J. Basic Microbiol. **42** (2002) 6, 434–443

([1]Department of Environmental Education, Sunchon National Universitu, Suncheon 540-742, Korea; [2]Research Institute for Basic Sciences, Cheju National University, Jeju 690-756, Korea; [3]Proteome Analysis Team, Korea Basic Science Institute, Daejon 305-333, Korea; and [4]Department of Life Science, Soonchunhyang University, Asan, Chung-Nam 336-600, Korea)

Evaluation of carbazole degradation by *Pseudomonas rhodesiae* strain KK1 isolated from soil contaminated with coal tar

BYOUNG-JUN YOON[2], DONG-HEON LEE[2], YOON-SUK KANG[2], DUCK-CHUL OH[2], SEUNG-IL KIM[3], KYE-HEON OH[4] and HYUNG-YEEL KAHNG[1, 2]*

(Received 12 February 2002/Accepted 26 April 2002)

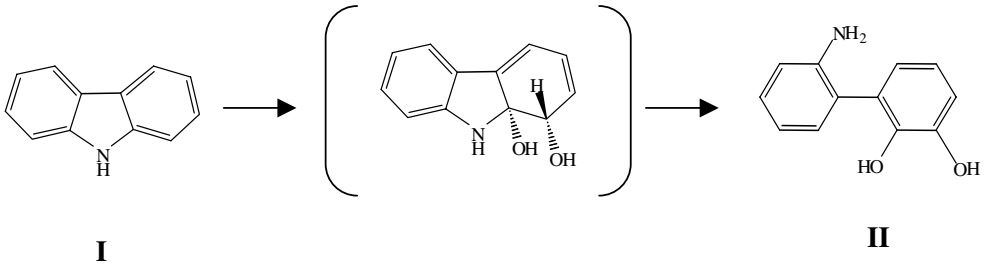
In this study, strain KK1 isolated from coal tar-contaminated soil was found to be able to mineralize carbazole as a sole source of carbon by radiorespirometric analysis. KK1 cells pregrown on phenanthrene were able to mineralize carbazole much more rapidly than cells pregrown on naphthalene, suggesting a possible close linkage between the pathways for carbazole and phenanthrene catabolism. Also, Rieske-type iron sulfur center sequence of dioxygenase from KK1 was analyzed to evaluate carbazole catabolism by KK1. A gene cloned out from KK1 using a universal dioxygenase primer set was found a dioxygenase for initial catabolism of carbazole based on deduced amino acid sequences. Northern hybridization using the putative carbazole dixoygenase gene fragment as a probe provided the information that catabolism of carbazole might be greatly activated in phenanthrene-grown cells. Analysis of PLFAs extracted from KK1 cells exposed to carbazole revealed that lipids 10:0 3OH, 17:0 cyclo, and 18:0 were representatives produced or significantly increased in response to carbazole. Strain KK1 was identified as *Pseudomonas* species with 94% confidence when BIOLOG system was applied, as *Pseudomonas* sp. with over 90% confidence by total cellular compositions of fatty acid, and as *Pseudomonas rhodesiae* with 99% confidence by 16S rRNA sequence. Accordingly, strain KK1 was identified as *Pseudomonas rhodesiae* based on combination of the data, and designated *Pseudomonas rhodesiae* KK1. The phylogenetic tree based on 16S rRNA suggested that strain KK1 was far away in the phylogenetic distance from the strains that can degrade carbazole.

The global pollution of soils, rivers, lakes, marshes, etc by polycyclic aromatic hydrocarons (PAHs) such as anthracene, chrysene, fluorene, naphthalene, phenanthrene, and pyrene has been of great concern to environmental microbiologists, because PAHs are considered serious pollutants that are hard to be degraded in the environment. However, little attention has been paid to carbazole that is a nitrogen heterocyclic aromatic compound, despite its toxicity (SANTODONATO and HOWARD 1981, WEST *et al.* 1986). Carbazole and its derivatives also have been used for the production of dyes, medicines, and plastics (OUCHIYAMA *et al.* 1993), and detected in diverse environments like atomospheric samples (SANTODONATO and HOWARD 1981), river sediment (WEST *et al.* 1986), and groundwater (PEREIRA *et al.* 1987).

Microorganisms play a primary role in the removal of many types of chemical pollutants including carbazole from the environment. Studies on biodegradation of carbazole by microorganisms such as *Pseudomonas*, *Ralstonia,* and *Sphingomonas* revealed the metabolic pathway for initial carbazole methabolism as shown in Fig. 1 (OUCHIYAMA *et al.* 1993, GIEG *et al.* 1996, SATO *et al.* 1997, SHEPERD and LLOYD-JONES 1998, OUCHIYAMA *et al.* 1998, KASUGA *et al.* 2001, NOJIRI *et al.* 2001).

* Corresponding author: Prof. H.-Y. KAHNG; e-mail: kahng@sunchon.ac.kr

 0233-111X/02/0612-0434 $ 17.50+.50/0



I **II**

Fig. 1
Proposed initial reaction for bacterial catabolism of carbazole by an angular dioxygenase. I, carbazole; II, 2'-aminobiphenyl-2,3-diol

Though similar enzymes for the initial dioxygenation of carbazole have been identified in a few microorganisms, the iron-sulfur center has two peculiar amino acid sequence motifs surrounding a region of amino acids whose sequence varies from enzyme to enzyme and the mechanism for carbazole degradation by microorganisms was not clear yet. In an effort to obtain more information about the bacterial degradation of carbazole, strain KK1 isolated from PAHs-contaminated soil was analyzed for carbazole-degradation potential using a dioxygenase universal primer-based PCR technique and radiorespirometry in this study. Physiological and genetic characterization of strain KK1 is also described.

Materials and methods

Carbazole extraction from soil sample: The coal tar-contaminated soil used in this study was collected from the depth of 0–2 m below surface at a former manufactured gas plant site in New Jersey. The soil was classified as loamy sand, consisting of 78% sand, 11% silt, and 11% clay. The soil was transferred to a 50 ml Teflon centrifuge tube and centrifuged at 18,600 × *g* for 15 min. After removing the supernatant, 10 ml each of dichloromethane and acetone were added to the soil and the soil-solvent suspension was shaken (200 rpm) for 48 hr at 30 °C for the extraction of PAHs including carbazole. The tube was then centrifuged at 18,600 × *g* for 15 min and the solvent mixture was transferred to a 50-ml test tube. After removing excess water (upper layer; ca. 2 ml) by pipetting, 4 g of anhydrous sodium sulfate were mixed with the PAHs-containing solvent to remove residual water completely from solvent. The concentration of PAHs in the water layer was less than the detection limit of the analytical procedure used in this study. The extract was then concentrated to 1 to 2 mL using an evaporator (Buchi Rotavapor; BUCHLER Instruments Inc., Fort Lee, NJ) for further analysis. By this procedure, PAHs in the 0–2 m layer were recovered. The extract was passed through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter to remove any particulate present and analyzed by a gas chromatography (GC) equipped with a flame ionization detector (VARIAN Star 3500; VARIAN Chromatography Systems, Walnut, CA). The GC was installed with a Rtx-5 silica column cross bounded with 5% diphenyl and 95% dimethylpolysiloxane (30 m × 0.53 mm interior diameter; RESTEK Corporation, Bellefonte, PA). The oven temperature was programmed at 40 °C for 6 min, followed by a linear increase of 10 °C per minute to 300 °C, and then the temperature was held for 15 min. Injector and detector temperatures were maintained at 300 °C. Two microliters of the extract were injected and nitrogen was used as a carrier gas.

Enrichment culture: PAHs-degrading bacteria were isolated from PAH-contaminated soil at the MGP (Manufactured Gas Plant) site through enrichment culture technique. Five-gram samples of MGP soil were incubated with a mixture of PAHs in 100 ml of inorganic salts solution (0.10 g $CaCl_2 \cdot 2\,H_2O$, 0.01 g $FeCl_3$, 0.10 g $MgSO_4 \cdot 7\,H_2O$, 0.10 g NH_4NO_3, 0.20 g KH_2PO_4, and 0.80 g K_2HPO_4/l of dH_2O; pH 7.0) at 30 °C for two weeks. PAHs including anthracene, benzo[a]pyrene, chrysene, phenanthrene, and pyrene were dissolved in methanol (10 mg/ml for the first three compounds and 1 mg/ml for the others), and the PAHs were used as substrates for the enrichment.

After two weeks of incubation, 10 ml of the supernatant were collected and incubated for two more weeks as described above. By this procedure, a consortium capable of degrading a variety of PAHs was obtained and used for isolation of pure bacterial strains that were able to degrade PAHs. Serial dilutions of the enrichment consortium (10^{-1} to 10^{-3}) were transferred to solid PAH media containing anthracene (10 μg/ml), naphthalene (100 μg/ml), phenanthrene (100 μg/ml), and then the fast-growing colonies of PAH-utilizing microorganisms were screened. Strain KK1 was selected from the colonies and tested for carbazole degradation potential in this study.

Physiological and biochemical tests for strain KK1: The pattern of carbon source utilization by strain KK1 was determined using the GN2 MicroPlate™ (BIOLOG, Hayward, CA). A single KK1 colony grown on a TSA plate was streaked onto BUG (BIOLOG Universal Growth) agar medium containing 5% sheep blood and incubated overnight at 30 °C. Cells were suspended in normal saline (0.15% NaCl) and 150 μm of the suspension was inoculated into the GN2 MicroPlate™. After 24 hr of incubation the utilization pattern was read using the Biolog automated Micro-Station™ instrument.

Analysis of fatty acids methyl ester (FAME): Phospholipid ester-linked fatty acids in the strain KK1 were analyzed in the form of fatty acids methyl ester (FAME) using MIDI system (Microbial Insights, Inc., Newark, DE). Extraction of fatty acids has been done according to the instruction provided by the manufacturer with some modification. Briefly, cells harvested following the 24 hr of growth on TSA (Tryptic Soy Broth Agar) were heated to 100 °C with NaOH-methanol to saponify cellular lipids and the released fatty acids were methylated by heating with HCl-methanol at 80 °C. FAMEs were solvent-extracted, and analyzed by gas chromatography with flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). FAMEs were identified by comparing their retention times and mass spectra with those of authentic standards provided by the MIDI database. To examine the fatty acids shifted in response to carbazole, cells grown on TSB were collected and washed twice in potassium phosphate buffer (pH 7.0). The washed cells were incubated in mineral salts media containing 5 mg/ml of carbazole. After 24 hr of incubation at 30 °C, changes in the composition of FAMEs were analyzed as described above.

Determination of carbazole mineralization using radiorespirometry: Catabolic potential of strain KK1 for carbazole was determined by measuring the radioactivity of $^{14}CO_2$ evolved from mineralization of [^{14}C]-labeled carbazole. Cells were grown in TSB or carbazole to late exponential phase, harvested by membrane filtration, and washed twice with mineral salts solution (0.10 g $CaCl_2 \cdot 2\,H_2O$, 0.01 g $FeCl_3$, 0.10 g $MgSO_4 \cdot 7\,H_2O$, 0.10 g NH_4NO_3, 0.20 g KH_2PO_4, and 0.80 g K_2HPO_4/l of dH_2O; pH 7.0). Approx 10^5 cells was then inoculated to a mineral salts solution containing radiolabeled carbazole (10^5 dpm each; [7–^{14}C]; specific activity, 28.2 mCi/mmol) as a sole carbon source and incubated at 30 °C for 10 days with shaking (150 rpm). Carbazole was purchased from SIGMA Chemical Company (St. Louis, MO, U.S.A.). The 50-ml flask used for mineralization experiment was sealed with a Teflon-wrapped silicone stopper through which was placed an 18-gauge hypodermic needle and a 16-gauge steel cannula. From the cannula was suspended a small vial containing 1.0 ml of 0.5 N NaOH to trap $^{14}CO_2$ released by mineralization. The flask was then incubated at 30 °C with shaking (150 rpm) and $^{14}CO_2$ formation was determined for 10 days by periodically removing the NaOH and replacing it with fresh solution. The radioactivity was measured by a liquid scintillation counter (LS 5000 TD; BECKMAN Instruments, Inc., Fullerton, CA).

Molecular analysis of carbazole degradation potential: To detect and amplify dioxygenase genes from the total genomic DNA of KK1, we used degenerate oligonucleotide primers that were designed based on the conserved RIESKE iron-sulfur motif of dioxygenases found in many bacterial species capable of degrading neutral aromatic hydrocarbons (CIGOLINI 2000). PCR amplification of dioxygenase gene fragment from the strain KK1 was performed in a total volume of 50 μl using the PERKIN ELMER reagents (PERKIN ELMER, Branchburg, NJ). PCR reactions were performed for 1 min at 95 °C, cycled 33 times (1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C), and then extended 10 min at 72 °C. The PCR products were inserted into pGEM-T vector, and transformed into *E. coli* JM109. A portion of 200 ηg of the double stranded DNA was used as a template for sequencing together with both the T7 and SP6 primers. Nucleotide sequencing was carried out using an ALF express automated sequencer. Sequence analysis was performed with Lasergene software (DNA STAR, Inc., Madison, WI) and BLAST searches of the databases.

Northern hybridization: In order to analyze the expression pattern of dioxygenases at the transcriptional level, cells were grown overnight in TSB to the mid-log phase (O.D. 600 = 0.8 ~ 1.0). At this time point cells were collected, and transferred to the medium containing either carbazole, or carbazole plus KNO_3, or glucose plus KNO_3, or phenanthrene, or phenanthrene plus KNO_3, and incubated for 6 hr. Cells were then harvested, and washed twice with the mineral salts solution. Approx 10^5 cells/ml were transferred to the medium containing 5 mg/ml of carbazole, and incubated for 12 hr at 30 °C. Total RNA was extracted from the KK1 cells using a Nucleospin RNA extraction kit according to the procedure provided by the manufacturer (CLONTECH Lab., Inc., Palo Alto, CA). DNA fragment for the probe in Northern hybridization was labeled by the random priming method provided by PROMEGA (PROMEGA, Madison, WI). Five milligrams of total RNA were used for Northern hybridization with each representative probe from four dissimilar dioxygenase groups obtained from KK1. Other procedures were performed as described previously (KAHNG *et al.* 2001).

Nucleotide sequence accession number: The 16S rDNA sequence data obtained through this study have been deposited in the GenBank data library under the accession number AY043360.

Results and discussion

Mineralization of carbazole by strain KK1

Analysis of the top layer (0–2 m below surface) at the MGP soil revealed that it was seriously contaminated with various PAHs such as anthracene, benzo(a)pyrene, chrysene, naphthalene, phenanthrene, and pyrene. Seven hundred grams of carbazole were extracted from the surface soil in this study. Strain KK1 was tested for its ability to degrade carbazole in mineral salts media under aerobic conditions. However, it could not degrade benzo[a]-pyrene, chrysene, and pyrene during an equivalent incubation. When 10^5 cells/ml grown in TSB were used, approximately 12% of carbazole was mineralized at the 10-day incubation time point (Fig. 2).

KK1 cells pregrown on naphthalene or phenanthrene were evaluated for carbazole mineralization. Interestingly, KK1 cells pregrown on phenanthrene exhibited much quicker and stronger catabolic potential for the substrate carbazole, while naphthalene-grown cells made

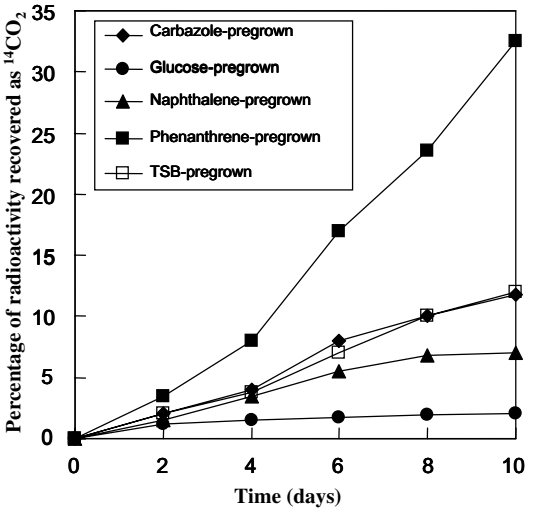


Fig. 2
Comparative analysis of carbazole mineralization rate by KK1 cells pregrown on glucose, carbazole, naphthalene, or phenanthrene

no great effect on carbazole degradation as shown in Fig. 2. Rate of carbazole mineralization increased approx 2.8-times in phenanthrene-grown cells. Chemical structure of carbazole is similar with that of phenanthrene, suggesting the possibility that expression of genes encoding carbazole dioxygenase might be stimulated by phenanthrene. It is yet unclear to interpret much higher mineralization of carbazole at 10-days incubation time point in phenanthrene-pregrown cells than carbazole-pregrown cells. However, similar data were obtained in the recently published paper on cellular responses of *Pseudomas* sp. strain KK1 to naphthalene (KAHNG 2002). Naphthalene dioxygenase could be more strongly induced and expressed by phenanthrene than naphthalene. It was suggested that 1,2-dihydroxynaphthalene from phenanthrene degradation can be further catabolized using naphthalene pathway. These results provided the possibility that expression of enzymes for lower metabolic pathway could be more stimulated by the substrate in upper pathway (KAHNG 2002). KIM *et al.* (2002) demonstrated that aniline could stimulate gene expression of catechol catabolism in *Acinetobacter lwoffii* K24. Our previous studies reported that expression of genes in the *tbu* and *tbc* operons from *Ralstonia pickettii* PKO1 and *Burkholderia* sp. strain JS150, respectively can be similarly stimulated by either benzene, toluene, ethylbenzene, or xylene (BTEX) (KAHNG *et al.* 2000, 2001). These findings suggested that similar compounds in chemical structure might make an effect on expression of the gene, as well as different chemicals experiencing similar catabolic pathway might be degraded by the genes in an operon.

PCR amplification and detection of a dioxygenase for carbazole metabolism

To investigate catabolic potential for initial catabolism of carbazole by KK1 cells we analyzed total DNA extracted from strain KK1 for the presence of dioxygenases capable of hydroxylating unactivated aromatic nuclei using a specific PCR primer set. The PCR amplification of the Rieske iron-sulfur motif region from dioxygenases found in KK1 strain revealed that strain KK1 has diverse dioxygenase genes for catabolism of neutral aromatic hydrocarbons (Data not shown). The microbial degradation of mono- and polycyclic aromatic hydrocarbons is often initiated by ring hydroxylating dioxygenase enzymes. The ring hydroxylating dioxygenases thus far identified are soluble multicomponent enzymatic systems comprised of a short electron transport chain and terminal oxygenase (CERNIGLIA *et al.* 1994, MASON and COMMACK 1992). Typically, the terminal dioxygenase is composed of two dissimilar subunits, large (or α) and small (or β) subunits. It is considered that the large subunit is the catalytic core of the enzyme and is responsible for the recognition of aromatic hydrocarbons. Every large subunit of a dioxygenase enzyme contains a REISKE-type iron-sulfur center (BATIE *et al.* 1987, GEARY *et al.* 1984, GURBIEL *et al.* 1989, MASON 1988). The iron-sulfur center has two peculiar amino acid sequence motifs surrounding a region of amino acids whose sequence varies from enzyme to enzyme. Accordingly, identifying amino acid sequences of the REISKE-type iron-sulfur motif regions in strain KK1 was of significance, considering that we have very limited information about the role of dioxygenase for degradation of PAHs. PCR process and DNA sequencing were attempted to obtain information about the dioxygenase for carbazole catabolism in strain KK1. One dioxygenase clone (5'-CRHRGATVCEHKKGKTNSFVCCYHGW) was closest to carbazole dioxygenase (Fig. 3).

The clone showed extensive similarity with CarAa from *Pseudomonas* sp. CA10 or *Sphingomonas* sp. CB3, NidA from *Mycobacterium* sp. PYR-1, PsbAb from *Rhodopseudomonas palustris* No.7, DxnA1 from *Sphingomonas* sp. RW1, and DntAc from *Burkholderia cepacia* R34. Though the putative carbazole dioxygenase shared high homology with known naphthalene dioxygenases, it was assumed that it is not responsible for naphthalene catabolism, in that another clone that shared 100% similarity in deduced amino acid sequences (5'-CRHRGKTLVSVEAGNAKGFVCCYHGW) with a naphthalene dioxygenase was found in strain KK1. Furthermore, dioxygenases for catabolism of *p*-cumate, or benzo-

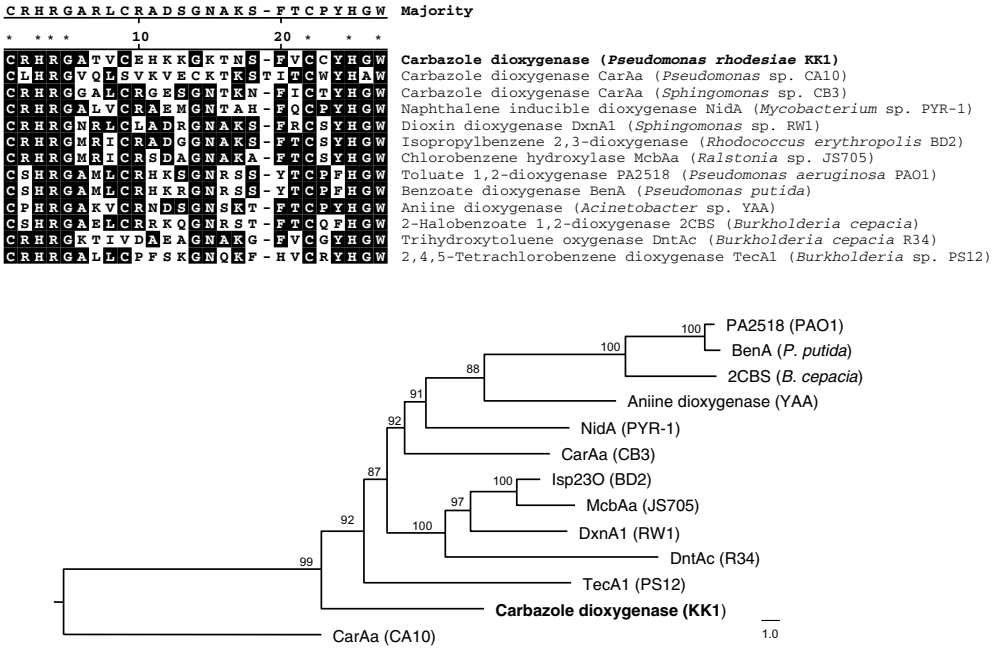
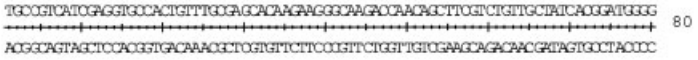


Fig. 3
Multialignment of the putative carbazole dioxygenase and the phylogenetic tree based on deduced amino acid sequences. Boostrap values at nodes of the dendrogram indicate the percentage of occurrence of the branching order in 500 boostraped trees (only values of 50 or above are shown). The bar scale represents 1 nucleotide substitution per 100 nucleotides

A. KK1-diox 24 nucleotide sequence used for a probe for northern blot



B. Northern hybridization with mRNAs extracted from cells exposed to carbazole

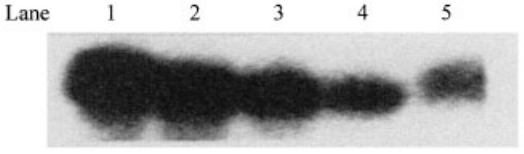


Fig. 4
Transcriptional analysis of putative carbazole dioxygenase under different conditions. KK1 cells were pregrown on phenanthrene plus KNO_3 (1ane 1), or phenanthrene (lane 2), or carbazole plus KNO_3 (lane 3), or naphthalene KNO_3 (lane 4), or glucose plus KNO_3 (lane 5). 10^5 cells collected from each medium were transferred to minimal media containing carbazole, and incubated for 24 hr, followed by extraction of total RNA. 5 μg of total RNA was used for northern hybridization

ate were found to exist separately in the strain. Substrate utilization test revealed that strain KK1 could not utilize dioxin, dihydroxy nitrotoluene, 2,4,5-trichlorophenoxy acetic acid, 1,2,4,5-tetrachlorobenzene, etc. (Data not shown). These findings suggested that the clone sequence, 5'-CRHRGATVCEHKKGKTNSFVCCYHGW might be a part of the carbazole dioxygenase in KK1.

Transcriptional expression of the putative carbazole dioxygenase

Northern hybridization was carried out using the putative carbazole dioxygenase gene fragment and mRNA isolated from KK1 cells grown on carbazole. Strongest positive signal was obtained for cells pregrown on phenanthrene, while glucose-pregrown cells gave weakest signal in response to carbazole, suggesting that the enzymes for carbazole catabolism might be activated very efficiently by phenanthrene (Fig. 4).

However, weaker signal in glucose-grown cells suggested that expression of carbazole dioxygenase might be repressed by glucose. Data obtained from northern hybridization were consistent with that found in carbazole mineralization using radiorespirometry as shown in Fig. 2. The fact that sequence 5'-CRHRGATVCEHKKGKTNSFVCCYHGW hybridized with mRNA extracted from cells grown in carbazole provided additional information that it might originate from the enzyme responsible for cabazole catabolism in strain KK1.

Shift in cellular fatty acid composition of KK1 cells for carbazole

The total cellular fatty acids of KK1 were comprised of 11 C-even and 2 C-odd fatty acids (fatty acids <0.2% in abundance were not considered in this calculation). The predominant lipid 16:0 made up 34% of total cellular fatty acids for cells grown on complex medium (TSA), but decreased slightly to 32% when cells were exposed to carbazole (Fig. 5).

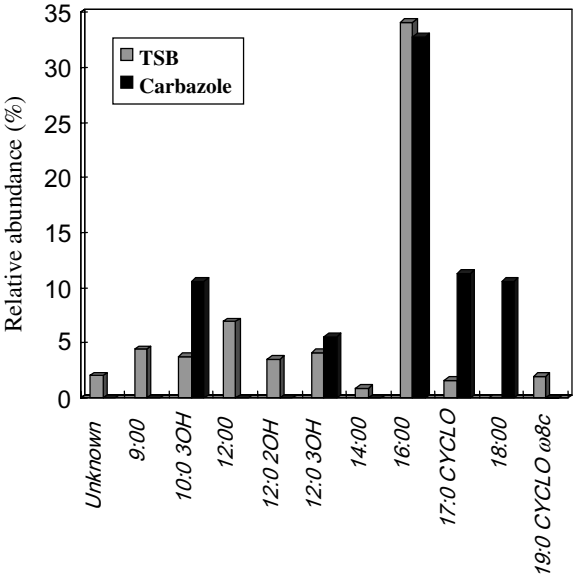


Fig. 5
Shifts of fatty acid compositions in strain KK1 cells in response to carbazole. Cells grown on TSA or carbazole for 24 hr were used for extraction of total cellular fatty acids, and the lipids were identified based on the retention of authentic references

Lipids 10:0 3OH, 12:0 3OH, 17:0 cyclo, and 18:0 increased in response to carbazole, while lipids 12:00, 12:0 2OH, and 19:0 cyclo ω8c were no longer detectable, suggesting that the total cellular fatty acid composition of strain KK1 was greatly affected by exposure to carbazole. Notably, lipid 18:0 that was not detectable on TSA-grown cells increased to 10% abundance for carbazole. Exposure of KK1 cells to carbazole resulted in changes of the total cellular fatty acid composition (Fig. 5). It is notable that lipids 17:0 cyclo and 18:0 significantly increased following exposure to carbazole. Many reports of a similar nature have suggested that conversion of unsaturated fatty acids from *cis* to *trans* has been linked to prevention of membrane damage by decreasing membrane fluidity (WARTH 1991). PINKART *et al.* (1996) reported that solvent- tolerant and solvent-sensitive *Pseudomonas putida* strains were able to produce *trans*-unsaturated fatty acids following exposure to *o*-xylene. It was considered that changes of these fatty acids in response to different substrates might affect cell's survival tolerance or cell's ability to utilize the substrate. This finding was consistent with our previous study that several *cis*-unsaturated fatty acids in *Bukholderia* sp. HY1 increased in response to aniline, along with increase of some saturated fatty acids (KAHNG *et al.* 2000). These facts suggest that shifts from *cis*- to *trans*-fatty acids (or *vice versa*), or from unsaturated to saturated fatty acids (or *vice versa*) in KK1 cells might result from cell response for both survival and use of substrate in the presence of carbazole.

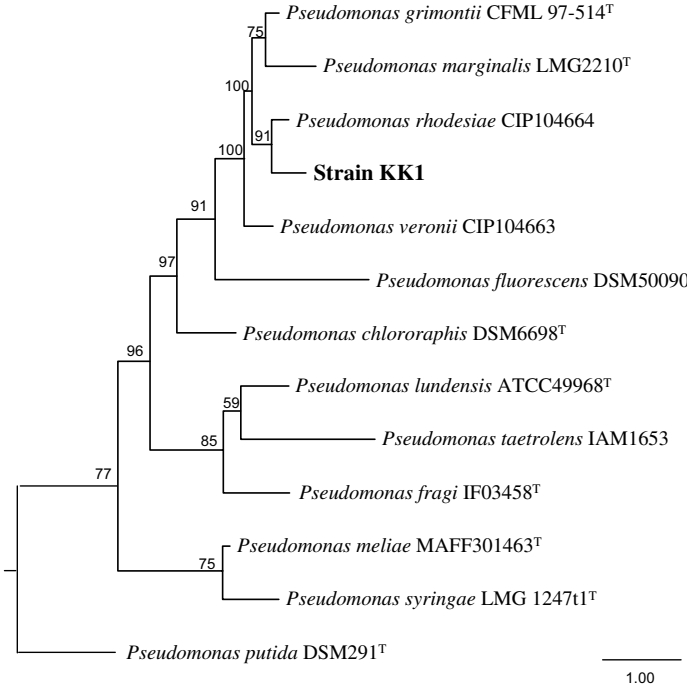


Fig. 6
Phylogenetic tree of *Pseudomonas rhodesiae* strain KK1 and representative bacteria of the Proteobacteria. The tree was built based on 1521 bases of 16S rRNA from strain KK1. Boostrap values at nodes of the dendrogram indicate the percentage of occurrence of the branching order in 500 boostraped trees (only values of 50 or above are shown). The bar scale represents 1 nucleotide substitution per 100 nucleotides

Physiological, biochemical and genitic characterization of strain KK1

The result obtained through BIOLOG substrate utilization analysis indicated that strain KK1 was a member of the *Pseudomonas* species cluster, with 94% matching similarity (Data not shown). Analysis of total cellular fatty acids by GC-FID also placed strain KK1 as a *Pseudomonas* species with over 90% confidence. Analysis of a 1521-bp fragment of the amplified 16S rDNA sequence from strain KK1 and comparative multiple alignment of this sequence with others in the 16S rRNA database indicated that strain KK1 was placed among five major clonal types in the Proteobacteria, including *Pseudomonas rhodesiae*, *Pseudomonas grimontii*, *Pseudomonas marginalis,* and *Pseudomonas veronii, Pseudomonas fluorescens***.** The greatest similarity was to *Pseudomonas rhodesiae*, with 99% similarity (Fig. 6).

Based on combination of these analyses, we designate strain KK1 as *Pseudomonas rhodesiae* KK1. The phylogenetic tree based on 16S rRNA was shown in Fig. 6, suggesting that strain KK1 was far away in the phylogenetic distance from the strains that can degrade polycyclic aromatic compounds.

Acknowledgement

This work was supported by Korea Research Foundation Grant (KRF-2001-050-D00033) to H.-Y. KAHNG.

References

BATIE, C. J., BALLOW, D. P. and CORRELL, C. C., 1991. Phthalate dioxygenase redutase and related flavin-iron-sulfur containing electron transferase. In: F. Muller (ed.), Chemistry and Biochemistry of Flavoenzymes. CRC Press, Inc., Boca Raton, Fla., pp. 546–556.

CERNIGLIA, C. E., GIBSON, D. T. and DODGE, R. H., 1994. Metabolism of benz[a]anthracene by the filamentous fungus *Cummminghamella elegans*. Appl. Environ Microbiol., **60**, 3931–3938.

GEARY, P. J., SABOOWALLA, F., PATIL, D. S. and COMMACK, R., 1984. An investigation of the iron-sulpur proteins of benzene dioxygenase from *Pseudomonas putida* by electron-spin-resonance spectroscopy. Biochem. J., **217**, 667–673.

GIEG, L. M., OTTER, A. and FEDORAK, P. M., 1996. Carbazole degradation by *Pseudomonas* sp. LD2: Metabolic characterization of a fluorene-degrading bacterium: identification of some metabolites. Envrion. Sci. Technol., **30**, 575–585.

GURBIEL, R. J., BATIE, C. J., SIVARAJA, M., TRUE, A. E. and FEE, J. A., 1989. Electron-nuclear double resonance spectroscopy of 15N-enriched phthalate dioxygenase from *Pseudomonas cepacia* proves that two histidines are coordinated to the [2Fe-2S] RIESKE-type clusters. Biochemistry, **28**, 4861–4871.

KAHNG, H.-Y., 2002. Cellular responses of *Pseudomonas* sp. KK1 to two-ring polycyclic aromatic hydrocarbon, naphthalene. J. Microbiol., **40**, 38–42.

KAHNG, H.-Y., BYRNE, A. M., OLSEN, H. O. and KUKOR, J. J., 2000. Characterization and role of *tbuX* in utilization of toluene by *Ralstonia pickettii* PKO1. J. Bacteriol., **182**, 1232–1242.

KAHNG, H.-Y., KUKOR, J. J. and OH, K.-H., 2000. Physiological and phylogenetic analysis of *Burkholderia* sp. HY1 capable of aniline degradation. J. Microbiol. Biotechnol., **10**, 643–650.

KAHNG, H.-Y., MALINVERNI, J. C., MAJKO, M. M. and KUKOR, J. J., 2001. Genetic and functional analysis of the *tbc* operons for catabolism of alkyl-and chloroaromatic compound in *Burkholderia* sp. strain JS150. Appl. Environ. Microbiol., **67**, 4805–4816.

KASUGA, K., HABE, H., CHUNG, J.-S., YOSHIDA, T., NOJIRI, H., YAMANE, H. and OMORI, T., 2001. Isolation and characterization of the genes encoding a novel oxygenase component of angular dioxygenase from the gram-positive dibenzofuran-degrader *Terrabacter* sp. strain DBF63. Biochem. Biophys. Res. Commun., **283**, 195–204.

KIM, S.-I., KIM, S.-J., NAM, M. H., KIM, S., HA, K.-S., OH, K.-H., YOO, J.-S. and PARK, Y.-M., 2001. Proteome analysis of aniline-induced proteins in *Acinetobacter lwoffii* K24. Curr. Microbiol., **44**, 61–66.

MASON, J. R. and COMMACK, R., 1992. The electron transport proteins of hydroxylating bacterial dioxygenase. Annu. Rev. Microbiol., **46**, 277–305.

MASON, J. R., 1988. Oxygenase catalyzed hydroxylation of aromatic compounds: simple chemistry by complex enzymes. Int. Ind. Biotechnol., **46**, 277–305.

NOJIRI, H., SEKIGUCHI, H., MAEDA, K., URATA, M., NAKAI, S.-I., YOSHIDA, T., HABE, H. and OMORI, T., 2001. Genetic characterization and envolutionary implication of a *car* Gene clucter in the carbazole degrader *Pseudomonas* sp. strain CA10. J. Bacteriol., **183**, 3663–3679.

OUCHIYAMA, N., MIYACHI, S. and OMORI, T., 1998. Cloning and nucleotide sequencing catabolic genes from *Pseudomonas stutzeri* strain OM1, isolated from activated sludge. J. Gen. Appl. Microbiol., **44**, 57–63.

OUCHIYAMA, N., ZHANG, Y., OMORI, T. and KODAMA, T., 1993. Biodegradation of carbazole by *Pseudomonas* spp. CA06 and CA10. Biosci. Biotechnol. Biochem., **57**, 455–460.

PINKART, H. C., WOLFRAM, J. W., ROGERS, R. and WHITE, D. C., 1996. Cell envelope changes in solvent-sensitive *Pseudomonas putida* strains following exposure to *o*-xylene. Appl. Environ. Microbiol., **62**, 1129–1132.

RIESKE, J. S., HANSE, R. E. and ZAUGG, W. S., 1964. Studies on the electron transfer system. LVIII. Properties of a new oxidation-reduction component of the respiratory chain as studied by electron paramagnetic resonance spectroscopy. J. Biol. Chem., **239**, 3017–3021.

SANTODONATO, J. and HOWARD, P. H., 1981. Azaarenes: sources, distribution, environmental impact, and health effects. In: J. SAXENA and F. FISHER (Editors), Hazard Assessment of Chemicals: Current Developments. Academic Press, New York. pp. 421–440.

SATO, S.-I., NAM, J.-W., KASUGA, K., NOJIRI, H., YAMANE, H. and OMORI, T., 1997b. Identification and characterization of genes encoding carbazole 1,9a-dioxygenase in *Pseudomonas* sp. strain CA10. J. Bacteriol., **179**, 4850–4858.

SATO, S.-I., OUCHIYAMA, N., KIMURA, T., NOJIRI, H., YAMANE, H. and OMORI, T., 1997a. Cloning of genes involved in carbazole degradation of *Pseudomonas* sp. strain CA10: Nucleotide sequences of genes and characterization of *meta*-cleavage enzymes and hydrolase. J. Bacteriol., **179**, 4841–4849.

SHEPARD, J. M. and LLOYD-JONES, G., 1998. Novel carbazole degradation genes of *Sphingomonas* CB3: sequence analysis, transcription, and molecular ecology. Biochem. Biophys. Res. Commun., **247**, 123–135.

WACKETT, L. P., KWART, L. D. and GIBSON, D. T., 1988. Benzylic monooxygenation catalyzed by toluene dioxygenase from *Pseudomonas putida*. Biochemistry, **27**, 1360–1367.

WARTH, A. D., 1991 Effect of benzoic acid on glycolytic metabolite levels and intracellular pH in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol., **57**, 3415–3417.

WEST, W. R., SMITH, P. A., BOOTH, G. M. and LEE, M. L., 1986. Determination and genotoxicity of nitrogen heterocycles in a sediment from the Black River. Environ. Toxicol. Chem., **5**, 511–519.

Mailing address: Dr. HYUNG-YEEL KAHNG, Department of Environmental Education, Sunchon National University, Suncheon 540-742, Korea
Telephone: +82-61-750-3385
Fax number: +82-61-750-3308
E-mail: kahng@sunchon.ac.kr