, BS III, and BS IV (Figure 5A). According to previous research,
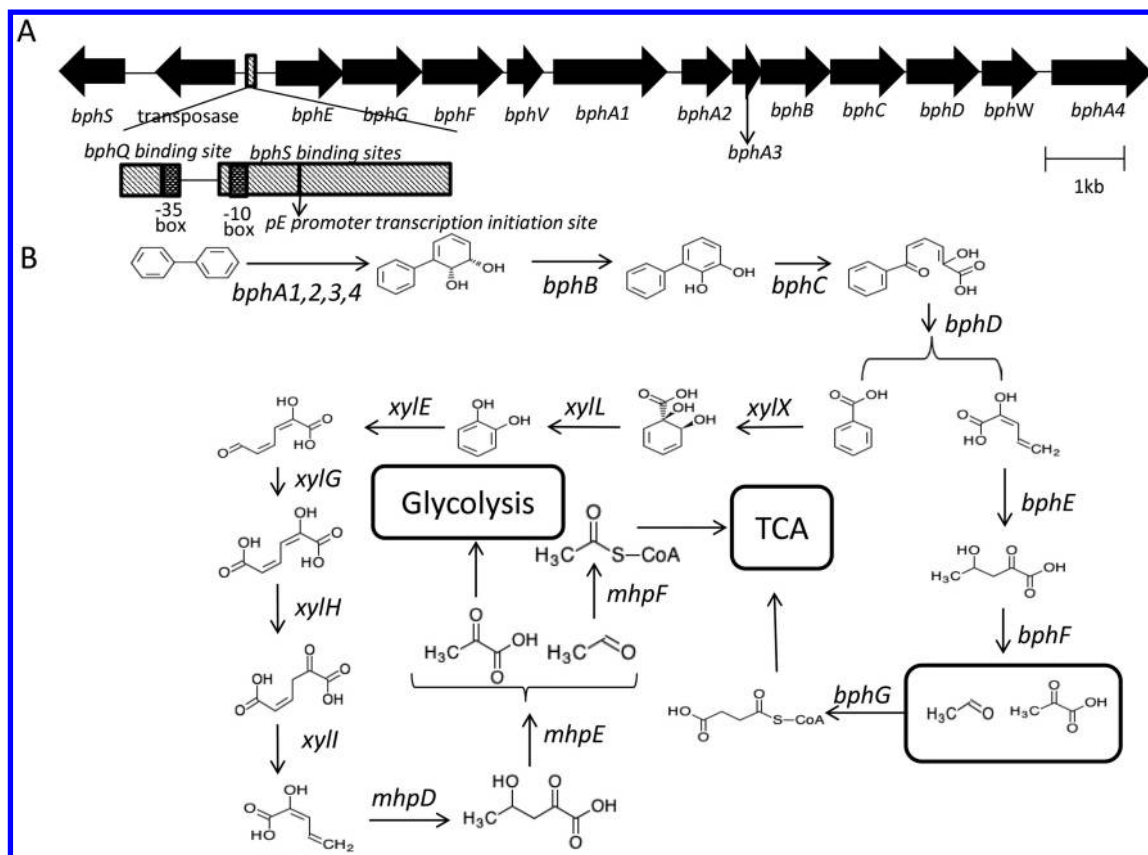
Figure 5. (A) Organization of the *bph* operon and (B) the proposed catabolic pathway for biphenyl.

the *bphS*-encoded protein binds to BS I and BS II cooperatively, as well as BS III and BS IV, to prevent access by RNA polymerase.[27] A *bphQ* binding site was observed adjacent to BS I, although the *bphQ* gene was not detected. We inferred that the *bphQ* gene might be located in another operon, a conclusion that is supported by previous research.[26] *bphQ* can activate the *pE* promoter to enhance expression of the *bph* operon. Although we found no evidence of positive regulation by the BPHQ activator, our results suggest that the protein encoded by *bphS*-mediated repression mechanisms had a major role in regulating biphenyl and PCB degradation via the *bph* operon identified in the present study.

The *bph* operon identified in this study contains the *bphABCDEFG* gene cluster, which is responsible for both biphenyl degradation and the aerobic open-ring metabolisms of PCBs.[13] The encoding enzymes can transfer (chloro)biphenyl to (chloro)benzoate and 2-hydroxypenta-2,4-dienoate[41] followed by catechol and β-ketoadipate pathways.[58,59] Because the initial dehalogenation of highly chlorinated PCBs does not involve the ring-opening of biphenyl skeleton[18,19] and incorporates no ^{13}C into microbial components, this *bph* operon is normally used as a biomarker for PCBs biodegradation.[21−23] For instance, the bioremediation potential and capability of PCBs were assessed by the abundance and diversity of *bphA* gene.[60,61] Although there was a lack of experiments directly addressing PCBs degradation in the present study, our findings give an indirect evidence and new insights of in situ PCBs metabolisms in soils via SIP.

Based on tetranucleotide signature characteristics, the identified *bph* operon might originate from *Ralstonia*, consistent with a similar tendency between *bphA* gene copy numbers and

Ralstonia abundance. Interestingly, the *bph* operon was first isolated from *Acidovorax* sp. KKS102 and then detected in *Comamonas testosteroni* P19,[57] hinting at the possible horizontal gene transfer of this operon among species. Further evidence of horizontal transfer is the location of the *bph* operon on an integrative and conjugative element (ICE) in *Acidovorax* sp. KKS102.[62] Typical of mobile elements found in bacterial chromosomes, ICEs are capable of conjugating and spreading genes horizontally.[63] Given the possibility of horizontal transfer, the *bph* operon might be shared by different biphenyl and PCBs degraders and thus play an important role in biphenyl and PCBs degradation. The high concentrations of organic pollutants and heavy metals in e-waste-contaminated soils may provide selective pressure and promote horizontal gene transfer, thereby improving biphenyl and PCB degradation efficiency. It has been reported that PAHs could increase gene-transfer efficiency by inhibiting degradation of free DNA[64] and that heavy metal stress might enhance the possibility of horizontal gene transfer.[65] According to BLAST results, the function of the *bph* operon has only been confirmed in two cultivable strains. In this study, we confirmed its involvement in PCBs degradation in situ using a cultivation-independent approach.

In the present study, three dominant biphenyl-degrading bacteria were identified in e-waste-recycling soil via DNA-SIP as *Ralstonia, Cupriavidus*, and the novel bacterium DA101. This is the first time that *Ralstonia* and *Cupriavidus* have been identified as biphenyl degraders in situ and the first link of DA101 to degradation of organic pollutants. A ^{13}C-labeled biphenyl degradation operon containing the *bphA* gene was amplified and characterized and, based on analysis of its
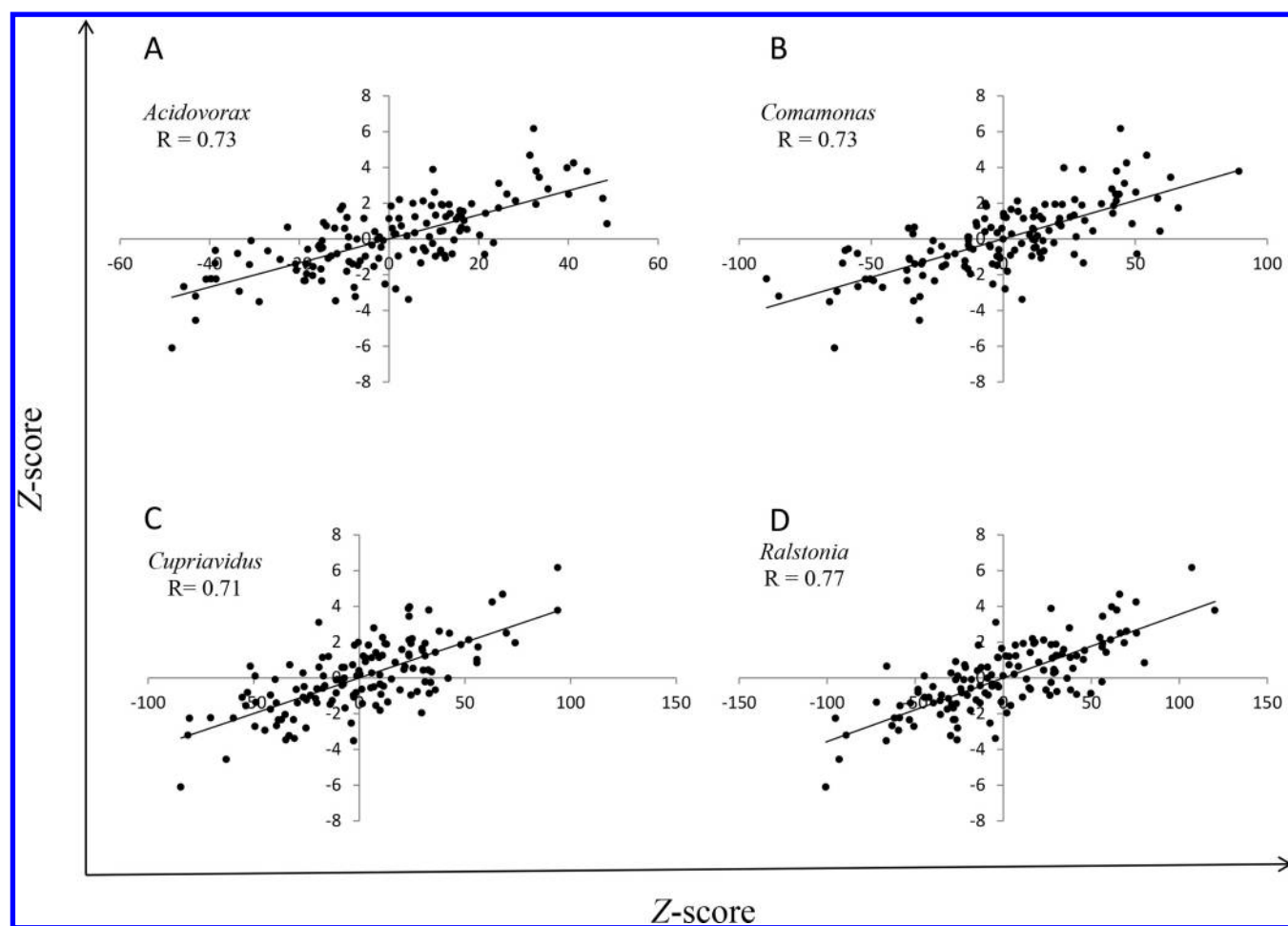
Figure 6. Relationships of tetranucleotide usage patterns between the *bph* operon and (A) *Acidovorax*, (B) *Comamonas,* (C) *Cupriavidus,* and (D) *Ralstonia.*

tetranucleotide signature, may belong to *Ralstonia.* Our results suggest that *Ralstonia* and *Cupriavidus* can occupy broader ecological niches, making them promising candidates for biphenyl and PCB bioaugmentation, due to the benefits of metabolic capability for degrading various organic pollutants and of tolerating heavy metals in e-waste-contaminated soils. In addition, the mobility of the biphenyl degradation operon may provide outstanding potential applications as a gene resource for biphenyl and PCB remediation.

ASSOCIATED CONTENT

⑤ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b06647.

Figure showing the relationship between biphenyl concentration and GC/MS peak area, a hylogenetic tree of *bphA*, copy numbers of the *bphA* gene, and tetranucleotide patterns. Tables showing PAHs, PBDEs, Cu, Pb, and Zn concentrations, primers used to amplify the flanking sequence of the *bphA* gene, and the taxonomic identification of rare degraders in microcosms and their relative abundances in samples. (PDF)

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Notes

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REFERENCES

(1) Zhang, K.; Schnoor, J. L.; Zeng, E. Y. E-Waste Recycling: Where Does It Go from Here? *Environ. Sci. Technol.* **2012**, *46* (20), 10861−10867.

(2) Leung, A. O. W.; Luksemburg, W. J.; Wong, A. S.; Wong, M. H. Spatial distribution of polybrominated diphenyl ethers and poly-chlorinated dibenzo-p-dioxins and dibenzofurans in soil and

combusted residue at Guiyu, an electronic waste recycling site in southeast China. *Environ. Sci. Technol.* **2007**, *41* (8), 2730−2737.

(3) Wong, M. H.; Wu, S. C.; Deng, W. J.; Yu, X. Z.; Luo, Q.; Leung, A. O. W.; Wong, C. S. C.; Luksemburg, W. J.; Wong, A. S. Export of toxic chemicals - A review of the case of uncontrolled electronic-waste recycling. *Environ. Pollut.* **2007**, *149* (2), 131−140.

(4) Ma, J.; Kannan, K.; Cheng, J.; Horii, Y.; Wu, Q.; Wang, W. Concentrations, Profiles, And Estimated Human Exposures for Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans from Electronic Waste Recycling Facilities and a Chemical Industrial Complex in Eastern China. *Environ. Sci. Technol.* **2008**, *42* (22), 8252−8259.

(5) Zhang, Q.; Ye, J. J.; Chen, J. Y.; Xu, H. J.; Wang, C.; Zhao, M. R. Risk assessment of polychlorinated biphenyls and heavy metals in soils of an abandoned e-waste site in China. *Environ. Pollut.* **2014**, *185*, 258−265.

(6) Wang, Y.; Luo, C. L.; Li, J.; Yin, H.; Li, X. D.; Zhang, G. Characterization of PBDEs in soils and vegetations near an e-waste recycling site in South China. *Environ. Pollut.* **2011**, *159* (10), 2443−2448.

(7) Tian, M.; Chen, S. J.; Wang, J.; Shi, T.; Luo, X. J.; Mai, B. X. Atmospheric Deposition of Halogenated Flame Retardants at Urban, E-Waste, and Rural Locations in Southern China. *Environ. Sci. Technol.* **2011**, *45* (11), 4696−4701.

(8) Zhang, J. Q.; Jiang, Y. S.; Zhou, J.; Wu, B.; Liang, Y.; Peng, Z. Q.; Fang, D. K.; Liu, B.; Huang, H. Y.; He, C.; Wang, C. L.; Lu, F. N. Elevated Body Burdens of PBDEs, Dioxins, and PCBs on Thyroid Hormone Homeostasis at an Electronic Waste Recycling Site in China. *Environ. Sci. Technol.* **2010**, *44* (10), 3956−3962.

(9) Zheng, X. B.; Luo, X. J.; Zeng, Y. H.; Wu, J. P.; Mai, B. X. Chiral Polychlorinated Biphenyls (PCBs) in Bioaccumulation, Maternal Transfer, and Embryo Development of Chicken. *Environ. Sci. Technol.* **2015**, *49* (2), 785−791.

(10) Abramowicz, D. A. Aerobic and Anaerobic Biodegradation of PCBs - a Review. *Crit. Rev. Biotechnol.* **1990**, *10* (3), 241−249.

(11) Pieper, D. H. Aerobic degradation of polychlorinated biphenyls. *Appl. Microbiol. Biotechnol.* **2005**, *67* (2), 170−191.

(12) Sakai, M.; Ezaki, S.; Suzuki, N.; Kurane, R. Isolation and characterization of a novel polychlorinated biphenyl-degrading bacterium, Paenibacillus sp KBC101. *Appl. Microbiol. Biotechnol.* **2005**, *68* (1), 111−116.

(13) Furukawa, K.; Fujihara, H. Microbial degradation of polychlorinated biphenyls: Biochemical and molecular features. *J. Biosci. Bioeng.* **2008**, *105* (5), 433−449.

(14) Hosokawa, R.; Nagai, M.; Morikawa, M.; Okuyama, H. Autochthonous bioaugmentation and its possible application to oil spills. *World J. Microbiol. Biotechnol.* **2009**, *25* (9), 1519−1528.

(15) Barbato, M.; Mapelli, F.; Magagnini, M.; Chouaia, B.; Armeni, M.; Marasco, R.; Crotti, E.; Daffonchio, D.; Borin, S. Hydrocarbon pollutants shape bacterial community assembly of harbor sediments. *Mar. Pollut. Bull.* **2016**, *104* (1−2), 211−220.

(16) Song, M. K.; Luo, C. L.; Li, F. B.; Jiang, L. F.; Wang, Y.; Zhang, D. Y.; Zhang, G. Anaerobic degradation of Polychlorinated Biphenyls (PCBs) and Polychlorinated Biphenyls Ethers (PBDEs), and microbial community dynamics of electronic waste-contaminated soil. *Sci. Total Environ.* **2015**, *502*, 426−433.

(17) Tang, X. J.; Qiao, J. N.; Chen, C.; Chen, L. T.; Yu, C. N.; Shen, C. F.; Chen, Y. X. Bacterial Communities of Polychlorinated Biphenyls Polluted Soil Around an E-waste Recycling Workshop. *Soil Sediment Contam.* **2013**, *22* (5), 562−573.

(18) Garrido-Sanz, D.; Manzano, J.; Martin, M.; Redondo-Nieto, M.; Rivilla, R. Metagenomic Analysis of a Biphenyl-Degrading Soil Bacterial Consortium Reveals the Metabolic Roles of Specific Populations. *Front. Microbiol.* **2018**, *9*.10.3389/fmicb.2018.00232

(19) Fennell, D. E.; Nijenhuis, I.; Wilson, S. F.; Zinder, S. H.; Haggblom, M. M. Dehalococcoides ethenogenes strain 195 reductively dechlorinates diverse chlorinated aromatic pollutants. *Environ. Sci. Technol.* **2004**, *38* (7), 2075−2081.

(20) Vezina, J.; Barriault, D.; Sylvestre, M. Diversity of the C-terminal portion of the biphenyl dioxygenase large subunit. *J. Mol. Microb Biotech* **2008**, *15* (2−3), 139−151.

(21) Wang, H.; Hu, J. X.; Xu, K.; Tang, X. J.; Xu, X. H.; Shen, C. F. Biodegradation and chemotaxis of polychlorinated biphenyls, biphenyls, and their metabolites by Rhodococcus spp. *Biodegradation* **2018**, *29* (1), 1−10.

(22) Uhlik, O.; Jecna, K.; Mackova, M.; Vlcek, C.; Hroudova, M.; Demnerova, K.; Paces, V.; Macek, T. Biphenyl-Metabolizing Bacteria in the Rhizosphere of Horseradish and Bulk Soil Contaminated by Polychlorinated Biphenyls as Revealed by Stable Isotope Probing. *Appl. Environ. Microb* **2009**, *75* (20), 6471−6477.

(23) Leigh, M. B.; Pellizari, V. H.; Uhlik, O.; Sutka, R.; Rodrigues, J.; Ostrom, N. E.; Zhou, J. H.; Tiedje, J. M. Biphenyl-utilizing bacteria and their functional genes in a pine root zone contaminated with polychlorinated biphenyls (PCBs). *ISME J.* **2007**, *1* (2), 134−148.

(24) Petric, I.; Hrsak, D.; Fingler, S.; Udikovic-Kolic, N.; Bru, D.; Martin-Laurent, F. Insight in the PCB-degrading functional community in long-term contaminated soil under bioremediation. *J. Soils Sediments* **2011**, *11* (2), 290−300.

(25) Koubek, J.; Mackova, M.; Macek, T.; Uhlik, O. Diversity of chlorobiphenyl-metabolizing bacteria and their biphenyl dioxygenases in contaminated sediment. *Chemosphere* **2013**, *93* (8), 1548−1555.

(26) Ohtsubo, Y.; Goto, H.; Nagata, Y.; Kudo, T.; Tsuda, M. Identification of a response regulator gene for catabolite control from a PCB-degrading beta-proteobacteria, Acidovorax sp KKS102. *Mol. Microbiol.* **2006**, *60* (6), 1563−1575.

(27) Ohtsubo, Y.; Delaware, M.; Kimbara, K.; Takagi, M.; Ohta, A.; Nagata, Y. BphS, a key transcriptional regulator of bph genes involved in polychlorinated biphenyl/biphenyl degradation in Pseudomonas sp KKS102. *J. Biol. Chem.* **2001**, *276* (39), 36146−36154.

(28) Zhou, Y.; Wei, J. S.; Shao, N. M.; Wei, D. Z. Construction of a genetically engineered microorganism for phenanthrene biodegradation. *J. Basic Microbiol.* **2013**, *53* (2), 188−194.

(29) Peng, R. H.; Fu, X. Y.; Zhao, W.; Tian, Y. S.; Zhu, B.; Han, H. J.; Xu, J.; Yao, Q. H. Phytoremediation of Phenanthrene by Transgenic Plants Transformed with a Naphthalene Dioxygenase System from Pseudomonas. *Environ. Sci. Technol.* **2014**, *48* (21), 12824−12832.

(30) Sul, W. J.; Park, J.; Quensen, J. F.; Rodrigues, J. L. M.; Seliger, L.; Tsoi, T. V.; Zylstra, G. J.; Tiedje, J. M. DNA-Stable Isotope Probing Integrated with Metagenomics for Retrieval of Biphenyl Dioxygenase Genes from Polychlorinated Biphenyl-Contaminated River Sediment. *Appl. Environ. Microbiol.* **2009**, *75* (17), 5501−5506.

(31) Dick, G. J.; Andersson, A. F.; Baker, B. J.; Simmons, S. L.; Yelton, A. P.; Banfield, J. F.; Thomas, B. C. Community-wide analysis of microbial genome sequence signatures. *Genome Biol.* **2009**, *10* (8), R85.

(32) Jiang, B.; Jin, N.; Xing, Y.; Su, Y.; Zhang, D. Unraveling uncultivable pesticide degraders via stable isotope probing (SIP). *Crit. Rev. Biotechnol.* **2018**, 1−24.

(33) Song, M.; Luo, C. L.; Jiang, L. F.; Zhang, D. Y.; Wang, Y. J.; Zhang, G. Identification of Benzo[a]pyrene-Metabolizing Bacteria in Forest Soils by Using DNA-Based Stable-Isotope Probing. *Appl. Environ. Microbiol.* **2015**, *81* (21), 7368−7376.

(34) Liu, Y. G.; Chen, Y. High-efficiency thermal asymmetric interlaced PCR for amplification of unknown flanking sequences. *BioTechniques* **2007**, *43* (5), 649−656.

(35) Teeling, H.; Meyerdierks, A.; Bauer, M.; Amann, R.; Glockner, F. O. Application of tetranucleotide frequencies for the assignment of genomic fragments. *Environ. Microbiol.* **2004**, *6* (9), 938−947.

(36) Ohtsubo, Y.; Maruyama, F.; Mitsui, H.; Nagata, Y.; Tsuda, M. Complete Genome Sequence of Acidovorax sp Strain KKS102, a Polychlorinated-Biphenyl Degrader. *J. Bacteriol.* **2012**, *194* (24), 6970−6971.

(37) Schloss, P. D. A High-Throughput DNA Sequence Aligner for Microbial Ecology Studies. *PLoS One* **2009**, *4* (12), e8230.

(38) Caporaso, J. G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F. D.; Costello, E. K.; Fierer, N.; Pena, A. G.; Goodrich, J. K.; Gordon, J. I.; Huttley, G. A.; Kelley, S. T.; Knights, D.; Koenig, J.

E.; Ley, R. E.; Lozupone, C. A.; McDonald, D.; Muegge, B. D.; Pirrung, M.; Reeder, J.; Sevinsky, J. R.; Turnbaugh, P. J.; Walters, W. A.; Widmann, J.; Yatsunenko, T.; Zaneveld, J.; Knight, R. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, 7 (5), 335−336.

(39) Edgar, R. C.; Haas, B. J.; Clemente, J. C.; Quince, C.; Knight, R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **2011**, 27 (16), 2194−2200.

(40) Langille, M. G. I.; Zaneveld, J.; Caporaso, J. G.; McDonald, D.; Knights, D.; Reyes, J. A.; Clemente, J. C.; Burkepile, D. E.; Thurber, R. L. V.; Knight, R.; Beiko, R. G.; Huttenhower, C. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* **2013**, 31 (9), 814−821.

(41) Leewis, M. C.; Uhlik, O.; Leigh, M. B. Synergistic Processing of Biphenyl and Benzoate: Carbon Flow Through the Bacterial Community in Polychlorinated-Biphenyl-Contaminated Soil. *Sci. Rep.* **2016**, 6, 22145.

(42) Bedard, D. L.; Unterman, R.; Bopp, L. H.; Brennan, M. J.; Haberl, M. L.; Johnson, C. Rapid Assay for Screening and Characterizing Microorganisms for the Ability to Degrade Polychlorinated-Biphenyls. *Appl. Environ. Microbiol.* **1986**, 51 (4), 761−768.

(43) Bedard, D. L.; Haberl, M. L.; May, R. J.; Brennan, M. J. Evidence for Novel Mechanisms of Polychlorinated Biphenyl Metabolism in Alcaligenes-Eutrophus H850. *Appl. Environ. Microbiol.* **1987**, 53 (5), 1103−1112.

(44) Jalilnejad, E.; Vahabzadeh, F. Models for the Biodegradation Kinetics of Naphthalene by Ralstonia Eutropha. *Polycyclic Aromat. Compd.* **2013**, 33 (5), 451−466.

(45) Hundt, K.; Wagner, M.; Becher, D.; Hammer, E.; Schauer, F. Effect of selected environmental factors on degradation and mineralization of biaryl compounds by the bacterium Ralstonia picketii in soil and compost. *Chemosphere* **1998**, 36 (10), 2321−2335.

(46) Chen, W. M.; Chang, J. S.; Wu, C. H.; Chang, S. C. Characterization of phenol and trichloroethene degradation by the rhizobium Ralstonia taiwanensis. *Res. Microbiol.* **2004**, 155 (8), 672−680.

(47) Louie, T. M.; Webster, C. M.; Xun, L. Y. Genetic and biochemical characterization of a 2,4,6-trichlorophenol degradation pathway in Ralstonia eutropha JMP134. *J. Bacteriol.* **2002**, 184 (13), 3492−3500.

(48) Samanta, S. K.; Bhushan, B.; Chauhan, A.; Jain, R. K. Chemotaxis of a Ralstonia sp SJ98 toward different nitroaromatic compounds and their degradation. *Biochem. Biophys. Res. Commun.* **2000**, 269 (1), 117−123.

(49) Kuppusamy, S.; Thavamani, P.; Megharaj, M.; Lee, Y. B.; Naidu, R. Polyaromatic hydrocarbon (PAH) degradation potential of a new acid tolerant, diazotrophic P-solubilizing and heavy metal resistant bacterium Cupriavidus sp MTS-7 isolated from long-term mixed contaminated soil. *Chemosphere* **2016**, 162, 31−39.

(50) Ben Salem, I.; Mezni, M.; Boulila, A.; Hamdi, M.; Saidi, M. Removal of Penicillin G and Erythromycin with Ionizing Radiation Followed by Biological Treatment. *Curr. Microbiol.* **2016**, 73 (4), 582−586.

(51) Basu, S.; Chowdhury, P. P.; Deb, S.; Dutta, T. K. Degradation Pathways of 2-and 4-Nitrobenzoates in Cupriavidus sp Strain ST-14 and Construction of a Recombinant Strain, ST-14::3NBA, Capable of Degrading 3-Nitrobenzoate. *Appl. Environ. Microbiol.* **2016**, 82 (14), 4253−4263.

(52) Wang, S.; Bai, N. L.; Wang, B.; Feng, Z.; Hutchins, W. C.; Yang, C. H.; Zhao, Y. H. Characterization of the molecular degradation mechanism of diphenyl ethers by Cupriavidus sp WS. *Environ. Sci. Pollut. Res.* **2015**, 22 (21), 16914−16926.

(53) Kim, S.; Picardal, F. Microbial growth on dichlorobiphenyls chlorinated on both rings as a sole carbon and energy source. *Appl. Environ. Microbiol.* **2001**, 67 (4), 1953−1955.

(54) Jiang, L. F.; Song, M. K.; Luo, C. L.; Zhang, D. Y.; Zhang, G. Novel Phenanthrene-Degrading Bacteria Identified by DNA-Stable Isotope Probing. *PLoS One* **2015**, 10 (6), e0130846.

(55) Moreira, H.; Pereira, S. I. A.; Marques, A. P. G. C.; Rangel, A. O. S. S.; Castro, P. M. L. Selection of metal resistant plant growth promoting rhizobacteria for the growth and metal accumulation of energy maize in a mine soil - Effect of the inoculum size. *Geoderma* **2016**, 278, 1−11.

(56) Rojas-Rojas, F. U.; Huntemann, M.; Clum, A.; Pillay, M.; Palaniappan, K.; Varghese, N.; Mikhailova, N.; Stamatis, D.; Reddy, T. B.; Markowitz, V.; Ivanova, N.; Kyrpides, N.; Woyke, T.; Shapiro, N.; Ibarra, J. A.; Estrada-de Los Santos, P. Draft Genome Sequence of Heavy Metal-Resistant Cupriavidus alkaliphilus ASC-732T, Isolated from Agave Rhizosphere in the Northeast of Mexico. *Genome Announc* **2016**, 4 (5), e01013-16.

(57) Kimbara, K.; Hashimoto, T.; Fukuda, M.; Koana, T.; Takagi, M.; Oishi, M.; Yano, K. Isolation and Characterization of a Mixed Culture That Degrades Polychlorinated-Biphenyls. *Agric. Biol. Chem.* **1988**, 52 (11), 2885−2891.

(58) Pieper, D. H.; Seeger, M. Bacterial metabolism of polychlorinated biphenyls. *J. Mol. Microb Biotech* **2008**, 15 (2−3), 121−138.

(59) Harwood, C. S.; Parales, R. E. The beta-ketoadipate pathway and the biology of self-identity. *Annu. Rev. Microbiol.* **1996**, 50, 553−590.

(60) Layton, A. C.; Lajoie, C. A.; Easter, J. P.; Jernigan, R.; Sanseverino, J.; Sayler, G. S. Molecular Diagnostics and Chemical-Analysis for Assessing Biodegradation of Polychlorinated-Biphenyls in Contaminated Soils. *J. Ind. Microbiol.* **1994**, 13 (6), 392−401.

(61) Hoostal, M. J.; Bullerjahn, G. S.; McKay, R. M. L. Molecular assessment of the potential for in situ bioremediation of PCBs from aquatic sediments. *Hydrobiologia* **2002**, 469 (1−3), 59−65.

(62) Ohtsubo, Y.; Ishibashi, Y.; Naganawa, H.; Hirokawa, S.; Atobe, S.; Nagata, Y.; Tsuda, M. Conjugal Transfer of Polychlorinated Biphenyl/Biphenyl Degradation Genes in Acidovorax sp Strain KKS102, Which Are Located on an Integrative and Conjugative Element. *J. Bacteriol.* **2012**, 194 (16), 4237−4248.

(63) Banuelos-Vazquez, L. A.; Tejerizo, G. T.; Brom, S. Regulation of conjugative transfer of plasmids and integrative conjugative elements. *Plasmid* **2017**, 91, 82−89.

(64) Kang, F. X.; Gao, Y. Z.; Wang, Q. A. Inhibition of Free DNA Degradation by the Deformation of DNA Exposed to Trace Polycyclic Aromatic Hydrocarbon Contaminants. *Environ. Sci. Technol.* **2010**, 44 (23), 8891−8896.

(65) Navarro, C. A.; von Bernath, D.; Jerez, C. A. Heavy Metal Resistance Strategies of Acidophilic Bacteria and Their Acquisition: Importance for Biomining and Bioremediation. *Biol. Res.* **2013**, 46 (4), 363−371.