Expression in *Escherichia coli* of Biphenyl 2,3-Dioxygenase Genes from a Gram-Positive Polychlorinated Biphenyl Degrader, *Rhodococcus jostii* RHA1

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*Rhodococcus jostii* RHA1 is a polychlorinated biphenyl degrader. Multi-component biphenyl 2,3-dioxygenase (BphA) genes of RHA1 encode large and small subunits of oxygenase component and ferredoxin and reductase components. They did not express enzyme activity in *Escherichia coli*. To obtain BphA activity in *E. coli*, hybrid BphA gene derivatives were constructed by replacing ferredoxin and/or reductase component genes of RHA1 with those of *Pseudomonas pseudoalcaligenes* KF707. The results obtained indicate a lack of catalytic activity of the RHA1 ferredoxin component gene, bphAc, in *E. coli*. To determine the cause of inability of RHA1 bphAc to express in *E. coli*, the bphAc gene was introduced into Rosetta (DE3) pLacI, which has extra tRNA genes for rare codons in *E. coli*. The resulting strain abundantly produced the bphAc product, and showed activity. These results suggest that codon usage bias is involved in inability of RHA1 bphAc to express its catalytic activity in *E. coli*.

**Key words:** polychlorinated biphenyl (PCB); Rhodococcus; biphenyl 2,3-dioxygenase; ring-hydroxylating dioxygenase; codon usage

Polychlorinated biphenyls (PCBs) are toxic, biologically recalcitrant compounds, and are classified into one of 12 groups of compounds named Persistent Organic Pollutants (POPs). Contamination of PCBs in the environment causes serious problems despite the fact that the use of PCBs is banned now. Many bacteria that degrade PCBs aerobically have been isolated and characterized.1-6) These bacteria degrade PCBs by catabolism, and PCBs are degraded through the biphenyl catabolic pathway in the presence of biphenyl. Degradation of PCBs by microorganisms is regarded as a promising approach for the removal of low-concentration PCBs from a widely polluted environment.

In the aerobic biphenyl metabolic pathway, biphenyl is transformed to benzoate and 2-hydroxypenta-2,4-dienoate by the sequential action of a biphenyl 2,3-dioxygenase (BphA), a dihydrodiol dehydrogenase (BphB), a 2,3-dihydroxybiphenyl dioxygenase (BphC), and a 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (BphD) (Fig. 1). Benzoate and 2-hydroxypenta-2,4-dienoate are further metabolized through the benzoate pathway and the lower biphenyl pathway, respectively.

BphA is a multi-component enzyme composed of oxygenase, ferredoxin, and ferredoxin reductase components (Fig. 1).7,8) The oxygenase component consists of large and small subunits, and the former subunit contains a mononuclear iron center and a Rieske-type [2Fe–2S] cluster, which are involved in catalytic reaction and electron transfer from ferredoxin, respectively. The ferredoxin and ferredoxin reductase components are responsible for electron transfer from NADH to the large subunit of an oxygenase component.

BphA is a key enzyme in determining the substrate spectrum of a PCB degrader. The substrate specificities and the regiospecificities of several BphAs have been characterized.9-11) The three-dimensional structure of the oxygenase component of BphA of *Rhodococcus jostii* RHA1 was solved using an enzyme prepared from the Rhodococcal host strain,7) *Rhodococcus erythropolis* IAM1399, and the substrate specificity of RHA1 BphA was characterized in strain IAM1399.12) In order to examine BphA activity in detail and to develop an engineered BphA, it is preferable to use *E. coli* as a host. However, Masai et al. concluded that RHA1 bphAAAbAcAd do not express enzyme activity in *E. coli*, based on the following results: *E. coli* JM109 containing RHA1 bphAAAbAcAdCIBI genes did not transform biphenyl to a yellow meta-cleavage product, although that containing RHA1 bphCIBI genes and the bnaA1A2A3A4 genes of *Pseudomonas putida* BE-81 transformed biphenyl to a yellow meta-cleavage product.13) BE-81 bnaA1A2A3A4 genes correspond to RHA1 bphAAAbAcAd. Similarly, Mackay et al. and Taguchi et al. reported results indicating difficulty in expression of enzyme activity of Rhodococcal BphA in *E. coli*.14,15) In addition, there are many reports suggest-
Fig. 1. The Degradation Pathway of Biphenyl.

The upper degradation pathway from biphenyl to benzoate and 2,5-dihydroxy-biphenyl with proposed electron transfer reactions of BipA is illustrated. Compounds: I, biphenyl; II, cis-2,3-dihydroxy-2,3-dihydroxybiphenyl; III, 2,3-dihydroxybiphenyl; IV, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate; V, benzoate; VI, 2-hydroxyxypentanoic-2,4-dienoate.

Materials and Methods

Bacterial strains, plasmids, and culture media. The strains and plasmids used in this study are listed in Table 1. The strains used were grown in Luria-Bertani (LB) medium (Bacto Tryptone 10 g per liter, yeast extract, 5 g per liter, NaCl, 5 g per liter). Strains RHA1, Pseudomonas pseudoalcaligenes KF707, and its derivatives were grown in W minimal medium containing 0.1% biphenyl. The strains were grown at 30°C, except for the cases indicated. Antibiotics were added when needed as follows: ampicillin, 100 μg/mL; tetracycline, 10 μg/mL; chloramphenicol, 30 μg/mL. Growth was monitored by measuring the optical density at 600 nm (OD600).

DNA manipulation and analysis. All the DNA techniques used, including gene cloning, nucleotide sequencing, electrophoresis (electrophoresis), and computer analysis of DNA sequences, have been described previously. DNA extraction from agarose gel was carried out using EASYTRAP Ver. 2, following the supplier’s instructions (Takara Bio, Tokyo, Japan).

Introduction of recombinant plasmids. Introduction of recombinant plasmids into P. pseudoalcaligenes KF730 and R. erythropolis IAM1399 was done by electroporation. Cells were grown in 10 mL of LB medium at 30°C to OD600 = 1.0, harvested by centrifuging 10 min at 1,300 × g, and washed 3 times with ice-cold 0.3 M sucrose. The cells were resuspended in ice-cold 0.5 M sucrose. One hundred μL of the cell suspension was mixed with plasmid DNA, and incubated 30 min on ice. The mixture was transferred to pre-chilled 1-mm cuvettes and subjected to electroporation (12 kV/cm, 800 Ω, 25 μF) (Bio-Rad Laboratories, Richmond, CA). SOC medium (Bacto Tryptone, 20 g per liter, yeast extract, 5 g per liter, NaCl, 0.5 g per liter, MgCl2·6H2O, 2.03 g per liter, MgSO4·7H2O, 2.46 g per liter, and glucose, 3.60 g per liter) were added to the mixture, and the suspension was incubated at 30°C for 12 h. The suspension was plated onto LB agar medium containing tetracycline and incubated at 30°C for 3 d.

Construction of hybrid BipA genes. A pK19mob sacB derivative, pMRF1, carrying RHA1 biphaAAbAcAcAd, which was inserted between the upstream segment of KF707 biphaA and the downstream segment of KF707 biphaA, was introduced into KF730, and a recombinant strain, KF730R1, with the replacement of KF730 biphaA::Tsx-B21-bipha2orf2orf3orf4 by RHA1 biphaAAbAcAcAd, was selected against sucrose tolerance conferred by the loss of sacB (Fig. 2A). A pK19mob sacB derivative, pMRF2, carrying RHA1 biphaAAbAcAcAd, which was inserted between the upstream segment of KF707 biphaA and the downstream segment of KF707 biphaA, was introduced into KF730, and a recombinant strain, KF730R2, with the replacement of KF730 biphaA::Tsx-B21-biphaA2 by RHA1 biphaAAbAcAcAd, was selected in the same manner (Fig. 2A).

Growth of recombinant strains on biphenyl. Strains RHA1, KF707, and KF730R2 were grown on biphenyl in W minimal medium, and KF730R1 was grown in LB medium. Each bacterial strain was inoculated at OD600 = 0.4 in W minimal agar medium containing 0.1% biphenyl. Growth was monitored by measuring OD600.

Quantitative determination of BipA activity. For quantitative determination of BipA activity, cells grown in LB medium were washed twice with 20 mM sodium phosphate buffer (pH 7.0) and suspended in the same buffer at a concentration of 50 mg of wet cells/mL. Half-mL aliquots of the cell suspension were incubated with 1 mM of biphenyl in a sealed tube at 30°C for 30 min. Biphenyl was added as a 100 mM solution in DMSO. After centrifugation at 18,800 × g at 4°C for 5 min, BipA activity was determined by monitoring the increase in absorbance at 306 nm resulting from the formation of cis-2,3-dihydroxy-2,3-dihydroxybiphenyl in the supernatant using a spectrophotometer (model DU-7500; Beckman Coulter, Fullerton, CA). A molar extinction coefficient of 15,500 M−1 cm−1 was used to quantify cis-2,3-dihydroxy-2,3-dihydroxybiphenyl. Activity was expressed as the amount (nmol) of cis-2,3-dihydroxy-2,3-dihydroxybiphenyl formed per h per mg of wet cells.
Preparation of crude extract containing BphAc. E. coli strains BL21 (DE3) and Rosetta (DE3) pLac containing pERA3 were grown in 10 mL of LB medium at 20°C to OD600 = 1.5, and BphAc expression was induced by adding 0.2 mm of isopropyl-β-D-thiogalactopyranoside (IPTG). After 3 h of incubation at 20°C, cells were harvested by centrifugation, washed twice with 50 mm Tris–HCl (pH 8.0), and resuspended in 2 mL of the same buffer. The cells were disrupted by sonication. Cell debris was removed by centrifugation, washed twice with 50 mm Tris–HCl (pH 8.0), and the supernatant was subjected to Tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (Tricine-SDS–PAGE) and NADH-cytochrome c oxidoreductase activity measurement. The concentration of protein in each sample was determined with a protein assay kit (Bio-Rad Laboratories, Richmond, CA).

Tricine-SDS–PAGE. Tricine-SDS–PAGE was done following Schägger and von Jagow26 using Mini-Protean III cells (Bio-Rad Laboratories, Richmond, CA). The total percentage concentrations of acrylamide were 16.5% for the separating gel, 10% for the spacer gel, and 4% for the stacking gel, and the percentage concentration of bisacrylamide relative to the total concentration was 3% for each gel. Fifteen micrograms of protein was applied to each lane, and electrophoresis was performed using a voltage-stepped procedure with 30 V initially, followed by 50 V. The proteins were stained with Coomassie Brilliant Blue R-250 (Fluka Chemie, Buchs, Switzerland).

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<tr>
<th>Strains and plasmids</th>
<th>Relevant characteristicsa</th>
<th>Reference or origin</th>
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<tr>
<td><strong>Strains</strong></td>
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<tr>
<td><em>Rhodococcus jostii</em> RHA1</td>
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<td>6) IAM culture collectiona</td>
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<td><em>Rhodococcus erythropolis</em> I31599</td>
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<td><em>P. pseudoalcaligenes</em> KF730</td>
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<td>Mutant derivative of KF730; ΔbphA1A2A3A4 by RHA1 bphaAbAcAd insertion, BPH+</td>
<td>This study</td>
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<td>This study</td>
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<td><em>Escherichia coli</em> JM109</td>
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<td><strong>Plasmids</strong></td>
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<td>pBR322 ori, oriT sacB Km'</td>
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<td>pMRF1</td>
<td>pK19mob sacB with a 7.3-kb Xbal-HindIII fragment carrying bphAABHAIAbHAIACAIHAACAIHA inserted between N-terminal segment of bphAI# and C-terminal segment of bphAI#</td>
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<td>pRT1</td>
<td>Gram positive/Gram negative shuttle vector containing the replication origins of pRC1 and RK2, TeC</td>
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<td>Expression vector, ApC, T7 T7 promoter</td>
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<td>pERA3</td>
<td>pET-17b with 330-bp PCR fragment carrying bphAc of RHA1</td>
<td>This study</td>
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**Results**

Expression of RHA1 BphA dioxygenase

Mckay et al. succeeded in detecting BphA activity of the bphaA1A2A3A4 genes of *R. globulus* P6 not in *E. coli*, but in a *Pseudomonas* host strain.14) Hence we first examined the expression of RHA1 bphaAbAcAd in strain KF730, a mutant derivative of a Gram-negative
PCB-degrading bacterium, *P. pseudoalcaligenes* KF707. In KF730, the *bphA1* gene (equivalent to *bphAa*) was disrupted by insertion of a transposon, Tn5-B21. RHA1 *bphAaAbAcAd* was introduced in KF730 by replacing KF730 *bphA1*:Tn5-B21-A2A3A4 and *bphA1*:Tn5-B21-A2 with RHA1 *bphAaAbAcAd* to construct strains KF730R1 and KF730R2, respectively. KF730R1 contains RHA1 *bphAaAbAcAd* plus KF730 (KF707) *bphBC*, and KF730R2 contains RHA1 *bphAaAbAcAd* plus KF730 (KF707) *bphA3A4BC* (Fig. 2A). KF730R1 did not grow on biphenyl (data not shown), but KF730R2 grew better than KF707 (Fig. 2B). These results suggest that KF707 *bphA3A4* made up for the lack of activity of KF730 *bphAcAd* in KF730R2.

The growth on biphenyl of KF730R2 containing RHA1 *bphAaAbAcAd* and KF730 *bphA3A4* suggests that a hybrid dioxygenase encoded by RHA1 *bphAaAb* and KF730 *bphA3A4* can support the transformation of biphenyl and PCBs in *E. coli*. Hence we constructed a series of hybrid dioxygenase genes between RHA1 *bphAaAbAcAd* and KF730 *bphA3A4* to determine the best combination of heterologous subunits originating from RHA1 and KF707.

pRAR carrying RHA1 *bphAaAbAcAd* and pRKA4 carrying RHA1 *bphAaAbAc* and KF707 *bphA4* exhibited BphA dioxygenase activity as measured by the production of dihydriodiol from biphenyl only in *R. erythropolis* IAM1399. They did not show BphA dioxygenase activity in *E. coli* JM109. pRFA carrying RHA1 *bphAaAb* and KF707 *bphA3A4* and pRKA32 carrying RHA1 *bphAaAbAd* and KF707 *bphA3* exhibited BphA dioxygenase activity not only in IAM1399 but also in JM109 (Fig. 3). These results suggest that the catalytic activity of RHA1 *bphAc* was not expressed in *E. coli*, and prevented the RHA1 BphA genes from expressing enzyme activity in *E. coli*. IAM1399 harboring a plasmid, pRA124, carrying RHA1 *bphAaAbAd*, lacked BphA enzyme activity, indicating that no innate catalytic activity equivalent to the *bphAc* product is present in IAM1399 (data not shown). The results for pRKA4 and pRKA32 in IAM1399 indicate that heterologous combinations of electron transfer components between RHA1 and KF707 are functional in activating the RHA1 *bphAaAb*-coding terminal dioxygenase component. The activity of IAM1399, harboring pRKA32, was much higher than that of IAM1399 harboring pRKA4, suggesting that electron transfer from *bphAd*-coding reductase to *bphA3*-coding ferredoxin is more efficient than that from *bphA4*-coding reductase to *bphAc*-coding ferredoxin. The results for pRAR and pRFA in IAM1399, however, indicate that homologous combinations of electron transfer components are more effective than heterologous combinations in activating the RHA1 *bphAaAb*-coding terminal dioxygenase component.

**Effect of the Shine-Dalgarno (SD) sequence on the bphAc gene expression in *E. coli***

To address the cause of the difficulty in expressing the catalytic activity of RHA1 *bphAc* in *E. coli*, the SD sequence of RHA1 *bphAc* was replaced with that of KF707 *bphA5* (Fig. 4A). The SD sequences of RHA1 *bphAc* and of KF707 *bphA5* and the polypeptide coding sequences of RHA1 *bphAc* and KF707 *bphA5* were connected and inserted between *bphAaAb* and *bphAd* of RHA1, and the resulting BphA genes were introduced in *E. coli* JM109 (Fig. 4B). The BphA activity of JM109 harboring pSDK1, which contained the SD sequence of KF707 *bphA3* and the coding sequence of RHA1 *bphAc*,
Fig. 3. BphA Activities of *R. erythropolis* IAM1399 and *E. coli* JM109 Cells Carrying Hybrid BphA Gene Derivatives between the RHA1 bphAaAbAcAd and KF707 bphA3A4 Genes.

BphA gene organizations are displayed on the left and the corresponding BphA activities in IAM1399 (black bars) and JM109 (white bars) on the right. The RHA1 and KF707 bph genes are indicated by black and gray arrows, respectively. Each gene has the original upstream nucleotide sequence containing the start codon and the Shine-Dalgarno sequence. The activities of hybrid enzymes were determined by measuring the amount of cis-2,3-dihydro-2,3-dihydroxybiphenyl formed from biphenyl, as described in the Materials and Methods section.

Fig. 4. Effect of the SD Sequence on Heterologous Expression in *E. coli* of RHA1 bphAc.
A. Comparison of the upstream sequences from the start codons of ferredoxin-coding genes in pSDR1, pSDK1, pSDRAK1, and pRKA32. Putative SD sequences are underlined. Start codons are indicated by boxes. B. BphA activities of *E. coli* JM109 cells with hybrid BphA gene derivatives, including ferredoxin-coding genes, with replacement of their SD sequences. BphA gene organizations are displayed on the left and the corresponding BphA activities on the right. The RHA1 and KF707 bph genes are indicated by black and gray arrows, respectively. Black and gray boxes represent the SD sequences of the RHA1 and KF707 ferredoxin-genes, respectively. The activities of hybrid enzymes were determined as described in Fig. 3.
was as low as that of JM109 harboring pSDR1, which contained the original SD sequence and the coding sequence of RHA1 bphAc. The BphA activity of JM109 harboring pSDRAK1, which contained the SD sequence of RHA1 bphAc and the coding sequence of KF707 bphA3, was as high as that of JM109 harboring pRKA32, which contained the original SD sequence and the coding sequence of KF707 bphA3. This indicates that the SD sequence is not involved in the inability of expressing the catalytic activity of RHA1 bphAc in E. coli.

Functional expression of RHA1 bphAc in E. coli Rosetta (DE3) pLacI

To address the cause of the inability of RHA1 bphAc to express catalytic activity in E. coli, the codon usage bias of E. coli was examined using an E. coli host strain, Rosetta (DE3) pLacI, which has extra tRNA genes for rare codons in E. coli. A plasmid, pERA3, containing RHA1 bphAc in pET-17b vector, was constructed and introduced into Rosetta (DE3) pLacI. The resulting recombinant strain abundantly produced a polypeptide in the crude extract corresponding to the deduced molecular mass (11,536 Da) of the RHA1 bphAc product (Fig. 5, lane 2). In contrast, strain BL21 (DE3), containing pERA3, poorly produced the same polypeptide (Fig. 5, lane 4). In contrast, strain BL21 (DE3), containing pERA3, poorly produced the same polypeptide in the crude extract (Fig. 5, lane 2).

Next BphAc activity, the NADH-cytochrome c oxidoreductase activity of Rosetta (DE3) pLacI harboring pERA3, was measured using cytochrome c as an electron acceptor. The crude extract of Rosetta (DE3) pLacI containing pERA3 showed strong activity (Fig. 6). In contrast, that of BL21 (DE3), containing pERA3, showed only slight activity.

These results indicate that reduction of the codon usage bias in E. coli enabled RHA1 bphAc to express its product abundantly with distinct activity, suggesting that the codon usage bias is the cause of the inability of RHA1 bphAc to express the catalytic activity in E. coli.

Discussion

Based on the results of Makay et al.,[4] RHA1 bphAcAAbAcAAd was introduced into P. pseudoalcaligenes KF730, but the resulting recombinant strain, KF730R1, did not express BphA activity. The hybrid BphA complex composed of large and small subunits of the oxygenase component coded by RHA1 bphAaAb plus ferredoxin and reductase components coded by KF707 bphA3A4 appears to have shown good activity in KF730R2. The growth of KF730R2 was superior to that of KF707, suggesting strong expression of hybrid BphA in a Pseudomonas host.

Replacement of RHA1 bphAc with KF707 bphA3 conferred BphA activity on E. coli recombinant strains, but BphA activity in E. coli of the hybrid enzyme complex with KF707 bphA3A4 was half of that in a Rhodococcus host strain, IAM1399. Considering the expression of BphA induced in E. coli by IPTG, the expression of RHA1 BphA genes in IAM1399 appeared to be strong.

In IAM1399, all the hybrid enzyme derivatives constructed by the replacement of RHA1 bphAc with KF707 bphA3, RHA1 bphAd with KF707 bphA4, and RHA1 bphAcAd with KF707 bphA3A4, yielded BphA activity. Similarly, expression of the activity of hybrid ring-hydroxylating dioxygenase complexes constructed by the replacement of heterologous ferredoxin30 ferredoxin reductase,12,31–34) or ferredoxin plus ferredoxin reductase34–36) components of heterologous origin has been reported. In some cases, replacement of the ferredoxin component with one of heterologous origin failed to yield activity, although replacement of the
reductase component with one of heterologous origin conferred activity. Thus, hybrid dioxygenase complexes constructed with a ferredoxin component of heterologous origin appears scarcely to confer activity. The corresponding BphA components of RHA1 and KF707 showed good amino acid identity to each other (Table 2). Such similarity appears to confirm expression of the activity of the hybrid dioxygenase complex between heterologous components of RHA1 and KF707, even in the case of hybrid dioxygenases constructed by replacement of a ferredoxin component of heterologous origin.

Using hybrid enzyme derivatives, we concluded that only the bphAc among the bphAaAbAcAd of RHA1 did not express activity in JM109. The bphAc gene is located in a gene cluster composed of bphAaAbAcAdC1B1 genes, which have been found to be transcribed as a single operon by RT-PCR analysis. Thus the inability to express catalytic activity in E. coli appears to originate in a problem in translation, perhaps SD sequence or codon usage unsuitable for the host strain. Saito et al. reported that optimization of the SD sequences of phenanthrene dioxygenase genes, phdABCD of Nocardioides sp. KP7, resulted in efficient expression of them in E. coli. The optimized SD sequence they used was AAGGAG. The original SD sequence of RHA1 is TAGGAG, which was replaced by that of KF707 bphA3, AAGAAAG in this study. The SD sequences of both RHA1 bphAc and KF707 bphA3 appear to function well in E. coli, because they are similar to the optimized SD sequence, and the connection of each of them to the coding sequence of KF707 bphA3 resulted in expression of the BphA dioxygenase complex with equal activity.

Expression of the genes for cytochrome P450 of Streptomyces coelicolor and pyruvate decarboxylase of Sarcina ventriculi was achieved using E. coli host strains with extra copies of rare codon tRNA genes in E. coli. In this study, strain Rosetta (DE3) pLacI with extra tRNA genes for rare codons in E. coli enabled RHA1 bphAc to express its catalytic activity. This strain has extra copies of rare codon tRNA genes, including AGG-Arg, AGA-Arg, ATA-Ile, CTA-Leu, CCC-Pro, and GGA-Gly. Among these rare codons, RHA1 bphAc contains three GGAs, one ATA, and one CCC. However, these rare codons are also found in RHA1 bphAc, bphB1, bphC1, and in KF707 bphA3 and bphA4 (Table 3). Especially RHA1 bphAd and bphC1 contain all six rare codons. The catalytic activities of RHA1 bphAc, bphAb, and bphAd, and of KF707 bphA3 and bphA4 were expressed in E. coli, as indicated in Fig. 3, and RHA1 bphB1 and bphC1 have been reported to confer their enzyme activities on E. coli. Hence it is inappropriate to conclude that the rare codons in RHA1 bphAc simply prevented its expression. Translation-coupled folding of a polypeptide chain or translation-coupled incorporation of a [2Fe–2S] cluster may have rendered RHA1 bphAc incapable of expressing its activity in E. coli.

Many reports have stated that no or poor ring-hydroxylating dioxygenase activities of gram-positive bacteria such as Rhodococcus were obtained when the responsible dioxygenase genes were introduced into E. coli to express their enzyme activities. Biphenyl dioxygenase genes, bphaAEGF of R. globulus P6, dibenzofuran dioxygenase genes, dfdA1A2A3A4 of Terrabacter sp. YK3, and those of Rhodococcus sp. HA01 expressed no enzyme activity in E. coli but no reports have described the details of the reason they were scarcely expressed. This study addressed one of the possible reasons. The results obtained indicate that the rare codons in RHA1 bphA3 prevented its expression in E. coli.

In conclusion, we succeeded in obtaining activity of RHA1 BphA as a hybrid complex of RHA1 bphAaAb and KF707 bphA3A4 gene products. The heterologous expression systems established in this study are expected to be useful in establishing an efficient degradation system of PCBs.

**Acknowledgments**

We thank Dr. James M. Tiedje for providing a broad-host-range shuttle vector, pRT1. This work was supported in part by the Program for the Promotion of Basic Research Activity in Innovative Bioscience (PROBRAIN), and by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Genomics for Agricultural Innovation, QTL-GMB0005).

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**Table 2.** Comparison between the BphA Subunits of RHA1 and KF707

<table>
<thead>
<tr>
<th>Gene product</th>
<th>No. of residues</th>
<th>Identity between products of RHA1 and KF707 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large subunit</td>
<td>460 458</td>
<td>50.6</td>
</tr>
<tr>
<td>Small subunit</td>
<td>187 213</td>
<td>57.9</td>
</tr>
<tr>
<td>Ferredoxin subunit</td>
<td>107 109</td>
<td>53.3</td>
</tr>
<tr>
<td>Ferredoxin reductase subunit</td>
<td>413 408</td>
<td>52.1</td>
</tr>
</tbody>
</table>

**Table 3.** Nos. of AGA, AGG, ATA, CTA, CCC, and GGA Codons in bphaABC Genes of RHA1 and bphaA3A4 Genes of KF707

<table>
<thead>
<tr>
<th>Codon</th>
<th>bphaAa</th>
<th>bphaAb</th>
<th>bphaAc</th>
<th>bphaAd</th>
<th>bphaB1</th>
<th>bphaC1</th>
<th>bphaA3</th>
<th>bphaA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA-Arg</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>AGG-Arg</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>GGA-Gly</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ATA-Ile</td>
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<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>CTA-Leu</td>
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<td>1</td>
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<td>0</td>
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</tr>
<tr>
<td>CCC-Pro</td>
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<td>1</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>5</td>
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References