APPLIED MICROBIAL AND CELL PHYSIOLOGY

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Degradation of polycyclic aromatic hydrocarbons by a newly isolated dibenzofuran-utilizing *Janibacter* sp. strain YY-1

Abstract The dibenzofuran (DF)-utilizing bacterium strain YY-1 was newly isolated from soil. The isolate was identified as *Janibacter* sp. with respect to its 16S rDNA sequence and fatty acid profiles, as well as various physiological characteristics. In addition to DF, strain YY-1 could grow on fluorene and dibenzothiophene as sole sources of carbon and energy. It was also able to cometabolize a variety of polycyclic aromatic hydrocarbons including dibenzo-*p*-dioxin, phenanthrene, and anthracene. The major metabolites formed from DF, biphenyl, dibenzothiophene, and naphthalene were identified by using gas chromatography-mass spectrometry as 2,3,2′-trihydroxybiphenyl, biphenyl-dihydrodiol, dibenzothiophene 5-oxide, and coumarin, respectively. These results indicate that strain YY-1 can catalyze angular dioxygenation, lateral dioxygenation, and sulfoxidation.

Introduction

Microbes capable of degrading dibenzofuran (DF) are of increasing interest due to their potential to cometabolize polychlorinated dibenzo-*p*-dioxin (DD) and DF. Many bacteria that utilize DF as a growth substrate have been isolated and characterized. Some of these, such as *Sphingomonas* sp. strain RW1 (Wilkes et al. 1996; Keim et al. 1999; Hong et al. 2002), *Sphingomonas* sp. HL7 (Fukuda et al. 2002), *Rhodococcus opacus* SAO101 (Kimura and Urushigawa 2001), and *Terrabacter* sp. DBF63 (Habe et al. 2001), can cooxidize mono- to tetra-chlorinated DD and DF.

Several excellent reviews describe bacterial DF degradation pathways (Wittich 1998; Bressler and Fedorak 2000; Nojiri et al. 2001). The initial step in the biodegradation of DF is categorized as angular dioxygenation and lateral dioxygenation. Angular dioxygenation, which is found in DF- or DD-utilizing bacteria, occurs at the 4 and 4a carbon atoms of DF and is followed by spontaneous cleavage of an ether bridge to yield a 2,3,2′-trihydroxybiphenyl (THB) compound. In contrast, lateral dioxygenation mainly by naphthalene- or biphenyl-utilizing bacteria occurs at the 1, 2, the 2, 3 and the 3, 4 positions, producing DF-dihydrodiols (Cerniglia et al. 1979; Resnick and Gibson 1996; Seeger et al. 2001). However, there are few reports of lateral dioxygenation by DF-utilizing bacteria (Fuse et al. 2003).

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants also of concern because of their ubiquitous distribution and their potentially harmful effects on human health. The biodegradation of PAHs has been elucidated and is well documented in reviews (Cerniglia 1992; Kanaly and Harayama 2000). PAH metabolic pathways are similar to those of DF. Some DF-degrading bacteria can transform other low-molecular-weight (LMW) PAHs (Grifoll et al. 1995; Takagi et al. 2002). However, less is known about transformation of high-molecular-weight (HMW) PAHs by DF-degrading bacteria.

In this study, we isolated and characterized the new DF-utilizing bacterium *Janibacter* sp. YY-1, which utilizes fluorene (FLU) and dibenzothiophene (DBT) as well as DF, as sole sources of energy and carbon. We further tested the degradation activity of strain YY-1 by using 22 PAHs and showed its versatile cometabolic activity for PAHs.

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Materials and methods

Media

For isolation and culture of DF-degrading bacteria, we modified slightly the carbon-free mineral medium (CFMM) described by Monna et al. (1993); our CFMM (pH 7.0) contained (per liter): 2.2 g Na_2HPO_4, 0.8 g KH_2PO_4, 3.0 g NH_4NO_3, 0.2 g $MgSO_4$, 10 mg $FeSO_4·6H_2O$, 10 mg $CaCl_2·2H_2O$, and 10 mg yeast extract. Solid media contained 16 g Bacto noble agar per liter.

Isolation and identification of DF-degrading bacteria

Soil samples collected from agricultural fields and forests in Japan were added to CFMM liquid medium supplemented with DF crystals (1 g/l), and incubated aerobically at 30°C on a reciprocal shaker at 150 rpm. Aliquots were transferred weekly to fresh medium and incubated under the same conditions; this process was repeated at least three times. Pure cultures were obtained by plating the enrichment culture on CFMM agar and applying the substrate to the lids of inverted Petri dishes.

Total DNA was extracted from the strains by the benzylchloride method (Zhu et al. 1993). 16S rDNA sequence was determined by the direct sequencing method described by Anzai et al. (1997) and compared with those in the DDBJ DNA database by using BLAST search. Cellular fatty acid profiles were determined by the microbial identification system (MIDI; Microbial ID, Newark, Del.) according to the instructions provided. Three type strains of *Janibacter* spp.— *J. limosus* JCM 10980[T] (Martin et al. 1997), *J. terrae* JCM 10750[T] (Yoon et al. 2000), and *J. brevis* IAM 14781[T] (Imamura et al. 2000) were used as references.

Degradation of DF

Degradation experiments with DF were carried out in test tubes containing 5 ml CFMM. DF (1 g/l) was added from a dimethylsulfoxide (DMSO) stock solution (DMSO does not support the growth of strain YY-1.). The test tubes were incubated at 30°C on a reciprocal shaker at 300 rpm. Bacterial growth was verified by measuring an increase in the bacterial protein content concomitant with a decrease in the DF concentration. The protein content was determined by Bradford assays using bovine serum albumin as a standard (Bradford 1976). The residual DF in the test tubes was extracted twice with an equal volume of hexane, and the DF concentration was measured by gas chromatography-mass spectrometry (GC-MS). All measurements were determined periodically by assaying individual test tubes; data are shown as the means of triplicate experiments. Uninoculated tubes and tubes without substrate served as controls.

Isolation and identification of metabolites from DF

To isolate metabolites from DF, 3-l growing cell culture and washed cell suspensions were set up. For washed cell suspensions, cells were grown on CFMM with DF until late exponential phase. Cells were harvested by centrifugation at 8,000 rpm for 10 min, washed twice with 50 mM sodium phosphate buffer (pH 7.0), and resuspended in the same buffer. For the detection of metabolites after initial dioxygenation, washed cell suspensions with DF were incubated in presence of a well-known *meta*-cleavage inhibitor. 3-chlorocatechol (0.1 mM concentration) (Wittich et al. 1992). Metabolites were extracted three times from the supernatants of cultures with an equal volume of ethyl acetate (neutral fraction) and acidified condition (pH 2.0) with 12 *N* HCl (acidic fraction). The extracts were dried with anhydrous Na_2SO_4 and concentrated to dryness in an evaporator. The dried samples were treated with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) or trimethylsilyldiazomethane and analyzed for trimethylsilyl (TMS) or methyl derivatives by GC-MS.

Growth and transformation of other aromatic compounds

Growth of strain YY-1 on other aromatic compounds (1 g/l) was tested in the same manner mentioned above. The 22 aromatic compounds used in this experiment are listed in Table 1.

Transformation of aromatic compounds by strain YY-1 was performed by using washed cell suspensions pre-grown with DF. After 1 day, the supernatant was analyzed by GC-MS. Heat-inactivated (80°C, 30 min) cells were used as controls to correct for adsorption of PAHs to cells.

Analytical method

The extracts were measured with a Shimadzu GC-MS model QP-5050A (ionization voltage, 70 eV;) (Shimadzu, Kyoto, Japan) equipped with a DB-1 column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm) (J&W Scientific, Folsom, Calif.) and helium as the carrier gas. The following temperature program was used for the identification of metabolites: 80°C for 3 min, 80 to 280°C at a rate of 5°C/min; that for monitoring the disappearance of aromatic compounds was: 100°C for 2 min, 80 to 170°C at a rate of 30°C/min, and 170 to 280°C at a rate of 30°C/min.

Table 1 Degradation activity of polycyclic aromatic hydrocarbons (PAHs) and related compounds by *Janibacter* sp. strain YY-1. Data values are averages of triplicate experiments

Substrate	Removal (%)	
	Single	Mixture
Naphtalene	100	94
Biphenyl	100	100
Diphenyl ether	82	35
Acenaphtene	55	26
Dibenzofuran[a]	94	100
Dibenzo-*p*-dioxin	100	100
Fluorene[a]	100	100
Xanthene	100	99
Fluoren-9-one	100	50
Dibenzothiophene[a]	100	94
Phenanthrene	85	87
Anthracene	100	91
Carbazole	100	100
Xanthone	100	66
2-Hydroxydibenzofuran	100	100
Anthrone	56	21
Fluoranthene	58	19
2,4,8,-Trichlorodibenzofuran	63	14
Pyrene	36	11
Benzo(*a*)anthracene	57	5
Chrysene	16	0
Perylene	42	2

[a]Compounds that support the growth of YY-1

Chemicals

Chemicals were of the highest purity commercially available. The derivatization reagents MSTFA and trimethylsilyldiazomethane were purchased from GL Sciences (Tokyo, Japan). DF, 9-fluorenone, FLU, DBT, and phenanthrene were purchased from Kanto (Tokyo, Japan), 2-hydroxydibenzofuran and 2,4,8-trichlorodibenzofuran were from Sigma-Aldrich (Tokyo, Japan), and carbazole and 2,3,2′-trihydroxybiphenyl were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were obtained from Tokyo Chemical Industry (Tokyo, Japan).

Nucleotide sequence accession number

16S rDNA sequence of strain YY-1 is deposited in DDBJ, EMBJ, and GenBank nucleotide sequence databases under the accession number AB089480.

Results

Isolation and characterization of *Janibacter* sp. strain YY-1

Strain YY-1 was isolated from soil of an agricultural field by the enrichment method using DF as sole sources of carbon and energy. After the third enrichment, the culture with DF gradually yielded a yellow turbidity. Repeated subculturing on CFMM agar plates containing DF as a sole carbon source enabled us to obtain a pure culture. Strain YY-1 was a Gram-positive, aerobic, catalase-positive, nonmotile, cocci bacterium. The 16S rDNA sequence (1,490 bp) of strain YY-1 showed 97% homology to 16S rDNA from *J. brevis* IAM 14781[T] and *J. terrae* JCM 10750[T] and located the strain within the genus *Janibacter* (Fig. 1). We used the MIDI system to determine the cellular fatty acid composition of strain YY-1 and the three *Janibacter* type strains. The results retrieved from the database did not match with any of the species listed in the MIDI library. Strain YY-1 contained iso C16:0, C17:1 cis9, and C18:1 cis9 as major components, a profile very similar to those of *J. terrae* JCM 10750[T] and *J. brevis* IAM 14781[T] but markedly different from that of *J. limosus* JCM 10980[T]. Regarding the fatty acid profiles of strain YY-1, *J. terrae* JCM 10750[T], and *J. brevis* IAM 14781[T], only strain YY-1 contains trace amounts of C14:0 and 10-methyl C18:0 fatty acids, and only *J. terrae* JCM 10750[T] contains anteiso-C17:1 fatty acid. The similarity of 16SrDNA sequence and fatty acid profiles of strain YY-1 to those of *J. brevis* IAM 14781[T] and *J. terrae* JCM 10750[T] support the assignment of strain YY-1 to the genus *Janibacter*. However, differences in major fatty acids and 16SrDNA sequence similarity clearly distinguish strain YY-1 from *J. limosus* JCM 10980[T].
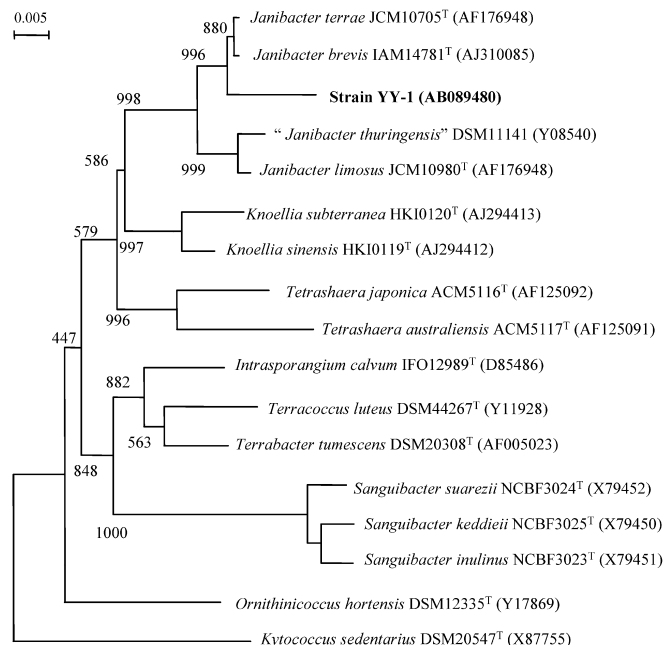

Fig. 1 Phylogenetic tree of *Janibacter* sp. strain YY-1 and related species. Bootstrap values are shown at the branch points

Degradation of DF by strain YY-1

Strain YY-1 can utilize DF as a sole source of carbon and energy (Fig. 2). The growth observed accompanied an increase in bacterial protein and a concomitant decrease in DF. The doubling time during exponential growth at 30°C was 13 h. Approximately 94% of the DF was degraded within 96 h. No growth was observed in the control test tubes (with DMSO only). During incubation of strain YY-1 with DF, the pH decreased from 7.0 to 6.5. The medium turned an orange-yellow color that had absorption maxima at 310, 410, and 466 nm and this color faded after 1 week.
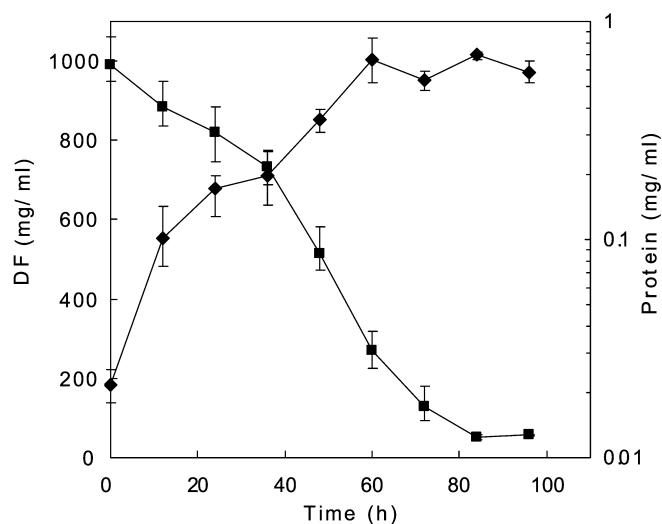

Fig. 2 Utilization of dibenzofuran (DF) as a sole source of carbon and energy by strain YY-1. Growth is shown as an increase in protein (♦). A decrease in DF (■) was monitored by gas chromatography-mass spectroscopy (GC-MS)

Identification of metabolites from DF and related compounds

We confirmed the ability of strain YY-1 to degrade DF by detection of intermediates extracted from DF-growing cell cultures and washed cell suspensions with DF. GC-MS analysis of supernatant extracts showed numerous peaks of metabolites (Fig. 3), which we identified by comparison of their mass spectra with those of authentic compounds, literature data, and the NIST (National Institute of Standards and Technology) library. On the basis of the identified metabolites, we propose metabolic pathways of DF by strain YY-1 (Fig. 4).

In 1-l growing cell culture, the medium color was changed to yellow, but no yellow metabolites were detected by GC-MS. Thus, to obtain sufficient quantities of metabolites, a 3-l scale culture was set up. Metabolites A, B, C, D, E, and F were extracted from the acidic fraction of growing cell culture, and their TMS and methyl derivatives were analyzed by GC-MS (Fig. 3a). The TMS derivative of metabolite A has a molecular ion at m/z 378 (M^+, 32) and fragment ions at m/z 363 (M^+-CH$_3$, 21), 293 (17), 261 (M^+-COOTMS, 100), 245 (M^+-COOTMS-O, 12), 193 (39), 171 (M^+-COOTMS-OTMS, 6), 147 (52), 73 (78). The TMS derivative of metabolite E has a molecular ion at m/z 450 (M^+, 2) and fragment ions at m/z 435 (M^+-CH$_3$, 15), 407 (4), 333 (M^+-COOTMS, 100), 257 (29), 147 (25), and 73 (81). The mass spectra of metabolites A and E were consistent with literature data for TMS

derivatives of 3-(chroman-4-on-2-yl) pyruvate and 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid (2′-OH-HOPDA), respectively (Kohler et al. 1993). The TMS derivative of metabolite B has a molecular ion at m/z 450 (20) and fragment ions at m/z 435 (11), 407 (9), 333 (45), 317 (3), 304(4), 147(16), and 73 (100), which is similar to that of metabolite E, indicating structural similarity. Based on the fragment ions at m/z 317 (M^+-COOTMS-O) and 304 (M^+-COOTMS-CO-H), consistent with the loss of keto and carboxyl groups, metabolite B was tentatively identified as the keto-enolic tautomerism compound of metabolite E, 2-oxo-6-(2′-hydroxyphenyl)-6-hydroxy-3,5-hexadienoic acid. Methylated metabolite D has a molecular ion (M^+, 5) at m/z 260 and fragment ions at m/z 201 (M^+-CO-OCH$_3$, 100), 186 (M^+-COOCH$_3$-CH$_3$, 33), and 158 (M^+-CO-OCH$_3$-CH$_3$-CO, 19). This mass spectrum is consistent with that previously reported for 2-oxo-4-(3′-hydroxy-benzofuran-2′-yl)-but-3-enoic acid (HOBB), which is a *meta*-cleavage product of 1,2-dihydroxydibenzofuran (Selifonov et al. 1991; Grifoll et al. 1995). Peak C and F had comparable mass spectra to TMS and methyl derivatives of metabolite D but with different intensities and different retention times. Therefore both metabolites were tentatively identified as cleavage products of dihydroxydibenzofuran.

GC-MS analysis of the neutral extract from the resting-cell reaction in the presence of DF and 3-chlorocatechol are shown in Fig. 3b. We identified metabolites G, H, and J as catechol, 2-hydroxydibenzofuran, and 2,3,2′-trihy-
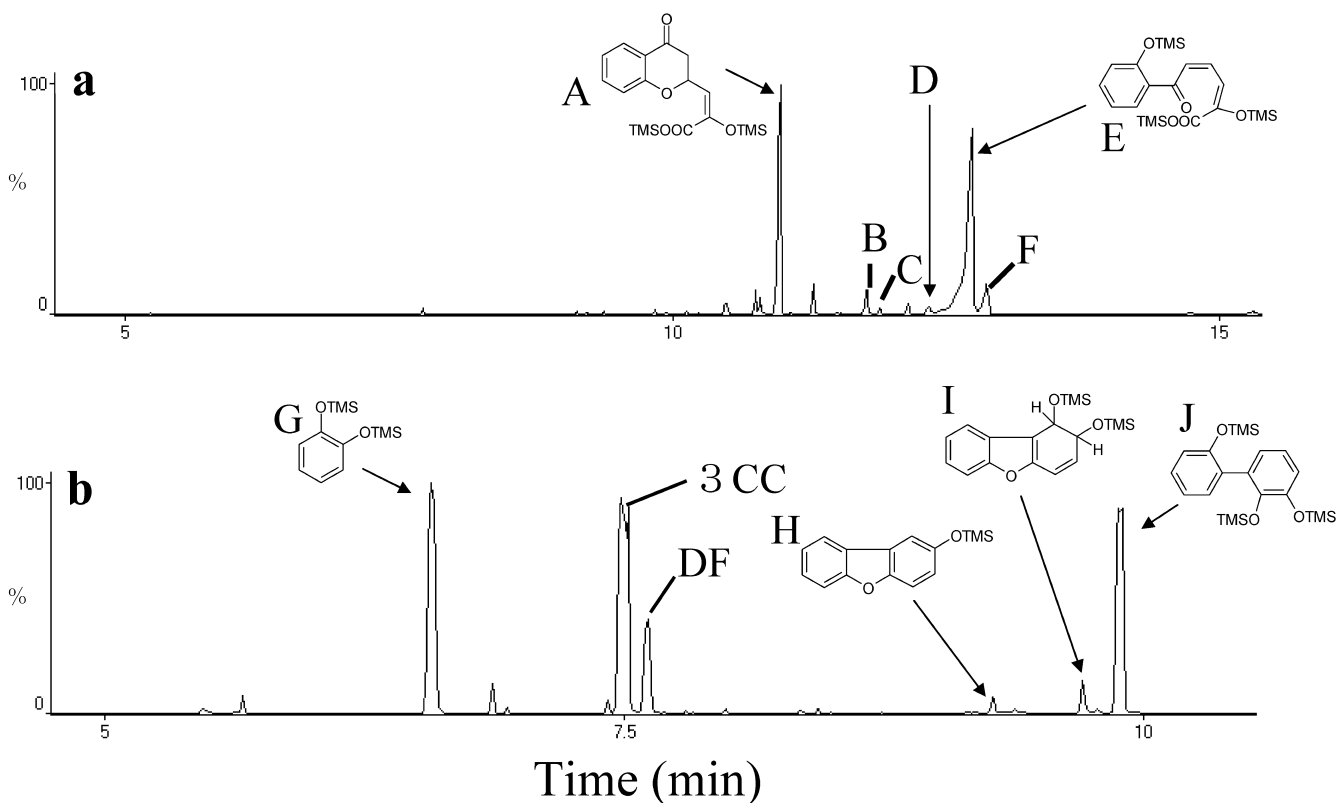

Fig. 3 GC profiles of trimethylsilyl (TMS) derivatives of acidified ethyl acetate extracts from 3-l growing cell cultures incubated with DF (**a**) and neutral ethyl acetate extracts from resting cell reactions incubated with DF in the presence of 3-chlorocatechol (3CC) (**b**)

Fig. 4 Proposed metabolic pathway of DF by *Janibacter* sp. YY-1. Compounds in brackets were not detected. Compounds in square dotted boxes were tentatively identified
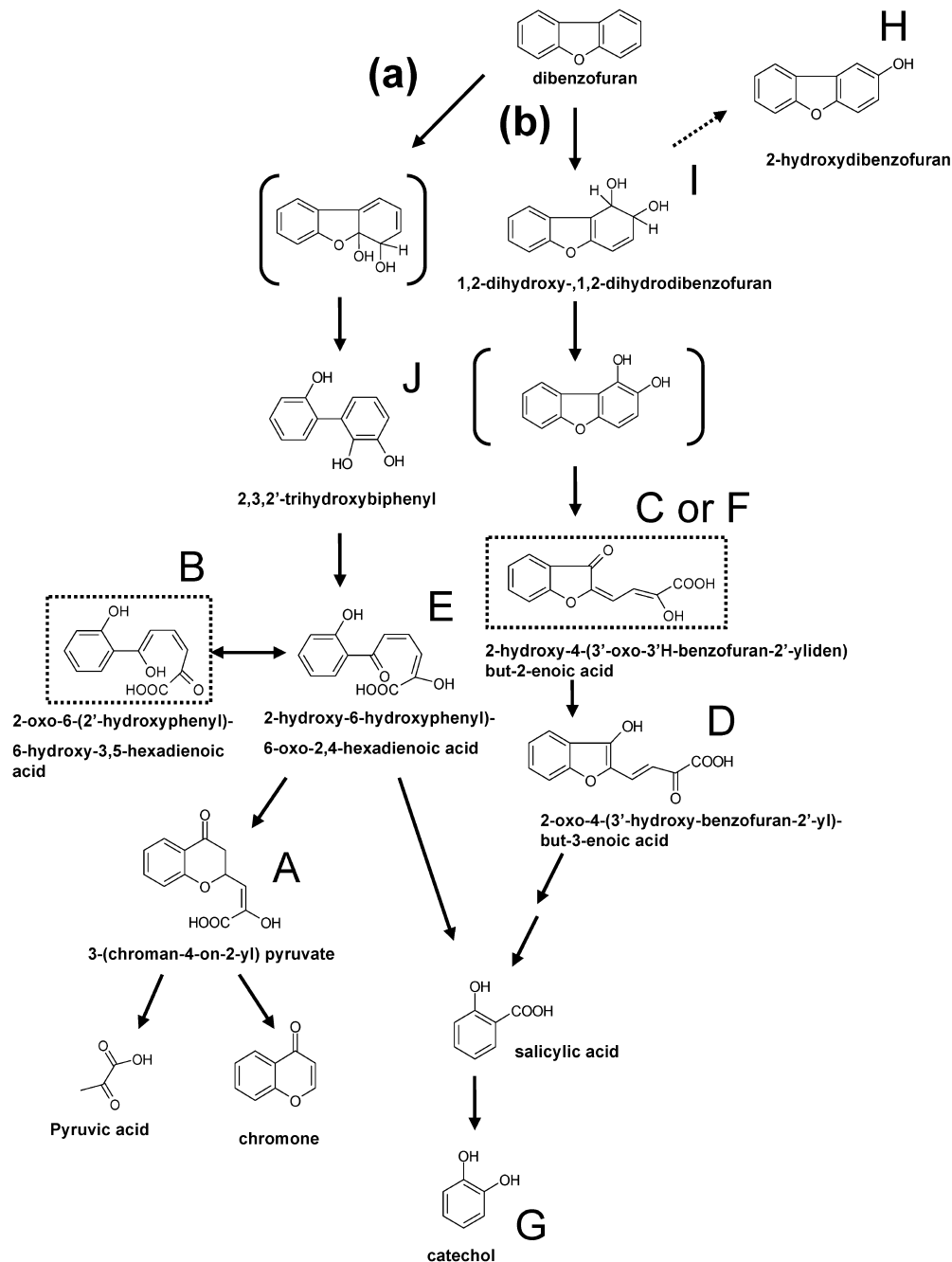
droxybiphenyl (2,3,2′-THB), respectively, by direct comparison of their retention times and mass spectra with those of available authentic standards. TMS derivative of metabolite I exhibited the mass spectrum [346 (M⁺, 82%), 315 (6%), 256 (13%), 241 (23%), 184 (16%), 168 (13%), 156 (22%) 147 (33%), 139 (8%) 73 (100%)]. This mass spectrum is identical to literature data on 1,2-dihydroxy-1,2-dihydrodibenzofuran (1,2-DHD) (Seeger et al. 2001). Another three metabolites from the resting cell reaction with DF were detected and identified as salicylic acid, pyruvic acid, and chromone, respectively, by their mass spectra comparing with authentic compounds or the NIST library.

Washed cell suspensions of strain YY-1 pre-grown on DF were incubated with DBT, biphenyl (BPH), and naphthalene (NAH). Metabolites were monitored by GC-MS analysis of neutral extracts. Four metabolites from DBT were identified as 2-hydroxybiphenyl, dibenzothiophene-5-oxide (DBT-5-O), monohydroxydibenzothiophene, and dibenzothiophene-dihydrodiol, respectively, by comparison with GC retention time and mass spectra of authentic samples or the literature (Nojiri et al. 1999). Five metabolites from BPH were identified as 2-hydroxybipheny, 3-hydroxybiphenyl, 2,3-dihydroxybiphenyl, and two different dihydrodiols, respectively, by comparison with GC retention times and mass spectra of their authentic samples or the literature (Nojiri et al. 1999). One

metabolite from NAH was identified as coumarin by comparison with the GC retention time and mass spectrum of authentic sample.

Monohydroxylated compounds detected in this experiment were considered to be formed by nonenzymatic dehydration from dihydrodiols (Nojiri et al. 1999).

Growth with, and cometabolic degradation of, other aromatic compounds

We determined the ability of YY-1 to grow on 22 aromatic compounds. Strain YY-1 grew on fluorene and DBT as well as DF. The color of CFMM supplemented with fluorene became yellow upon growth of YY-1, and CFMM supplemented with DBT became red. In addition to the aromatic compounds listed in Table 1, we tested benzene, toluene, phenol, 2,5-dichlorophenol, pentachlorophenol, and benzoic acid as growth substrate, but none of these compounds supported growth of strain YY-1.

We also investigated the cometabolic degradation activity of aromatic compounds by using washed cells of strain YY-1. In tests using each compound (10 mg/l) individually, NAH, BPH, DD, FLU, xanthene, 9-fluorenone, DBT, carbazole, xanthone, 2-hydroxydibenzofuran, and anthracene were completely removed in 1 day, whereas chrysene was not removed to any significant extent.

When the 22 aromatic compounds listed in Table 1 were provided in mixtures (10 mg/l each), the removal of each compound tended to become lower overall. In particular, 2,4,8-trichlorodibenzofuran, fluoranthene, pyrene, benzo[a]anthracene, chrysene, and perylene were hardly transformed when other aromatic compounds were present (Table 1). The most recalcitrant substrate for transformation by strain YY-1 seemed to be chrysene. In contrast, BPH, DF, DD, FLU, carbazole, and 2-hydroxydibenzofuran were still removed completely even under conditions in which one substrate might inhibit the degradation of another.

Discussion

DF-utilizing strain YY-1 was isolated from soil. It was identified as a member of the actinomycetes, *Janibacter* sp., with respect to 16S rDNA sequence and fatty acid composition. Many actinomycetes, including the genus *Terrabacter*, *Rhodococcus*, and *Mycobacterium*, have been reported to degrade PAHs (Churchill et al. 1999; Bastiaens et al. 2000; Dean-Ross et al. 2001; Iida et al. 2002). Bastiaens et al. (2000) reported that the hydrophobic property of the outer cell layer of actinomycetes may be important for interaction with, or uptake of, PAH.

The detection of key metabolites such as 2,3,2′-THB and 2′-OH-HOPDA reveals that DF degradation by strain YY-1 occurs via a pathway common to other DF-utilizing bacteria (Fortnagel et al. 1990; Monna et al. 1993; Schmid et al. 1997). In this route (Fig. 4a), after angular

dioxygenation at the 4 and 4a carbon atoms of DF, 2,3,2′-THB is spontaneously produced. Then 2,3,2′-THB is *meta*-cleaved and converted into 2′-OH-HOPDA. Salicylic acid, which is further converted to catechol, is produced from 2′-OH-HOPDA. 3-(Chroman-4-on-2′-yl) pyruvate is formed by Michael cyclization of 2′-OH-HOPDA (Strubel et al. 1991) and further converted into chromone and pyruvic acid. The accumulation of large amounts of 2,3,2′-THB and intermediates suggests that this route (Fig. 4a) is a leading pathway of DF degradation in strain YY-1.

An alternative initial step, via lateral dioxygenation at 1,2-carbon atom position, was also suggested (Fig. 4b) on the basis of detection of 1,2-DHD, 2-hydroxydibenzofuran, and HOBB. 2-Hydroxydibenzofuran is probably produced due to spontaneous conversion from 1,2-DHD. HOBB was produced after *meta*-cleavage of 1,2-dihydroxydibenzofuran and further converted into salicylic acid. This pathway was found in cometabolic degradation of DF by biphenyl-utilizing bacteria (Becher et al. 2000; Stope et al. 2002). We also detected two other metabolites, C and F, formed probably by either *ortho*- or *meta*-cleavage of dihydroxydibenzofuran. Traces of metabolites formed by a lateral dioxygenation pathway suggested that the activity of lateral dioxygenation is lower than that of angular dioxygenation in strain YY-1 (Fig. 3). There are a few reports of bacteria that transform DF by both angular and lateral dioxygenation (Seeger et al. 2001; Fuse et al. 2003).

Identification of DBT-5-O and 2-hydroxybiphenyl formed from DBT suggests that strain YY-1 degrades DBT through the known pathway initiated by sulfoxidation reported for DBT-desulfurizing bacteria (Izumi et al. 1994; Oldfield et al. 1997). The lateral dioxygenation pathway was also indicated by the detection of dihydrodiol and monohydroxydibenzothiophene from DBT. These degradation pathways were first reported in DF-utilizing bacteria.

The detection of metabolites from BPH and NAH confirms the activity of lateral dioxygenation by strain YY-1. Identification of two monohydroxy-biphenyls, two dihydrodiols, and biphenyl-2,3-diol from BPH suggests that lateral dioxygenation takes place on 2,3- and 3,4-carbon atoms in the degradation of BPH by strain YY-1. Formation of coumarin as a metabolite from NAH fits in the previous pathway of the bacterial naphthalene metabolism, indicating aromatic ring dioxygenation and *meta*-cleavage (Annweiler et al. 2000).

A number of bacteria capable of utilizing DF have been isolated and characterized. Some bacteria grow on DF and its analog compounds. *Terrabacter* sp. strain DBF63 (Monna et al. 1993) and DPO1361 (Engesser et al. 1989) grow on DF and FLU. *Sphingomonas* sp. RW1 grows on DF and DD (Wittich et al. 1992). In contrast, strain YY-1 grows on DF, FLU, and DBT. Recently Takagi et al. (2002) demonstrated the substrate specificity of dibenzofuran 4,4a-dioxyganase (DFDO) from *Terrabacter* sp. DBF63. This DFDO was reported to catalyze angular dioxygenation of DF, lateral dioxygenation of BPH and

NAH, and sulfoxidation of DBT. In addition to these reactions, strain YY-1 catalyzed lateral dioxygenation of DBT.

Washed cell suspensions of strain YY-1 also cometabolized a variety of aromatic compounds. When various bi- and tricyclic aromatic compounds were provided individually, strain YY-1 fully transformed them. Moreover, strain YY-1 also transformed 16% to 58% of HMW PAHs with more than four benzene rings when they were provided at 10 mg/l. The compound most recalcitrant to transformation by strain YY-1 is chrysene (Table 1). Because an increased number of rings in a PAH molecule increases its hydrophobicity and electrochemical stability, LMW PAHs are degraded more rapidly than are HMW PAHs (Shuttleworth and Cerniglia 1995; Kanaly and Harayama 2000).

When we added 22 compounds in mixture, the degradation of each substrate was typically lower than when the same PAH was provided individually. However, NAH, BPH, DD, FLU, DBT, xanthene, and carbazole were almost completely degraded even when provided in a mixture. These results indicate that the dioxygenase of strain YY-1 has high specificity for fluorene and its heteroatomoic analogs due to its high activity of angular dioxygenation. However, the dioxygenase of strain YY-1 shows lower transformation activity toward aromatic ketones such as xanthone, fluoren-9-one, anthrone, and 2,4,8-trichlorodibenzofuran in comparison with FLU and its heteroatomoic analogs. In contrast, perylene, chrysene, benzo[a]anthracene, and fluoranthene were degraded poorly when provided in a mixture. A possible explanation for the apparent recalcitrance to degradation of these compounds when supplied in a mixture of LMW- and HMW PAHs is that complex interactions might inhibit the degradation of HMW PAHs (Bouchez et al. 1995; Stringfellow and Aitken 1995).

Strain YY-1 showed broad metabolic versatility toward a variety of aromatic compounds. Characterization of the initial dioxygenation and *meta*-cleavage products from DF, DBT, BPH, and NAH indicates that strain YY-1 possesses activities of both angular and lateral dioxygenation. This ability may be due to the presence of multiple dioxygenase genes or to broad substrate specificity of the same dioxygenase. Elucidation of the dioxygenation mechanisms in PAH degradation requires further enzymatic and molecular genetic analyses.

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218

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