J Korean Soc Appl Biol Chem (2012) 55, 613–618
10.1007/s13765-012-2106-5

ORIGINAL ARTICLE

Multiple Pathways in the Degradation of Dibenzothiophene by *Mycobacterium aromativorans* Strain JS19b1^T

Jong-Su Seo

Received: 12 May 2012 / Accepted: 19 July 2012 / Published Online: 31 October 2012

Abstract *Mycobacterium aromativorans* JS19b1^T, isolated from a polycyclic aromatic hydrocarbon (PAH)-contaminated site in Hilo, Hawaii, USA, was studied for its degradation of dibenzothiophene (DBT) as a sole carbon source. Strain JS19b1^T degraded 100% of DBT (40 mg/L) within 11 days incubation through branched metabolic pathways, including dioxygenation on C-1,2 and C-3,4 positions and monooxygenation on sulfur atom. The metabolites were isolated and identified by gas chromatography-mass spectrometry. Dibenzothiophene-5,5'-dioxide was transformed from sulfur oxidation of DBT. *Cis*-dibenzothiophene dihydrodiols were detected as initial dioxygenation products. Two ring cleavage products of dibenzothiophene diols were detected as *trans*-4-(3-hydroxy-benzo[*b*]thiophen-2-yl)-2-oxobut-3-enoic acid and *trans*-4-(2-hydroxy-benzo[*b*]thiophen-3-yl)-2-oxobut-3-enoic acid, which could be produced by *meta*-cleavage of dibenzothiophene-1,2-diol and dibenzothiophene-3,4-diol, respectively. The detection of benzothienopyranone strongly supported that *M. aromativorans* JS19b1^T can degrade DBT through 1,2- and/or 3,4-dioxygenations followed by *meta*-cleavage. The ring cleavage products were further transformed into 2,2'-dithiosalicylic acid *via* 2-mercaptobenzoic acid. These results suggested that strain JS19b1^T has diverse lateral dioxygenase and metabolic enzyme systems. The diverse DBT metabolic pathways in JS19b1^T gave a new insight to the bacterial degradation of aromatic compounds.

Keywords dibenzothiophene · dioxygenation · *meta*-cleavage · monooxygenation · *Mycobacterium aromativorans* JS19b1^T

J.-S. Seo (✉)
Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology, 854 Samgokri, Munsaneup, Jinju, 660-844, Republic of Korea
E-mail: jsseo@kitox.re.kr

Introduction

Dibenzothiophene (DBT) is one of components of creosote, crude oils, and shale oils. Sulfur heterocycles such as DBT also can be found in some secondary metabolites of microorganisms and plants. DBT has been used as model compound to study the microbial desulfurization in fossil fuels (Bressler et al., 1998). It is well known that microorganisms play a primary role in the degradation of persistent organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) and heterocycle aromatic compounds in environmental matrixes. There are several reports of bacterial species that can degrade DBT including *Arthrobacter* sp. (Seo et al., 2006), *Burkholderia* sp. (Gregorio et al., 2004), *Mycobacterium* sp. (Okada et al., 2002; Li et al., 2003), *Pseudomonas* sp. (Monticello et al., 1985; Denome et al., 1993), *Rhodococcus* sp. (Izumi et al., 1994; Oldfield et al., 1997), *Sphingomonas* sp. (van Herwijnen et al., 2003; Gai et al., 2007), and *Stenotrophomonas* sp. (Papizadeh et al., 2011).

Degradation of DBT can occur via two pathways: ring cleavage by dioxygenation (Kodama et al., 1973; Seo et al., 2006) and desulfurization by monooxygenation (Kilbane II, 1990; Oldfield et al., 1997; Okada et al., 2002; Papizadeh et al., 2011). For ring cleavage pathway, Kodama et al. (1973) found *trans*-4[2-(3-hydroxy)-thianaphthenyl]-2-oxo-3-butenoic acid could be produced from the 1,2-dioxygenation of DBT followed by *meta*-cleavage of dibenzothiophene-1,2-diol. Seo et al. (2006) suggested that *Arthrobacter* sp. P1-1 could degrade DBT dibenzothiophene diols from 1,2- and 3,4-dioxygenation, which undergo *ortho*- and *meta*-cleavages to produce benzo[*b*]thiophene-2,3-dicarboxylic acid and hydroxyl-benzo[*b*]thiophene carboxylic acid, respectively. For desulfurization pathway, sulfur-specific metabolic pathway, known as 4S pathway, is of interest to researchers, because desulfurization takes place without aromatic ring cleavage. Oldfield et al. (1997) elucidated four enzymatic steps involved in degradation of DBT by *Rhodococcus* sp. strain IGTS8 *via* two sulfur oxidation

metabolites including dibenzothiophene 5-oxide and dibenzothiophene 5,5-dioxide catalyzed by DszC, 2-(2'-hydroxyphenyl)benzene sulphinate catalyzed by DszA, and 2-hydroxybiphenyl catalyzed by DszB.

Our previous study with *Mycobacterium aromativorans* JS19b1^T suggested that this strain was able to utilize various polycyclic aromatic hydrocarbons (PAHs) including phenanthrene, fluorene, fluoranthene, and pyrene as carbon and energy sources (Seo et al., 2007a). The present study was focused on the isolation and identification of metabolites of DBT by *M. aromativorans* JS19b1^T. In addition, detailed metabolic pathways of dibenzothiophene are proposed.

Materials and Methods

Chemicals. Dibenzothiophene (>98% purity) was purchased from Sigma-Aldrich (USA). All medium reagents were at the least of reagent grade. All organic solvents were of high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (USA). Diazomethane produced from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide in a diazomethane generator (Cat. No. Z411736, Aldrich) was used for the derivatization of DBT metabolites (Keum et al., 2006).

Biodegradation kinetics. The bacterial cells pre-grown in phenanthrene-supplied minimal medium (MM) (Bastiaens et al., 2000) to an optical density of 0.2 at 540 nm were washed with MM three times (3×10 mL). A 200-μL aliquot of DBT stock solution (1000 mg/L) dissolved in acetone was placed into a sterilized culture tube. After the solvent was evaporated with nitrogen gas, the MM (4 mL) and pre-grown cells (1 mL) were added. After the culture tubes were incubated at 28°C, 150 rpm in the dark, the cultured medium (5 mL) in a whole test tube was extracted with ethyl acetate (5 mL) at days 0, 1, 3, 6, 8, 11, and 14 for gas chromatography-flame ionization detector (GC-FID) analysis. The analysis was performed on a Hewlett Packard 5890 gas chromatograph equipped with a ZB-5 column (30 m, 0.25 mm film thickness, Phenomenex, Inc., USA). The column temperature started from 100°C (2 min), rose to 280°C at 10°C/min, and held at 280°C for 20 min. Injector and detector temperatures were 270 and 290°C, respectively. Cultures inoculated with boiled dead cells were controls. All experiments were carried out in triplicates.

Bacterial growth and metabolite extraction. Strain JS19b1^T was grown in MM supplied with DBT (300 mg/1.5 L) at 28°C and 150 rpm (C24 Rotary shaker, New Brunswick Scientific, USA). After incubation for 14 days, the cultures were filtered through glass wool to remove the un-reacted crystalline DBT and centrifuged (6,000 g, 10 min). The metabolites of DBT were extracted, derivatized, and analyzed according to the methods previously described (Seo et al., 2006). Briefly, the supernatant was extracted with ethyl acetate after acidified to pH 2–3 with 6 N HCl. The organic layer was extracted with 10 mM NaOH solution. The remaining organic phase was dried over anhydrous sodium sulfate and concentrated to 5 mL (neutral fraction). The aqueous NaOH extract was acidified to pH 2–3 and then extracted with ethyl acetate (acidic fraction).

Metabolites in the neutral fraction after derivatization or no derivatization were analyzed with a gas chromatograph-mass spectrometry (GC-MS). After removal of ethyl acetate from the neutral fraction, the residue was dissolved in acetone (10 mL) followed by addition of 50 mg of *n*-butylboronic acid. After refluxing for 30 min, the mixture was concentrated to 1 mL and analyzed by GC-MS. Metabolites in the acidic fraction were derivatized with diazomethane using a diazomethane-generator system and detected with GC-MS (Keum et al., 2006).

Analytical methods. The metabolites of DBT were analyzed on a Varian QP-5000 gas chromatograph-Saturn-2000 mass spectrometer (Varian, Inc., USA), equipped with a ZB-1 column (60 m, 0.25 mm film thickness, Phenomenex Inc.) (Seo et al., 2006). The carrier gas was helium at a rate of 2 mL/min. The column temperature started from 120°C (2 min), increased to 280°C at 2°C/min, and held at 280°C for 10 min. Injector and analyzer temperature were 270 and 280°C, respectively. The mass spectrometer was at electron impact mode (70 eV).

Results

Biodegradation kinetics. The degradation of DBT by *M. aromativorans* JS19b1^T was monitored at various time intervals (Fig. 1). The initial lag phase of DBT degradation was short. DBT (40 mg/L) was degraded rapidly after 3 days of incubation and completely decomposed by strain JS19b1^T in 11 days as compared with no DBT degradation in sterilized control (Fig. 1). These results suggested that strain JS19b1^T can utilize DBT as a sole carbon source.

Catabolism of dibenzothiophene. Dibenzothiophene was rapidly metabolized through multiple pathways. A total of eight DBT metabolites, covering *cis*-dihydrodiols of DBT to 2,2'-dithiosalicylic acid, were detected in culture supernatants and identified by retention times and mass fragmentation patterns (Table 1). Two metabolites in the neutral fraction were tentatively identified as *n*-butylboronate esters of *cis*-dibenzothiophene-1,2-dihydrodiol (D1a) and *cis*-dibenzothiophene-3,4-dihydrodiol (D1b). Mass spectra of both metabolites [*m/z* 284 (M^+), 227 (M^+-$(CH_2)_3$-CH_3), 200 (M^+-$(CH_2)_3$-CH_3-BO), 184 (M^+-$(CH_2)_3$-CH_3-BO-O)] were very similar with that of phenanthrene dihydrodiol (Table 1 and Fig. 2A) (Seo et al., 2007b). This result suggested that dibenzothiophene degradation by strain JS19b1^T started from 1,2- and 3,4-dioxygenations. Metabolite D2 had a molecular ion (M^+) at *m/z* 216 and fragment ions at *m/z* 200 and 184 produced by two sequential losses of O (M^+-16 and -32), and thus was identified as dibenzothiophene-5,5'-dioxide (Table 1). This result suggested that strain JS19b1^T has a monooxygenase system, in addition to a dioxygenase system.

There are four possible ring cleavage products from

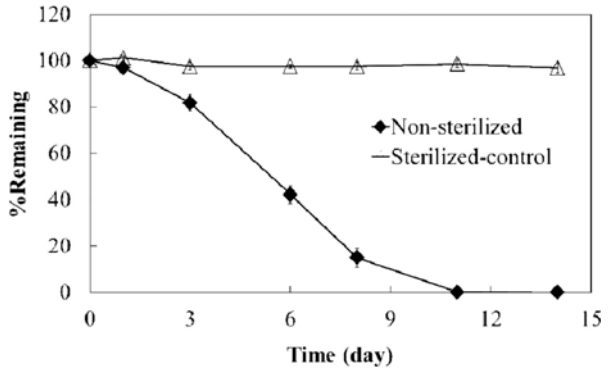

Fig. 1 Biodegradation kinetics of dibenzothiophene in *Mycobacterium aromativorans* JS19b1^T

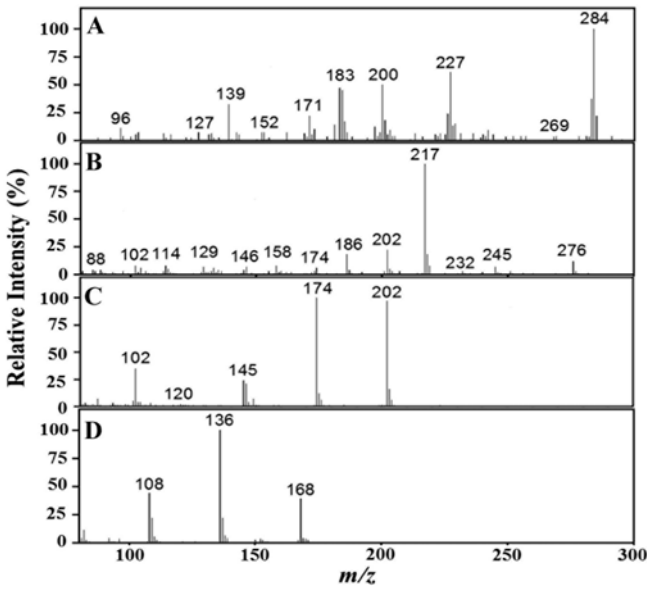

Fig. 2 Representative mass spectra of dibenzothiophene metabolites in *M.aromativorans* JS19b1^T. **A**, *n*-butylboronate of *cis*-dibenzothiophene dihydrodiol (D1); **B**, ring cleavage product of dibenzothiophene diol (D3); **C**, benzothienopyranone (D4); **D**, methyl ester of 2-mercaptobenzoic acid.

dibenzothiophene diols, having the same molecular ion and similar fragment ions (Seo et al., 2006). They are two carboxy-vinylbenzothiophene carboxylic acid (D3b and D3c in Fig. 3), which could be produced by *ortho*-cleavage of dibenzothiophene diols, and two *o*-hydroxybenzothiophenyl-α-oxobutenoates (D3a and D3d in Fig. 3), which could be produced by *meta*-cleavage of dibenzothiophene diols. In this study, two of four possible ring cleavage products were detected at different GC retention times (Table 1). Fragment ion *m/z* 217 [M^+-59 (-$COOCH_3$)] from D3 indicates that these two metabolites have one methylated carboxylic group (Table 1 and Fig. 2B). Therefore, two metabolites detected might be *trans*-4-(3-hydroxy-benzo[b]thiophen-2-yl)-2-oxobut-3-enoic acid (D3a) and *trans*-4-(2-hydroxy-benzo[b]thiophen-3-yl)-2-oxobut-3-enoic acid (D3d) (Fig. 3). This ring cleavage pattern is similar with *meta*-cleavage of phenanthrene diols (Seo et al., 2012).

One of two possible benzothienopyranone metabolite (D4a or D4b in Fig. 3) was detected with a molecular ion (M^+) at *m/z* 202 and fragment ions at *m/z* 174 and 146 (Table 1 and Fig. 2C), which was similar with the result reported by Frassinetti et al. (1998) and Seo et al. (2006). D4a and D4b might have derived from decarboxylation and cyclization of *trans*-4-(3-hydroxy-benzo[b]thiophen-2-yl)-2-oxobut-3-enoic acid (D3a) and *trans*-4-(2-hydroxy-benzo[b]thiophen-3-yl)-2-oxobut-3-enoic acid (D3d) (Fig. 3), respectively. The detection of benzothienopyranone from *o*-hydroxybenzothiophenyl-α-oxobutenoates is similar with that of benzocoumarin from *o*-hydroxynaphthaleneyl-α-oxobutenoates (Seo et al., 2012).

2-Mercaptobenzoic acid (D5) (Fig. 2D) and its dimmer form (D6), 2-mercaptobenzoic acid disulfide (or 2,2'-dithiosalicylic acid), were detected with methyl and dimethyl ester form (Table1), respectively. The mass fragmentation pattern of D6 was identical to that obtained by Bressler and Fedorak (2001).

Discussion

Catabolic pathways of dibenzothiophene by *M. aromativorans* JS19b1^T was proposed based on the metabolites detected and tentatively identified with their GC retention times and mass spectra (Table 1 and Fig. 3). The analysis of dibenzothiophene

Table 1 GC-MS retention times and mass spectral data of dibenzothiophene metabolites

ID	Chemical name[a]	RT[b] (min)	MS fragmentation pattern[c]
D1	*cis*-Dibenzothiophene dihydrodiols (BuB)	50.49	284(M^+, 100), 227(61), 200(50), 184(45), 183(47)
		53.10	284(M^+, 100), 227(34), 200(50), 184(30), 183(21)
D2	Dibenzothiophene-5,5'-dioxide	43.98	216(M^+, 49), 184(100), 152(18), 139(29)
D3	Ring cleavage products of dibenzothiophene diols	47.53	276(M^+, 15), 245(7), 217(100), 202(22), 185(7)
		51.72	276(M^+, 18), 245(9), 217(100), 202(28), 185(10)
D4	Benzothienopyranone	38.00	202(M^+, 87), 174(100), 146(21)
D5	2-Mercaptobenzoic acid (Me)	12.85	168(M^+, 36), 136(100), 108(35)
D6	2,2'-Dithiosalicylic acid (diMe)	64.24	334(M^+, 56), 167(100), 137(28), 118(39), 108(16)

[a]derivatives of; BuB, *n*-butylboronic acid; Me, methyl ester; diMe, dimethyl ester
[b]GC retention time
[c]values in parenthesis represent *m/z* of molecular ion (M^+) and relative abundance

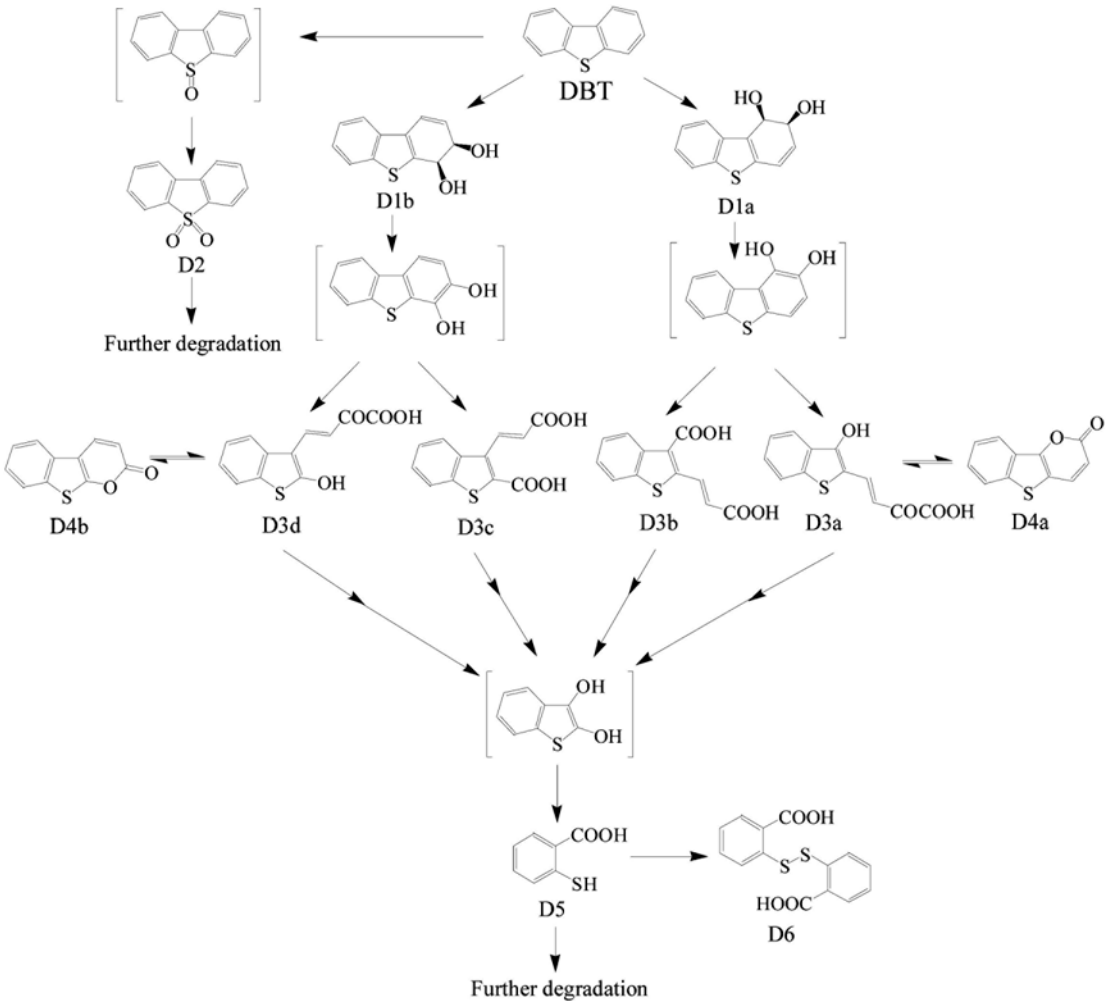

Fig. 3 Proposed catabolic pathways of dibenzothiophene by *Mycobacterium aromativorans* JS19b1^T. D1a, *cis*-dibenzothiophene-1,2-dihydrodiol; D1b, *cis*-dibenzothiophene-3,4-dihydrodiol; D2, dibenzothiophene-5,5'-dioxide; D3a, *trans*-4-(3-hydroxy-benzo[*b*]thiophen-2-yl)-2-oxobut-3-enoic acid; D3b, 2-(2-carboxy-vinyl)-benzo[*b*]thiophene-3-carboxylic acid; D3c, 3-(2-carboxy-vinyl)-benzo[*b*]thiophene-2-carboxylic acid; D3d, *trans*-4-(2-hydroxy-benzo[*b*]thiophen-3-yl)-2-oxo-but-3-enoic acid; D4a, benzo[4,5]thieno[3,2-*b*]pyran-2-one; D4b, 1-oxa-9-thia-fluoren-2-one; D5, 2-mercaptobenzoic acid; D6, 2,2'-dithiosalicylic acid.

metabolites suggested that the *M. aromativorans* JS19b1^T can decompose dibenzothiophene through multiple pathways including two dioxygenations and one monooxygenation pathways. Kodama et al. (1973) first reported 1,2-dioxygenation of dibenzothiophene in *Pseudomonas jianii* DDC 279. In general, this pathway is known as Kodama pathway. Gregorio et al. (2004) identified two sets of genes for the initial steps of the oxidative degradation of dibenzothiophene. In the present study, dibenzothiophene degradation by strain JS19b1^T started from 1,2- and 3,4-dioxygenations to produce *cis*-dibenzothiophene-1,2-dihydrodiol (D1a) and *cis*-dibenzothiophene-3,4-dihydrodiol (D1b), respectively, suggesting that the strain JS19b1^T have either a dioxygenase system with broad specificity or diverse dioxygenase systems (Fig. 3).

Cis-dibenzothiophene-1,2-dihydrodiol (D1a) was further transformed into *trans*-4-(3-hydroxy-benzo[*b*]thiophen-2-yl)-2-oxobut-3-enoic acid (D3a) through dibenzothiophene-1,2-diol produced by dihydrodiol dehydrogenation of D1a (Fig. 3). D3a could be produced by *meta*-cleavage of dibenzothiophene-1,2-diol as reported by Kodama et al. (1973) and Frassinetti et al. (1998). Eaton and Chapman (1992) reported that the compounds carrying a *trans*-α,β-unsaturated keto acid side chain and an adjacent hydroxyl group, *trans*-4-(3-hydroxy-2-thianaphthenyl)-2-oxobut-3-enoate, *trans*-4-(3-hydroxy-2-benzofuranyl)-2-oxobut-3-enoate, and *trans*-4-(3-hydroxy-2-thienyl)-2-oxobut-3-enoate, were metabolized by hydratase-aldolase. Being similar to D1a, D1b was transformed into *trans*-4-(2-hydroxy-benzo[*b*]thiophen-3-yl)-2-oxobut-3-enoic acid (D3d) through dibenzothiophene-3,4-diol produced by dihydrodiol dehydrogenation of D1b (Fig. 3). In addition, D1a and D1b might be degraded by *ortho*-cleavages of dibenzothiophen-1,2-diol and dibenzothiophene-3,4-diol into 2-(2-carboxy-vinyl)-benzo[*b*]thiophene-3-carboxylic acid (D3b) and 3-(2-carboxy-vinyl)-benzo[*b*]thiophene-2-carboxylic acid (D3c),

respectively (Fig. 3). This result is similar with the case of 1,2-dicarboxynaphthalene produced *via* intradiolic dioxygenation of 3,4-dihydrophenanthrene (Kim et al., 2005; Keum et al., 2006). The detection of two benzothienopyranone metabolites (D4a and D4b), one (benzothienopyran-2-one) of which was detected by Frassinetti et al. (1998), strongly supports that *M. aromativorans* JS19b1^T can degrade dibenzothiophene through 1,2- and 3,4-dioxygenations followed by *meta*-cleavage. This result is similar with the result of Pinyakong et al. (2000), who reported that 5,6- and 7,8-benzocoumarins were derived from 1,2- and 3,4-dioxygenations of phenanthrene, respectively.

Two carboxyvinylbenzothiophene carboxylic acids (D3b and D3c in Fig. 3) and two *o*-hydroxybenzothiophenyl-α-oxobutenoates (D3a and D3d in Fig. 3) converged into benzo[*b*]thiophene-2,3-diol, which was further metabolized into 2-mercaptobenzoic acid (D5) (Fig. 3). D5 was dimerized abiotically to form 2,2'-dithiosalicylic acid (D6), which agreed with the observation of Finkel'stein et al. (1997) and Bressler and Fedorak (2001). Finkel'stein et al. (1997) detected several dibenzothiophene metabolites including benzo[*b*]thiophene-2,3-diol, 2-mercaptobenzoic acid and 2,2'-dithiosalicylic acid from *Pseudomonas fluorescens* 17 and 26 cultures. Bressler and Fedorak (2001) suggested the formation of disulfides from abiotic reactions beginning with benzothiophene-2,3-dione.

Desulfurization pathways have been extensively studied in several bacterial genera including *Arthrobacter* (Lee et al., 1995; Seo et al., 2006), *Brevibacterium* (van Afferden et al., 1993), *Microbacterium* (Li et al., 2005), *Mycobacterium* (Okada et al., 2002; Li et al., 2003), and *Rodococcus* (Izumi et al., 1994; Oldfield et al., 1997). There are several degradation products of dibenzothiophene such as 2-hydroxylbiphenyl found through desulfurization pathway. However, dibenzothiophene-5,5'-dioxide (D2), which is involved in sulfur-specific process of biodesulfurization named as 4S pathway (Oldfield et al., 1997), was detected in the present study.

In summary, *M. aromativorans* JS19b1^T can degrade dibenzothiophene through three initial catabolic pathways, monooxygenation at sulfur atom, and 1,2- and 3,4-dioxygenations. The two dioxygenation pathways are converged in benzo[*b*]thiophene-2,3-diol through *ortho*- and *meta*-ring cleavages. Benzo[*b*]thiophene-2,3-diol is further transformed through 2-mercaptobenzoic acid into 2,2'-dithiosalicylic acid.

Acknowledgments I would like to thank Dr. Qing X. Li in University of Hawaii for his assistance and Dr. Young-Soo Keum in KonKuk University for providing valuable information.

References

Bastiaens L, Springael D, Wattiau P, Harms H, deWachter R, Verachtert H et al. (2000) Isolation of adherent polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using PAH-sorbing carriers. *Appl Environ Microbiol* **66**, 1834–43.

Bressler DC and Fedorak PM (2001) Identification of disulfides from the biodegradation of dibenzothiophene. *Appl Environ Microbiol* **67**, 5084–93.

Bressler DC, Norman JA, and Fedorak PM (1998) Ring cleavage of sulfur heterocycles: how does it happen? *Biodegradation* **8**, 297–311.

Denome SA, Stanley DC, Olson ES, and Young KD (1993) Metabolism of dibenzothiophene and naphthalene in Pseudomonas strains: complete DNA sequence of an upper naphthalene catabolic pathway. *J Bacteriol* **175**, 6890–901.

Eaton RW and Chapman PJ (1992) Bacterial metabolism of naphthalene: Construction and use of recombinant bacteria to study ring cleavage of 1,2-dihydroxynaphthalene and subsequent reactions. *J Bacteriol* **174**, 7542–54.

Finkel'stein ZI, Baskunov BP, Vavilova LN, and Golovleva LA (1997) Microbial transformation of dibenzothiophene and 4,6-dimethyldibenzothiophene. *Microbiologiya* **66**, 481–7.

Frassinetti S, Setti L, Coti A, Farrinelli P, Montevecchi P, and Vallini G (1998) Biodegradation of dibenzothiophene by a nodulating isolate of *Rhizobium meliloti*. *Can J Microbiol* **44**, 289–97.

Gai Z, Yu B, Li L, Wang Y, Ma C, Feng J et al. (2007) Cometabolic degradation of dibenzofuran and dibenzothiophene by a newly isolated carbazole-degrading *Sphingomonas* sp. strain. *Appl Environ Microbiol* **73**, 2832–8.

Gregorio SD, Zocca C, Sidler S, Toffanin A, Lizzari D, and Vallini G (2004) Identification of two new sets of genes for dibenzothiophene transformation in *Burkholderia* sp. DBT1. *Biodegradation* **15**, 111–23.

Izumi Y, Ohshiro T, Ogino H, Hine Y, and Shimao M (1994) Selective desulfurization of dibenzothiophene by *Rhodococcus erythropolis* D-1. *Appl Environ Microbiol* **60**, 223–6.

Keum YS, Seo JS, Hu Y, and Li QX (2006) Degradation pathways of phenanthrene by *Sinorhizobium* sp. C4. *Appl Microbiol Biotechnol* **71**, 935–41.

Kilbane II JJ (1990) Sulfur-specific microbial metabolism of organic compounds. *Resour Conserv Recy* **3**, 69–70.

Kim YH, Freeman JP, Moody JD, Engesser KH, and Cerniglia CE (2005) Effects of pH on the degradation of phenanthrene and pyrene by *Mycobacterium vanbaalenii* PYR-1. *Appl Microbiol Biotechnol* **67**, 275–85.

Kodama K, Umehara K, Shimizu K, Nakatani S, Minoda Y, and Yamada K (1973) Identification of microbial products from dibenzothiophene and its proposed oxidation pathway. *Agr Biol Chem* **37**, 45–50.

Lee MK, Senius JD, and Grossman MJ (1995) Sulfur-specific microbial desulfurization of sterically hindered analogs of dibenzothiophene. *Appl Environ Microbiol* **61**, 4362–6.

Li F, Xu P, Ma C, Zheng Y, and Qu Y (2003) Biodesulfurization of dibenzothiophene by a newly isolated bacterium *Mycobacterium* sp. X7B. *J Chem Eng Jpn* **36**, 1174–7.

Li W, Zhang Y, Wang MD, and Shi Y (2005) Biodesulfurization of dibenzothiophene and other organic sulfur compounds by a newly isolated *Microbacterium* strain ZD-M2. *FEMS Microbiol Lett* **247**, 45–50.

Monticello DJ, Bakker D, and Finnerty WR (1985) Plasmid-mediated degradation of dibenzothiophene by *Pseudomonas* species. *Appl Environ Microbiol* **49**, 756–60.

Okada H, Nomura N, Nakahara T, and Maruhashi K (2002) Analysis of dibenzothiophene metabolic pathway in *Mycobacterium* strain G3. *J Biosci Bioeng* **93**, 491–7.

Oldfield C, Pogrebinsky O, Simmonds J, Olson E, and Kulpa CF (1997) Elucidation of the metabolic pathway for dibenzothiophene desulphurization by *Rhodococcus* sp. strain IGTS8 (ATCC 53968). *Microbiology* **143**, 2961–73.

Papizadeh M, Ardakani MR, Motamedi H, Rasouli I, and Zarei M (2011) C-S targeted biodegradation of dibenzothiophene by *Stenotrophomonas* sp. NISOC-04. *Appl Biochem Biotechnol* **165**, 938–48.

Pinyakong O, Habe H, Supaka N, Pinpanichkarn P, Juntongjin K, Yoshida T et al. (2000) Identification of novel metabolites in the degradation of

phenanthrene by *Sphingomonas* sp. strain P2. *FEMS Microbiol Lett* **191**, 115–21.

Seo JS, Keum YS, Cho IK, and Li QX (2006) Degradation of dibenzothiophene and carbazole by *Arthrobacter* sp. P1-1. *Int Biodeterior Biodegradation* **58**, 36–43.

Seo JS, Keum YS, Harada RM, and Li QX (2007a) Isolation and characterization of bacteria capable of degrading polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides from PAH-contaminated soil in Hilo, Hawaii. *J Agric Food Chem* **55**, 5383–9.

Seo JS, Keum YS, Hu Y, Lee SE, and Li QX (2007b) Degradation of phenanthrene by *Burkholderia* sp. C3: initial 1,2- and 3,4-dioxygenation and *meta*- and *ortho*-cleavages of naphthalene-1,2-diol. *Biodegradation* **18**, 123–31.

Seo JS, Keum YS, and Li QX (2012) *Mycobacterium aromativorans* JS19b1^T degrades phenanthrene through C-1,2, C-3,4 and C-9,10 dioxygenation pathways. *Int Biodeterior Biodegradation* **70**, 96–103.

van Afferden M, Tappe D, Beyer M, Truper HG, and Klein J (1993) Biochemical mechanisms for the desulfurization of coal-relevant organic sulfur compounds. *Fuel* **72**, 1635–43.

van Herwijnen R, Wattiau P, Bastiaens L, Daal L, Jonker L, Springael D et al. (2003) Elucidation of the metabolic pathway of fluorine and cometabolic pathways of phenanthrene, fluoranthene, anthracene and dibenzothiophene by *Sphingomonas* sp. LB126. *Res Microbiol* **154**, 199–206.