Characterization of the Upper Pathway Genes for Fluorene Metabolism in *Terrabacter* sp. Strain DBF63

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Genes involved in the degradation of fluorene to phthalate were characterized in the fluorene degrader *Terrabacter* sp. strain DBF63. The initial attack on both fluorene and 9-fluorenol was catalyzed by DbfA to yield 9-fluorenol and 1,1a-dihydroxy-1-hydro-9-fluorenone, respectively. The FlnB protein exhibited activities against both 9-fluorenol and 1,1a-dihydroxy-1-hydro-9-fluorenone to produce 9-fluorenone and 2'-carboxy-2,3-dihydroxybiphenyl, respectively. FlnD is a heteromeric protein encoded by flnDI and ORF16, being a member of the class III two-subunit extradiol dioxygenase. FlnE was identified as a serine hydrolase for the metabolism products that yield phthalate.

Polycyclic aromatic hydrocarbons (PAHs) have toxic, mutagenic, and carcinogenic properties, and therefore, their long persistence in the environment is of major concern. Among the PAHs, fluorene (FN) has been classified as one of 16 priority pollutants by the U.S. Environmental Protection Agency because of its toxicity to organisms and abundance in the environment (18). For the purpose of bioremediation, the bacterial degradation of FN has been studied for many years (2, 3, 8, 9, 10, 11, 19, 26), and three major degradative pathways have been proposed (Fig. 1). Two of these pathways are initiated by a dioxygenation at the 1,2 (Fig. 1A) or 3,4 (Fig. 1B) position. The third route is initiated by monooxygenation at the C-9 position to give 9-fluorenol (VI), which is then dehydrogenated to 9-fluorenone (VII). Angular dioxygenation of 9-fluorenone then leads to 1,1a-dihydroxy-1-hydro-9-fluorenone (DHF, VIII) (6, 7, 23). Dehydrogenation of DHF produces a 2’-carboxy-2,3-dihydroxybiphenyl (CDB, IX; this metabolite is spontaneously transformed to 8-hydroxy-3,4-benzocoumarin [HBC, XI]), which is metabolized by the reactions analogous to those of biphenyl degradation, leading to the formation of phthalate (XI) (2, 9, 26). However, although many FN-utilizing bacteria have been isolated and characterized, very little is known about the specific enzymes involved in the catabolism of FN and especially the genes coding for these enzymes.

*Terrabacter* sp. strain DBF63 was originally isolated from a soil sample as a bacterium capable of utilizing dibenzofuran (DF) and FN as the sole source of carbon and energy (17, 19). Recently, novel terminal oxygenase genes of angular dioxygenase (dbfA1 and dbfA2), whose products can catalyze the angular dioxygenation of DF with the complementation of the nonspecific electron transport system of *Escherichia coli*, were isolated by a PCR-based strategy (16). The facts that *pht* genes for phthalate to protocatechuate cluster together with the *dbfA1A2* genes (12) and that both the *dbfA1A2* and *pht* genes were expressed in FN-grown DBF63 cells (20) suggested that the whole island could be involved in FN metabolism. The present report is the first on the characterization of the upper metabolic pathway genes for FN.

Nucleotide sequencing of the downstream region of *dbfA1A2* genes. We determined the nucleotide sequences of the 6,525-bp BamHI region (Fig. 2, shaded box) as described previously (12, 16), and seven ORFs (open reading frames; ORF3, ORF5, ORF15, ORF16, ORF17, ORF18, and ORF19) were found in that DNA region (Fig. 2). The deduced amino acid sequences of the respective ORFs were compared with those registered in the databases (Table 1).

Experiments in which FN was biotransformed to phthalate by a recombinant *E. coli* clone. pDF104 harboring *ORF4-dbfa1A2* and pDFS110 harboring *ORF16-ORF17* under the control of the lac promoter were constructed (Fig. 2; detailed information on the plasmid construction in this study is available on request), both plasmids were introduced into the same *E. coli* JM109 cells, and an FN biotransformation experiment was performed with the recombinant *E. coli* cells. Biotransformation experiments with *E. coli* clones and gas chromatography-mass spectrometry (GC-MS) analysis of the extracts from reaction mixtures were performed as described previously (12, 16). GC-MS analysis of the methylated extract revealed the presence of a product with a molecular ion M+ at *m/z* 194 [6] and fragmentation ions at *m/z* 163 [100], *m/z* 135 [5], *m/z* 133 [7], *m/z* 104 [6], *m/z* 92 [11], *m/z* 77 [38], and *m/z* 76 [24] (the retention time [RT] was 6.0 min; relative intensities expressed as percentages are given in brackets) (Fig. 2). The mass spectral properties were identical to those of authentic dimethylated phthalate.

On the other hand, we also constructed pDF109, in which a
Boring both pDF109 and pDFS110. GC-MS analysis of the

IX, CDB; X, HBC; XI, phthalic acid; XII, 1,2-dihydro-1,2-dihydroxy-

uorenone; VIII, DHF; IX shown have not been characterized yet. Compound designations: I, 

IX was indirectly identified because of its spontaneous transformation

teria. Metabolites I to XI were reported previously (2, 9). Compound

3.8-kb SacI-NotI fragment harboring ORF3 (flnE) and ORF15

(flndI) was deleted from pDF104 (Fig. 2). We then performed

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FIG. 1. Proposed pathways of fluorene catabolism by aerobic bac-
teria. Metabolites I to XI were reported previously (2, 9). Compound

IX was indirectly identified because of its spontaneous transformation
to compound X (20). The chemical structures of the other metabolites
shown have not been characterized yet. Compound designations: I, 2-indanone; II, 3-isochromanone; III, 1-indanone; IV, 3,4-dihydrocou-
amarin; V, salicylic acid; VI, 9-fluoreno; VII, 9-fluorenone; VIII, DHF; IX, CDB; X, HBC; XI, phthalic acid; XII, 1,2-dihydro-1,2-dihydroxy-
9-fluorenone. The corresponding enzymes in Terrabacter sp. strain

DBF63 are as follows: DbaA, 9-fluorenone 1,1a-dioxygenase (fluorene 
9-monoxygenase, DF 4,4a-dioxygenase); FlnB, DHF dehydrogenase;

FlnD, CDB 1,2-dioxygenase; FlnE, 2-hydroxy-6-oxo-6-(2’-carboxyphenyl)-hexa-2,4-dienoic hydrolase.

3.8-kb SacI-NotI fragment harboring ORF3 (flnE) and ORF15

(flndI) was deleted from pDF104 (Fig. 2). We then performed

an experiment to biotransform FN by using E. coli cells har-

boring both pDF109 and pDFS110. GC-MS analysis of the

methylated extract revealed the presence of another product
with a molecular ion M+ at m/z 226 [100] and fragmentation ions at m/z 211 [2], m/z 197 [4], m/z 195 [2], m/z 183 [44], m/z 168 [3], m/z 155 [17], m/z 139 [9], m/z 127 [34], m/z 113 [3], m/z 101 [9], m/z 77 [18], and m/z 63 [18] (the RT was 11.5 min), consistent with the notion that these are the methyl derivatives of HBC previously reported (9) (Fig. 2). We did not detect phthalate in this reaction mixture. Considering that CDB is spontaneously transformed to HBC, these results indicate that

the genes located downstream from ORF5 are the FN meta-
bolic genes in strain DBF63 and that ORF3 (flnE) and ORF15

(flndI) are involved in the degradation of CDB to phthalate.

9-Fluorenel dehydrogenase. We previously demonstrated that DbfA1A2 could catalyze the monooxygenation of FN to produce 9-fluorenel with ferredoxin and reductase from E. coli (16). In this study, since two genes encoding putative short-
chain dehydrogenase-reductase (SDR) family proteins, ORF4 (designated flnB) and ORF17 (designated flnC), were found within the FN degradative gene cluster (Fig. 2), we determined the 9-fluorenel-dependent reduction of NAD+ to assess the

9-fluorenel dehydrogenase activities of these enzymes. The 1,074- and 759-bp DNA fragments containing ORF4 (flnB) and ORF17 (flnC), respectively, were amplified by PCR with the forward primers containing an efficient Shine-Dalgarno (SD) sequence for E. coli (24) (detailed information on the primer sequences in this study is available on request). The PCR products were cloned with the pT7Blue (R) vector (Nov-

vagen), and the nucleotide sequences of PCR products were

confirmed by sequencing. The clones were cut at both the

HindIII and EcoRI sites (derived from the primer), and then

the fragment was cloned between the HindIII and EcoRI sites of pBluescriptII KS(—) to give pDF106 (containing flnB) and

pDF108 (containing flnC). Preparation of E. coli cell extracts

worked as described previously (21), and the 9-fluore-
nol dehydrogenase activities were measured as described by

Trenz et al. (26). As a control, E. coli JM109 harboring pBlue-
scriptII KS(B) was used. Compared to the NAD+ reduction by the cell extracts of E. coli JM109 harboring pBluescriptII KS(B) (4.22 ± 0.912 U mg of protein−1; the nonspecific dehydrogenase derived from E. coli acted on 9-fluorenel to produce 9-fluorenol), the cell extracts of E. coli harboring pDF106 (flnB) showed threefold higher activity with 9-fluore-
nol (12.8 ± 2.00 U mg of protein−1). By contrast, the cell

extracts of E. coli harboring pDF108 (flnC) showed the same

level of 9-fluorenel dehydrogenase activity (4.64 ± 0.402 U mg

of protein−1) as that harboring pBluescriptII KS(—). This ten-

dency was observed in several independent experiments. These

results indicated that FlnB catalyzed the dehydrogenation of

9-fluorenel to produce 9-fluorenol.

Angular dioxygenase for 9-fluorenel. We previously re-

ported that the angular dioxygenase component, DbfA1A2, 

converted 9-fluorenel to a cis-diol compound with the fer-

reoxin and reductase component from E. coli (16). However,

the cis-diol compound has not been determined to be DHF

(Fig. 1, compound VIII) or 1,2-dihydro-1,2-dihydroxy-9-

fluorenone (XII) (16, 19) because the

coli clone harboring the

dbfA1A2 genes did not yield enough of the compound for

nuclear magnetic resonance (NMR) analysis. When the resting

cells of E. coli harboring pDF32 (dbfA1A2) (100-ml culture)

were incubated with 9-fluorenel (0.1%, wt/vol), the accumu-

lation of a small amount of the cis-diol compound was ob-

served by reverse-phase high-pressure liquid chromatography

(HPLC) at an RT of 6.2 min (the HPLC conditions are de-

scribed below). By contrast, the resting cell reaction with E.

coli harboring both pDF32 (dbfA1A2) and pDFS112 (ORF16),

in which the 1.48-kb SmaI fragment containing flnc was dele-

ted from pDF110 (Fig. 2), gave a peak area of the cis-
diol compound four to five times that obtained with E. coli harboring

only pDF32 (dbfA1A2) (data not shown).
With *E. coli* containing both pDF32 and pDFS112, we tried to isolate the cis-diol compound. The recombinant *E. coli* cells were harvested by centrifugation of 8 liters of a 12-h culture. Collected cells were washed twice with 50 mM sodium-potassium phosphate buffer (pH 7.0) and resuspended in 2 liters of the same buffer. The washed cell suspension with 9-fluorenone (1 g/liter) was incubated for 18 h on a rotary shaker at 30°C (120 rpm). After incubation, the cells and the remaining 9-fluorenone were removed by centrifugation. First, rough purification of the compound by silica gel column chromatography was performed as described by Selifonov et al. (23), with some modifications. The residue was redissolved in an appropriate volume of methanol and further purified by HPLC. HPLC analysis was performed with a Waters 600E chromatograph (Waters Co., Milford, Mass.) equipped with an octadecyl silane 4253-D column (10 by 250 mm; Senshu Scientific Co., Ltd., Tokyo, Japan). The mobile phase was water-methanol (40:60, vol/vol), and the flow rate was 3 ml min⁻¹. The elutions were monitored at 254 and 275 nm by a Waters 996 photodiode array detector and collected every minute. The solvent of fractions collected at an RT of 6 to 7 min was evaporated under reduced pressure at 25°C. Finally, 3.1 mg of the yellowish solid was obtained from 8 liters of the *E. coli* culture. As this compound was chromatographically pure (thin-layer chromatography, HPLC) and showed the presence of a major UV absorption maximum (314 nm [in methanol]), the compound was subjected to NMR analysis. For ¹H-NMR analysis, the purified DHF sample was dissolved in an appropriate volume of CDCl₃. The ¹H-NMR spectrum of DHF was recorded with a JNM-A500 spectrometer (JEOL, Ltd., Tokyo, Japan) operating at 500 MHz with tetramethyl silane as the internal standard. A NAORAC H5X/FG probe was used. The 500-MHz ¹H-NMR spectrum of the compound showed the proton signals in the aromatic, olefinic, sp³-CH(OH) regions to be as follows (parts per million): 4.708 (ddd, H-1), 5.914 (dd, H-2), 6.189 (ddd, H-3), 6.570 (d, H-4), 7.433 (ddd, H-7), 7.673 (dd, H-6), 7.707 (dd, H-5), and 7.813 (dd, H-8). These ¹H-NMR proton signals were identical to those of DHF previously published by Selifonov et al. (23). GC-MS analysis of the TMS derivative of the compound revealed a molecular ion M⁺ at m/z 358 [21] and fragmentation ions at m/z 343 [4], m/z 270 [11], m/z 253 [30], m/z 223 [2], m/z 180 [2], m/z 147 [34], and m/z 73 [100] (the RT was 10.5 min). This mass spectral pattern was almost identical to that of the cis-diol compound previously reported by our group (16). From these results, we concluded that the cis-diol compound previously reported is DHF and...
TABLE 1. Homology search analyses of the fla genes and the neighboring region

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<th>Gene*</th>
<th>Probable function or product</th>
<th>Mol wt</th>
<th>Homologous protein</th>
<th>Source</th>
<th>Identity (%)</th>
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* Accession number AB095015.
† Homology searching was performed with the SwissProt and GenBank protein databases or the DDBJ, EMBL, and GenBank nucleotide sequence databases with the BLAST program (version 2.0.10) (1). Multiple sequence alignment was carried out with Clustal W version 1.6 (25).
Ω Hypothetical protein.
‡ The C-terminal region of the ORF3 product (FlnE) was longer than that of any other meta-cleavage product hydrolase by approximately 30 amino acids.
§ Accession number in the SwissProt and GenBank protein databases or the DDBJ, EMBL, and GenBank nucleotide sequence databases.
∥ The ORF product was compared with only the 90-amino-acid-long N-terminal portion of the ORF16 product.
* The ORF product was compared with only the 100-amino-acid-long C-terminal protein of the ORF16 product. This region was Revealed to be longer than other related [3Fe-4S] types of ferredoxin by approximately 30 amino acids.
| Accession number AB095015.

that DbfA1A2 can catalyze the angular dioxygenation of 9-fluorenone.

**DHF dehydrogenase.** Previously, Trenz et al. (26) indicated that the NAD⁺-dependent dehydrogenase with four identical 40-kDa subunits catalyzed the conversion of DHF to CDB; however, the gene sequence of this enzyme has not been reported. In order to elucidate whether flaB or flaC takes part in the conversion of DHF to CDB, we performed experiments in which purified DHF (approximately 0.7 mM final concentration) was biotransformed by E. coli JM109 cells harboring pBluescriptII KS(−) (control), pDF106 (flaB), or pDF108 (flaC), respectively. By GC-MS analysis, we identified HBC as a metabolite in E. coli cells harboring flaB. On the other hand, biotransformation experiments with E. coli JM109 cells harboring pBluescriptII KS(−) or pDF108 (flaC) did not produce HBC. As HBC was usually isolated as a lactone form of CDB (9, 26), this result indicates that FlnB is a DHF dehydrogenase. The deduced amino acid sequences of FlnB showed homology not to cis-dihydriodiol dehydrogenases involved in aromatic compound degradation but to the group of SDR family proteins (Table 1). Involvement of SDR-type enzymes in the metabolic steps, instead of the ordinary cis-dihydriodiol dehydrogenase, may be one of the features of bacterial 9-fluorenone degradation via angular dioxygenation. However, the C-terminal region of FlnB was approximately 70 amino acids longer than those of other, related, SDR-like enzymes (data not shown). By contrast, the role of another SDR family protein, FlnC, is still unknown.

**Novel two-subunit extradiol dioxygenase.** The 932-, 1,495-, and 1,201-bp DNA fragments containing ORF15 (flnD1), both ORF15 and ORF16, and both ORF15 and the 5’-terminal 273 bp of ORF16, respectively, were amplified by PCR with the forward primers containing an efficient SD sequence for E. coli (24). The DNA fragments were cut at both the HindIII and EcoRI sites (derived from the primer) and then cloned between the HindIII and EcoRI sites of pUC119 to give pDF501 (containing flnD1), pDF502 (containing flnD1 and ORF16) and pDF503 (containing flnD1 and the 5’-terminal 273-bp por-
First, we constructed only pDF501 and performed preliminary biotransformation experiments. However, *E. coli* JM109 harboring pDF501 could not transform 2,3-dihydroxybiphenyl (2,3-DHB) to 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (6-phenyl-HODA), and the reaction mixture did not show a yellow color (data not shown). As several enzymes belonging to class III of the extradiol dioxygenases are known to consist of two proteins (large and small subunits) (5, 15), we examined whether ORF15 is one of the subunits of extradiol dioxygenase. When an experiment in which 2,3-DHB was biotransformed was performed with pDF502 containing ORF15-ORF16, the reaction mixture exhibited a yellow color derived from 6-phenyl-HODA, indicating that the ORF16 product is necessary for the activity. However, we did not understand how the active extradiol dioxygenase is constituted, because ORF16 has a modular organization of an unknown ORF and a ferredoxin-encoding gene. Detailed comparison of the DNA region containing ORF15-ORF16 from *Terrabacter* sp. strain DBF63 with that containing *edi4-ORF6-ORF7* from *Rhodococcus* sp. strain YK2 (14; Fig. 3) revealed that although the sequence identity was more than 92%, they had different genetic organizations of the ORF16 portion (Fig. 3). The insertion of a single C base, at position 266 from the first base of the ORF16 start codon, caused the frame shift, and ORF16 of strain DBF63 was predicted to be formed as a fusion of two genes highly homologous to ORF6 and ORF7 from strain YK2 (Fig. 3). Hence, we hypothesized that the unknown N-terminal 90-amino-acid sequence of the ORF16 product is involved in the enzyme activity. We constructed pDF503 (Fig. 3) by PCR with the primer sets for amplifying both ORF15 and the 5′-terminal 273-bp region of ORF16, in whose reverse primer designation the single C base at position 266 is deleted and as a result there is a stop codon (Fig. 3). By a biotransformation experiment with *E. coli* harboring pDF503, we also confirmed the extradiol dioxygenase activity for 2,3-DHB by the yellow color of 6-phenyl-HODA. The extradiol dioxygenase activities for 2,3-DHB and 3-methylcatechol in the cell extracts of *E. coli* were assayed as described by Iwata et al. (15). Cell extracts from *E. coli* harboring pDF502 and pDF503 showed the extradiol dioxygenase activity for 2,3-DHB, and their enzymatic activities for 2,3-DHB were 208 ± 7.81 and 365 ± 4.36 U μg of
protein<sup>-1</sup>, respectively. Compared to those activities, almost negligible activities were observed with both extracts from E. coli harboring pUC119 (5.76 ± 1.53 U μg of protein<sup>-1</sup>) and pDFS501 (2.67 ± 1.15 U μg of protein<sup>-1</sup>). In addition, the activities of these extracts for 3-methylcatechol were the same as those of the control (E. coli harboring pUC119, 4.33 ± 1.53 U μg of protein<sup>-1</sup>, E. coli harboring pDFS501, 4.67 ± 2.89 U μg of protein<sup>-1</sup>, E. coli harboring pDFS502, 2.57 ± 0.577 U μg of protein<sup>-1</sup>). These results demonstrated that the ORF15 product and the N-terminal portion of the modular protein (ORF16 product) consist of a novel two-subunit extradiol dioxygenase. However, the enzymatic activity of the extract from E. coli harboring pDFS502 was only 57% of that of the extract from E. coli harboring pDFS503, probably owing to the unusual three-dimensional conformation of the ORF16 product. We also tried several methods to prepare CDB as a substrate for FlnD, e.g., accumulation of CDB by resting cells of FN-grown strain DBF63 with the extradiol dioxygenase inhibitor 3-chlorocatechol, but until now, all failed (data not shown). However, FlnD can catalyze the meta cleavage of CDB as described in biotransformation experiments. We designated ORF15 flnD1, encoding a large subunit of a two-subunit class III extradiol dioxygenase.

Since we were interested in the modular nature of ORF16, we sought to investigate whether this allele exists in other strains belonging to the genus Terrabacter. PCR experiments were performed with total DNAs of another two DF-degrading Terrabacter strains, DFA1 and DFA10, isolated from activated sludge (our unpublished data; the 16S rRNA gene sequences of strains DFA1 and DFA10 were 100 and 99% identical to that of strain DBF63, respectively). We can successfully amplify the whole DNA region of ORF16 homologues, and sequence analysis revealed that ORF16 homologues from strains DFA1 and DFA10 were 100 and 99% identical to the ORF16 sequence of strain DBF63, respectively (data not shown). Thus, in our survey, this modular nature of ORF16 was found in not only strain DBF63 but also other Terrabacter strains. It will be interesting to investigate how ORF16 really works in the FN or DF degradation of strain DBF63.

**meta-cleavage product hydrolase.** We investigated by E. coli biotransformation experiments whether the ORF3 product possesses hydrolase activities for meta-cleavage products of DHB or 2'-substituted DHB (2'-aminobiphenyl-2,3-diol). 2'-Aminobiphenyl-2,3-diol was prepared as described previously (15). The 1,018-bp DNA fragment containing ORF3 (flnE) was amplified with the forward primer containing an efficient SD sequence for E. coli (24). The DNA fragments were cut at both the HindIII and EcoRI sites (derived from the primer) and then cloned between the HindIII and EcoRI sites of pUC119 to give pDF27, and those of pSTV29 were modified to give pDFS27 (Fig. 2). E. coli JM109 was transformed with both plasmids pDFS27 and pDFS502. As a control, E. coli JM109 harboring both plasmids pDFS502 and pSTV29 was used. Transformation experiments and GC-MS analysis showed that only E. coli cells with both pDFS502 and pDFS27 could transform DHB to benzoate. Also, 2'-aminobiphenyl-2,3-diol was transformed to anthranilate (data not shown).

Enzymatic hydrolase activity of the ORF3 product was assayed as described by Nojiri et al. (21). Since the half-life of 6-(2'-aminophenyl)-HODA (meta-cleavage product of 2'-aminobiphenyl-2,3-diol) was too short (21), we could not measure the decrease in its absorbance. The enzymatic activities of the extracts from E. coli harboring pDF27 for 2-hydroxy-6-oxohepta-2,4-dienoic acid (6-methyl-HODA) and 6-phenyl-HODA were 0.391 ± 0.0512 and 3.73 ± 0.101 U μg of protein<sup>-1</sup>, respectively. No hydrolase activities were observed in the control experiments (0.0474 ± 0.0205 U μg of protein<sup>-1</sup>). After hydrolase activity measurement, the products in the reaction mixture were extracted with ethyl acetate and analyzed by GC-MS. As a result, benzoic acid was detected only from the reaction mixture with E. coli harboring pDF27 with 6-phenyl-HODA. This indicates that ORF3 encodes the meta-cleavage product hydrolase for 6-(2'-substituted phenyl)-HODA, and thus we designated ORF3 flnE.

The meta-cleavage product hydrolases belong to the α/β-hydrolase family, and most of them are classified into two major groups, I and III, proposed by Hernández et al. (13). However, FlnE did not form a cluster with the group I proteins including BphD but formed a branch with the group IV proteins including OhpC and CmtE (data not shown). OhpC and CmtE are involved in the degradation of monocyclic compounds with carboxyl group substitution, i.e., 3-(2-hydroxybiphenyl)propionate (22) and p-cumate (4), respectively. There may be some evolutionary relationship among these enzymes.

In conclusion, we characterized the dbf-fln genes of Terrabacter sp. strain DBF63, which are involved in the degradation of FN to phthalate (flnB-flnA1-flnA2-flnE-flnD1-ORF16), and several genes were found to be unusual in their sequence length, low homology to the known proteins, modular nature, and so on. This new information adds some knowledge to the genetics of bacterial PAH degradation, because although the genes involved in the upper pathway of naphthalene, phenanthrene, and anthracene metabolism have been studied extensively in recent decades, there have been no reports about the complete upper metabolic pathway genes for the other 16 priority PAHs. For better understanding of the pathway and mechanism of FN catabolism by strain DBF63, biochemical studies with the purified enzymes in each catalytic step are under way.

**Nucleotide sequence accession numbers.** The nucleotide sequence data reported here have been submitted to the DDBJ, EMBL, and GenBank nucleotide sequence databases and assigned accession number AB095015.

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