Use of a Novel Fluorinated Organosulfur Compound To Isolate Bacteria Capable of Carbon-Sulfur Bond Cleavage

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The vacuum residue fraction of heavy crude oils contributes to the viscosity of these oils. Specific microbial cleavage of C—S bonds in alkylsulfide bridges that form linkages in this fraction may result in dramatic viscosity reduction. To date, no bacterial strains have been shown conclusively to cleave C—S bonds within alkyl chains. Screening for microbes that can perform this activity was greatly facilitated by the use of a newly synthesized compound, bis-(3-pentafluorophenylpropyl)-sulfide (PFPS), as a novel sulfur source. The terminal pentafluorinated aromatic rings of PFPS preclude growth of aromatic ring-degrading bacteria but allow for selective enrichment of strains capable of cleaving C—S bonds. A unique bacterial strain, Rhodococcus sp. strain JVH1, that used PFPS as a sole sulfur source was isolated from an oil-contaminated environment. Gas chromatography-mass spectrometry analysis revealed that JVH1 oxidized PFPS to a sulfoxide and then a sulfone prior to further degradation. However, other examples of bacterial cleavage of alkyl C—S bonds within alkyl chains. The type of desulfurization displayed by JVH1 differs significantly from previously described reaction results.

Microbial methods of removing sulfur from organosulfur compounds are of interest to the petroleum industry for reducing sulfur emissions and, more recently, for reducing heavy oil viscosity. As conventional crude oils are consumed throughout the world, heavier oils are being exploited which, due to their high viscosity, cannot be transported from remote fields to refineries without adding diluents. The vacuum residue fraction of crude oils (boiling point ≥524°C [975°F]) contributes to viscosity, and recent models indicate that alkyl sulfides compose important bridges in the network of high-molecular-weight molecules in this fraction. Up to 40% of the sulfur in these fractions is in the form of alkyl sulfides; if these alkyl C—S bonds can be selectively cleaved using a biological catalyst, reductions in molecular size and viscosity could occur.

The first requirement for developing a biological process for heavy oil viscosity reduction is obtaining a microorganism capable of alkyl C—S bond cleavage without reducing the carbon value of the substrate. Precedence for this type of reaction with aromatic heterocycles can be found in the well-characterized 4S pathway that selectively removes sulfur from dibenzothiophene (DBT) (35). The dsz or sox operon (genes responsible for DBT desulfurization) (7, 38) in Rhodococcus sp. strain IGTS8 has been observed in a variety of genera that are apparently widespread in petroleum-contaminated environments (6, 8). Other genera capable of selective sulfur removal from DBT include Gordonia sp. strain CYKS1 (40), Arthrobacter sp. strain ECRD-1 (28), the thermophilic Paenibacillus sp. strain A11-2 (23, 24), and Bacillus subtilis WU-S2B (22). Strains that desulfurize alkylated DBTs (12, 13, 25, 28, 32, 33) and benzothiophene (14) have also been isolated. Recently, a metabolic pathway has been described for Rhodococcus sp. strain WU-K2R that can desulfurize naphthothiophene and benzothiophene (21).

However, there are no reports that conclusively illustrate the bacterial cleavage of alkyl C—S bonds by bacteria. Van Hamme et al. (45) have shown that a variety of white-rot fungi oxidize dibenzyl sulfide to dibenzyl sulf oxide and dibenzyl sulfone prior to further degradation. However, other examples of the microbial degradation of compounds containing this type of bond (e.g., 2-chloroethyl sulfide and thiodiglycol) give no evidence for direct sulfur oxidation followed by C—S bond cleavage without degradation of the alkyl or aromatic moieties (20, 29, 36, 40). For example, metabolism by Nocardiooides simplex of 1-(phytanylsulfanyl)-octadecane (used as a model compound for sulfide bridges in high-molecular-weight fractions of sulfur-rich petroleum) has been described previously (18); although the sulfur was oxidized, no C—S bond cleavage was observed. This bacterium oxidized the terminal alkyl groups in the model compound, which was subsequently degraded by β-oxidations.

The challenge in isolating a bacterial strain capable of cleaving C—S bonds within alkyl chains lies in finding an appropriate substrate. Commercially available sulfur-containing substrates typically have terminal alkyl or aromatic moieties that...
are susceptible to microbial attack. Several studies have evaluated and used fluorene carboxylic acids, including pentafluorobenzene, as conservative tracers in soil and ground water (2, 5, 17, 37) because of their chemical stability. Thus, we hypothesized that a short-chain alkyl sulfide that contained a stable pentafluorophenyl group at each terminus would be ideal for selecting microorganisms that cleave C=S bonds.

This report outlines the synthesis and use of bis-(3-pentafluoro-phenyl)propyl sulfide (PFPS) as a novel compound to select, screen, and characterize isolates capable of cleaving C=S bonds within alkyl chains that may occur in the residual fractions of heavy crude oils and bitumens. An isolate (Rhodococcus sp. strain JVH1) which is distinct from the known DBT-desulfurizing strains in that it cannot use DBT as a sulfur source is described. In addition, metabolites produced by JVH1 from PFPS are shown and a metabolic pathway involving specific sulfur oxidation and C=S bond cleavage is presented.

MATERIALS AND METHODS

Substrates and chemicals. The commercially unavailable compounds PFPS, bis-(3-pentafluoro-phenyl)propyl sulfide (PFPP), and pentafluorophenyl propane-1-ol (PFPP-OH) were synthesized as described below. Pentafluorophenyl propane-1-ol (PFPP-OH), 3-pentafluoro-phenylpropanoic acid (PFPP-acid), bis-(3-pentafluoro-phenyl)propyl sulfone (PFPSO), and bis-(3-pentafluoro-phenyl)propyl sulfone (PFPSO2) were synthesized as described below.

Benzothiophene, dimethyl sulfoxide (DMSO), diethyl sulfoxide, dibenzyl sulfide, dibenzyl sulfone, 1,4-dithiane, tetrahydrothiophene sulfone, DBT sulfone, pentafluorobenzene (Aldrich), dibenzyl sulfone, 1,4-dithiane, tetrahydrothiophene sulfone, DBT sulfone, pentafluorobenzene, 2-phenylpropylene sulfoxide (PFPSO), and perfluorophenyl propane. This reaction was carried out in DMSO with argon at room temperature according to the method of Lange and Göttaerd (27). Triphenyl phosphine (Aldrich) was dissolved following the addition of imidazole (Aldrich), and the mixture was allowed to stand for 30 min prior to the addition of iodoane. The reaction mixture was extracted with DCM, and filtered. The extract was concentrated and the purified iodide, a dark pink oil (1H-NMR [300 MHz] δ 3.00 [2H, m, CH2], 3.17 [2H, J = 9 Hz, CH2]), was used to make PFPS and PFPPS with the method of Landini and Rollo (26) and using the phase transfer catalyst hexadecyltrimethylphosphonium bromide (Aldrich) and Na2S3H2O. The reaction proceeded for 2 h with argon (in water purged with argon to remove residual oxygen). The two-phase reaction mixture was extracted with ether, dried with MgSO4, and concentrated using a vacuum to yield a viscous, cloudy, orange mixed product. Purified PFPP (62% yield) (1H-NMR [300 MHz] δ 1.83 [2H, m, CH2—CH2—], 2.53 [2H, J = 9 Hz, CH2], and 2.79 [2H, J = 9 Hz, CH2]) and PFPPS (2% yield) (1H-NMR [300 MHz] δ 1.96 [2H, m, CH2—CH2—], 2.68 [2H, J = 9 Hz, CH2], and 2.79 [2H, J = 9 Hz, CH2]) were viscous, slightly yellow, transparent liquids.

Synthesis of PFPP-acid. PFPP was synthesized (using the method of Prasad et al.) (39) from PFPS-OH. In this case, PFPS-OH, acetone, water, ethyl acetate, Na2O2, and RuCl3·H2O were mixed. The reaction was monitored using thin-layer chromatography, and (following the disappearance of the PFPP-OH) the mixture was filtered to remove the white precipitate. The filtrate was adjusted to pH 2 with HCl and extracted three times with ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and concentrated. The purified PFPP-acid (45%) was a fluffy, white, amor material (1H-NMR [300 MHz] δ 2.66 [2H, J = 7.5 Hz, CH2], 3.02 [2H, J = 7.5 Hz, CH2—COOH]).
Synthesis of PFPSO and PFPSO₂. For this synthesis, the method of Trost and Curran (43) was used. PFPS and methanol were cooled on ice prior to adding water containing 3-mol equivalents of Oxone (Aldrich) (potassium hydrogen persulfate; 2KHSO₅/H₂SO₄/K₂SO₄). The reaction was allowed to proceed at room temperature for 30 min. PFPSO (97% yield) (¹H-NMR [300 MHz] δ 2.13 [2H, m, CH₂OCH₂O], 2.87 [2H, d, J = 9 Hz, CH₃], and 2.97 [2H, d, J = 9 Hz, CH₃]) and PFPSO₂ (2% yield) (¹H-NMR [300 MHz] δ 2.07 [2H, m, CH₂OCH₂O–S], 2.65 [2H, m, CH₃], and 2.86 [2H, d, J = 9 Hz, CH₃]) were white solids.

RESULTS

Isolation of the PFPS-desulfurizing bacterium *Rhodococcus* sp. strain JVH1. To isolate microorganisms capable of cleaving C—S bonds within alkyl chains, PFPS (Table 1) was used as the sole sulfur source in enrichment cultures. Enrichment cultures derived from various petroleum-contaminated soils and slud-
ges and maintained by passage in medium without the addition of sulfate were used as inocula. Shake flasks were prepared with SFGA and 40 μM PFPS as the only added sulfur source. Cultures were grown in subcultures (4% [vol/vol]) weekly for 5 weeks to remove solids and any associated sulfur sources. Turbid cultures were diluted and spread on agar to obtain isolated colonies. A total of 70 pure isolates, including 3 filamentous fungal isolates and 1 yeast isolate, were screened for the ability to produce biomass and remove PFPS when PFPS was provided as the sole sulfur source at a limiting concentration (40 μM). Control cultures with no added sulfur were prepared for each isolate, as some strains were able to produce biomass on the trace sulfur found in medium components. Gas chromatograph–flame-ionization detector analysis showed that 4 of the 70 isolates oxidized PFPS to PFPSO₂, and 2 of the 70 were able to produce significant amounts of biomass with PFPS as the sole sulfur source.

One strain, JVH1, was chosen for further study on the basis of the extent of growth and PFPS removal. This organism is a gram-positive, branching rod-shaped bacterium 1 μm in width, with branches up to 5 μm in length. Strain JVH1 was sent to MIDI Labs (Newark, Del.) for full-length 16S rRNA gene sequence analysis; the strain was found to cluster with Rhodococcus, Corynebacterium, and Tsukamurella species. It had a 99% match to Rhodococcus opacus in GenBank (accession number Y11893); this isolate has been named Rhodococcus sp. strain JVH1.

**Strain JVH1 cleaves C—S bonds.** To find PFPS metabolites, strain JVH1 was grown in SFGA medium with PFPS (325 μM) as the sole sulfur source. Cultures were extracted with pentane at neutral pH and then ethyl acetate at pH <2 at various times during the growth and stationary phases. Concentrated extracts, and extracts derivatized with either N-O-bis-(trimethylsilyl)acetamide or diazomethane, were analyzed by GC-MS. Table 1 shows the mass spectra of PFPS and metabolites detected in culture extracts. In each case, spectra of the metabolites and derivatized metabolites were identical to those of authentic standards synthesized for this work. PFPSO and PFPSO₂ were detected in pentane extracts along with PFPP-OH. Ethyl acetate extracts yielded PFPP-acid. Both PFPP-OH and PFPP-acid were derivatized to produce trimethylsilyl derivatives, while PFPP-acid was converted to a methyl ester. The spectra of these novel metabolites and the sulfur source PFPS have not been reported previously.

Samples taken daily were analyzed for fluoride ion to check for possible aromatic ring attack. At no time did fluoride concentrations exceed the detection limit of 0.2 mg/liter.

**Strain JVH1 differs from known DBT-desulfurizing strains.** The detection of the sulfoxide and sulfone of PFPS indicated that strain JVH1 used a pathway similar to that described for the DBT-desulfurizing strain *R. erythropolis* IGTS8 and related organisms. To test the versatility of strain JVH1 regarding cleavage of C—S bonds, it was incubated with various organosulfur compounds. Table 2 shows that JVH1 was able to use linear alkyl sulfides ranging from DMSO to didodecyl sulfide as sole sulfur sources. However, diocatadecyl sulfide was not used by JVH1 as a sulfur source, presumably due to low solubility and to its crystalline state in aqueous medium. In addition, dibenzyl sulfide and its sulfur oxidation products, PFPS, 1,4-dithiane, and tetrahydrothiophene sulfone, were used as sulfur sources. In contrast, phenyl sulfide, benzothioiophene, and DBT and its sulfone were not used by JVH1 to produce biomass.

Because strain JVH1 could not utilize DBT as a sulfur source, further comparisons were made with four well-known DBT-desulfurizing bacteria. The dry weights of biomass produced during a 7-day incubation with various sulfur sources were determined. Figure 1 shows that JVH1 is distinct from four known DBT-desulfurizing strains in that DBT does not support its growth whereas PFPS cannot support growth of the DBT-desulfurizing strains. However, GC-MS analysis showed that strain IGTS8 was able to stoichiometrically convert PFPS to PFPSO₂ via PFPSO. Conversely, JVH1 was unable to oxidize DBT to DBT-sulfoxide or DBT-sulfone or to extract sul-

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**TABLE 2. Growth of Rhodococcus sp. strain JVH1 in the presence of various organosulfur compounds as sole sulfur source**

| Sulfur source | OD₆₀₀ |
|---------------|-------|
| None          | 0.5   |
| PFPS          | 7.1   |
| PFPSO         | 8.6   |
| PFPSO₂        | 3.4   |
| PFPS          | 2.4   |
| DMO           | 6.5   |
| Dibenzyl sulfide | 4.6 |
| Didodecyl sulfide | 7.5 |
| Dibocatadecyl sulfide | 0.0 |
| Dibenzyl sulfone | 5.9 |
| Dibenzyl sulfoxide | 6.1 |
| 1,4-Dithiane | 2.8   |
| Tetrahydrothiophene sulfone | 1.7 |
| Phenyl sulfide | 0.0   |
| Benzothioiophene | 0.0 |
| DBT           | 0.0   |
| Dibenzothioiophene sulfone | 0.0 |
| Thiophene     | 0.0   |

*The compounds were provided at 0.1 mmol of S per liter of SFGA. Growth was measured as turbidity after a 7-day incubation.*
fur from DBT-sulfone. All strains were able to grow well using DMSO and sulfate as sole sulfur sources.

Strain JVH1 degrades PFPS in batch culture. Growth of strain JVH1 corresponded to the disappearance of PFPS from the culture medium, as shown in Fig. 2. Growth caused an increase in pH, presumably from the consumption of acetate. After 14 days of incubation, JVH1 had removed all of the PFPS from the medium, with no accumulation of PFPSO or PFPSO2. Sulfate concentrations in the culture supernatant did not exceed that of the cell-free control at any time during the incubation.

Gray et al. (15) reported that the CO bond cleavage of DBT sulfone yielded 2-hydroxybiphenyl-2-sulfinate. In an analogous manner, we hypothesized that cleavage of the CO bond in PFPSO2 would yield PFPP-OH and a sulfinate (Fig. 3).

Subsequent hydrolysis of the sulfinate in the culture supernatant did not exceed that of the cell-free control (~10 μM sulfite) at any time during the incubation.

Gray et al. (15) reported that the C—S bond cleavage of DBT sulfone yielded 2'-hydroxybiphenyl-2-sulfinate. In an analogous manner, we hypothesized that cleavage of the C—S bond in PFPSO2 would yield PFPP-OH and a sulfinate (Fig. 3). Subsequent hydrolysis of the C—S bond in the sulfinate would also yield PFPP-OH. The sulfinate was not detected by our analytical methods, but up to 18 μM of PFPP-OH accumulated in the culture medium by day 4 and then disappeared by day 6 (Fig. 2). Trace amounts of PFPP-acid were also detected. Further degradation of the acid via beta-oxidation would form pentafluorobenzoic acid, but none of this proposed metabolite was detected.

DISCUSSION

PFPS allows for the efficient selection of strains capable of cleaving C—S bonds within alkyl chains. The relative ease with which isolates were obtained may indicate that this type of activity is not uncommon in the environment. However, to our knowledge this activity has not been described previously.

Based on the metabolites formed, the metabolic pathway in Fig. 3 is proposed. Here, sulfur is oxidized to produce first a sulfoxide and then a sulfone in a manner analogous to that of the 4S pathway observed with DBT-desulfurizing bacteria (35). Once activated in this manner, C—S bond cleavage occurs to produce an alcohol and, presumably, a sulfinate. In the 4S pathway for DBT desulfurization, DBT-sulfone (DBTO2) is oxidized to 2-(2'-hydroxybiphenyl)-benzene sulfinate via the action of DszA, which is a DBTO2-monooxygenase (15). If a sulfinate were formed from PFPSO2, it would likely be cleaved to yield sulfite, which would then spontaneously oxidize to sulfate prior to being reduced by the microorganism for growth. Gray et al. (15) showed that the desulfinate in the 4S pathway (DszB) produces 2-hydroxybiphenyl and sulfite from 2-(2'-hydroxybiphenyl)-benzene sulfinate. Similarly, both sulfite and sulfate were detected by Greene et al. (16) in cultures of a Variovorax sp. strain growing on tetrahydrothiophene sulfone and by Bressler et al. (3) in cultures of a Pseudonocardia sp. strain with the sulfones of benzothiophene and 3- and 5-methylbenzothiophene as sole carbon and sulfur sources. In the current report, culture sulfate concentrations did not increase above those of the cell-free controls, indicating that sulfate was rapidly consumed by the growing cells.

While this would complete the sulfur extraction process, JVH1 further oxidized PFPP-OH to PFPP-acid. PFPP-acid would be metabolized to pentafluorobenzoic acid, presumably,
but this metabolite has not been detected and the fate of the PFPP-acid is unknown. We have not pursued this question, because the primary goal of the study was C=S bond cleavage. Defluorination and aromatic ring opening prior to sulfate release are not included in the proposed metabolic pathway, because fluoride ion was not detected in the culture medium. Even if only 1 in 10 fluoride ions per molecule of PFPS had been removed, the fluoride ion concentration would have risen to 6.2 mg/liter (30 times higher than the detection limit). De-fluorination of oxidized aromatic rings has been previously reported among members of the Actinomycetales (9, 44) and Pseudomonadaceae (10, 41, 42), but (in general) fluorinated compounds are considered to be more recalcitrant than other halogenated organics (4, 11).

The novelty of the desulfurization activity exhibited by strain JVH1 is best illustrated by comparison to known DBT-desulfurizing bacteria unable to utilize alkyl sulfides other than DMSO as sulfur sources (19). Interestingly, Arensdorf et al. (1) reported that a Dsz-negative mutant of strain IGTS8 can transform DMSO, indicating that the DBT-desulfurization pathway is not required for this activity. In the results seen with this study, the detection of PFPSO in trace amounts (and the near stoichiometric recovery of PFPSO₂ from the four DBT-desulfurizing cultures) supports the hypothesis that these strains are unable to cleave C=S bonds in alkyl chains. Assuming that these strains employ the dsz operon for DBT desulfurization (6, 7, 31), it would appear that DszC, a sulfoxide-sulfone monoxygenase (30) that catalyzes the conversion of DBT to DBT-sulfoxide to DBT-sulfone (7, 38), has sufficiently broad substrate specificity to catalyze the first two steps of PFPS oxidation. This observation agrees with the work by Lei and Tu (30), who found that purified DszC from IGTS8 is able to oxidize benzil sulfide and benzyl sulfoxide to benzyl sulfone.

In contrast, it would appear that DszA is not active against PFPSO₂. These results are in agreement with recent work by Arensdorf et al. (1, 9), who used a chemostat to isolate mutants of strain IGTS8 that could utilize octyl sulfide, 5-methylbenzothiophene, and benzothiophene as sulfur sources. In their work, specific single-nucleotide changes in DszA were responsible for expanding the normally narrow substrate range of this enzyme. Once the enzymes involved in desulfurization by strain JVH1 are isolated, comparative studies can be undertaken.

In conclusion, the novel organosulfur compound PFPS was effective in selecting microorganisms capable of cleaving C=S bonds in alkyl chains. This activity will be valuable in desulfurization and viscosity reduction studies (provided that preferential use of more-bioavailable sulfur compounds can be controlled). To this end, reaction mechanisms involved in PFPS oxidation and cleavage are being investigated, and current work is focused on locating the novel desulfurization genes and enzymes in strain JVH1.

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REFERENCES

1. Arensdorf, J. J., A. K. Loomis, P. M. DiGrazia, D. J. Monticello, and P. T. Plunkos. 2002. Chemostat approach for the directed evolution of biosulfurization gain-of-function mutants. Appl. Environ. Microbiol. 68:691–698.
2. Becker, M. W., and A. M. Shipton. 2000. Tracer transport in fractured crystalline rock: evidence of nondiffusive breakthrough tailing. Water Resour. Res. 36:1677–1686.
3. Bresciani, J. F., B. K. Leskiw, and P. M. Fedorak. 1991. Biodegradation of benzenethiophene sulfones by a filamentous bacterium. Can. J. Microbiol. 45:360–368.
4. Carvalho, M. F., C. T. Alves, M. I. M. Ferreira, P. De Marco, and P. M. L. Castro. 2002. Isolation and initial characterization of a bacterial consortium able to mineralize fluorobenzene. Appl. Environ. Microbiol. 68:102–105.
5. Dahan, O., A. Nativ, E. A. Adar, B. Berkowitz, and Z. Ronen. 1999. Field observation of flow in fracture intersecting unsaturated chalk. Water Resour. Res. 35:3315–3326.
6. Denis-Larose, C., D. Labbé, H. Bergeron, A. M. Jones, C. W. Greer, J. Al-Hawari, M. J. Grossman, B. M. Sankey, and P. C. K. Lau. 1997. Conservation of plasmid-encoded dibenzothiophene desulfurization genes in several rhodococci. Appl. Environ. Microbiol. 63:2915–2919.
7. Denome, S. A., C. Oldfield, L. I. Nash, and K. D. Young. 1994. Characterization of the desulfurization genes from Rhodococcus sp. strain IGTS8. J. Bacteriol. 176:6707–6716.
8. Duarte, G. F., A. S. Rosado, L. Seldin, W. de Araujo, and J. D. van Elsas. 2001. Analysis of bacterial community structure in sulfuric-oil-containing soils and detection of species carrying dibenzothiophene desulfurization (dsz) genes. Appl. Environ. Microbiol. 67:1052–1062.
9. Engresser, K. H., M. A. Rubio, and D. W. Ribbons. 1988. Bacterial metabolism of side chain fluorinated aromatics: cometabolism of 4-trifluoromethyl-(TFM)-benzoate by 4-isopropylbenzoate grown Pseudomonas putida JT strains. Arch. Microbiol. 149:195–206.
10. Engresser, K. H., R. B. Cain, and H. J. Knackmuss. 1998. Bacterial metabolism of side chain fluorinated aromatics: cometabolism of 3-trifluoromethyl-(TFM)-benzoate by Pseudomonas putida (avirile) mt-2 and Rhodococcus rhodop reincens N657. Arch. Microbiol. 160:188–197.
11. Fetnazar, S., and F. Lingens. 1994. Bacterial dehalogenases: biochemistry, genetics, and biotechnological applications. Microbiol. Rev. 58:641–685.
12. Folsom, B. R., D. R. Schieche, P. M. DiGrazia, J. Werner, and S. Palmer. 1999. Microbial desulfurization of alkylated dibenzothiophenes from a hydrodesulfurized middle distillate by Rhodococcus erythropolis 1-19. Appl. Environ. Microbiol. 65:4967–4972.
13. Furuya, T., K. Kinumori, K. Kino, and S. Usami. 2001. Thermophilic biodegradation of dibenzothiophene and its derivatives by Mycobacterium phlei WU-F1. FEMS Microbiol. Lett. 204:129–133.
14. Gilbert, S. C., J. Morton, S. Buchanan, C. Oldfield, and A. McRoberts. 1998. Isolation of a unique benzothiophene-desulfurizing bacterium, Gordona sp. strain 23E (NCIMB 1618), and characterization of the desulphurisation pathway. Microbiology 144:2545–2553.
15. Gray, K. A., O. S. Pugrebinsky, G. T. Trachko, L. Xi, D. J. Monticello, and C. H. Squires. 1996. Molecular mechanisms of biocatalytic desulfurization of fossil fuels. Nat. Biotechnol. 14:1705–1709.
16. Greene, E. A., P. H. Beauty, and P. M. Fedorak. 2000. Sulfolane degradation by mixed cultures and a bacterial isolate identified as a Variovorax sp. Arch. Microbiol. 174:111–119.
17. Jaynes, D. R. 1994. Evaluation of fluorobenzene tracers in surface soils. Ground Water 32:532–538.
18. Jenisch-Anton, A., P. Adam, W. Michaelis, J. Connan, D. Herrmann, M. Rohmer, and P. Albrecth. 2000. Molecular evidence for biodegradation of geomacromolecules. Geochim. Cosmochim. Acta 64:3525–3537.
19. Kayser, K. J., B. A. Bielaga-Jones, K. Jackowski, O. Odusun, and J. J. Kilbane. 1993. Utilization of organosulfur compounds by axenic and mixed cultures of Rhodococcus rhodochrous IGTS8. J. Gen. Microbiol. 139:3123–3129.
20. Kilbane, J. J., and K. Jackowski. 1996. Biocatalytic detoxification of 2-chloroethylethyl sulfide. J. Chem. Tech. Biotechnol. 65:370–374.
21. Kirmiruma, K., T. Furuya, R. Sato, Y. Ishii, K. Kino, and S. Usami. 2002. Biodesulfurization of napthothiophene and benzenothiophene through selective cleavage of carbon-sulfur bonds by Rhodococcus sp strain WU-K2R. Appl. Environ. Microbiol. 68:3867–3872.
22. Kirmiruma, K., T. Furuya, Y. Nishii, Y. Ishii, K. Kino, and S. Usami. 2001. Biodesulfurization of dibenzothiophene and its derivatives through the selective cleavage of carbon-sulfur bonds by a moderately thermophilic bacterium Bacillus subtilis WU-S2B. J. Biosci. Bioeng. 91:262–266.
23. Konishi, J., T. Onaka, Y. Ishii, K. Okamura, and M. Suzuki. 2000. Demonstration of the carbon-sulfur bond targeted desulfurization of benzothio-
phenes by thermophilic \textit{Pseudomonas} sp. strain A11–2 capable of desulfurizing dibenzothiophene. FEMS Microbiol. Lett. \textbf{187}:151–154.

24. Konishi, J., Y. Ishii, T. Onaka, K. Okumura, and M. Suzuki. 1997. Thermophilic carbon-sulfur bond-targeted biosulfurization. Appl. Environ. Microbiol. \textbf{63}:3164–3169.

25. Kropp, K. G., J. T. Anderson, and P. M. Fedorak. 1997. Biotransformation of three dimethyl dibenzothiophenes by pure and mixed bacterial cultures. Environ. Sci. Technol. \textbf{31}:1547–1554.

26. Landini, D., and F. Rollo. 1974. A convenient synthesis of primary and secondary dialkyl sulfides in the presence of phase-transfer catalysts. Synthesis \textbf{5}:565–566.

27. Lange, G. C., and C. Gottardo. 1980. Facile conversions of primary and secondary alcohols to alkyl iodides. Synth. Commun. \textbf{20}:1473–1479.

28. Lee, M. K., J. D. Senius, and M. J. Grossman. 1995. A convenient synthesis of primary and secondary dialkyl sulfoxides monooxygenase. J. Bacteriol. \textbf{178}:5699–5705.

29. Li, M. Z., C. H. Squires, D. J. Monticello, and J. D. Childs. 1996. Genetic analysis of the \textit{diz} promotor and associated regulatory regions of \textit{Rhodococcus erythropolis} IGT8. J. Bacteriol. \textbf{178}:6409–6418.

30. MacPherson, T., C. W. Greer, E. Zhou, A. M. Jones, G. Wisse, P. C. K. Lau, B. Sankey, M. J. Grossman, and J. Hawari. 1998. Application of SPME/GC-MS to characterize metabolites in the biodesulfurization of organosulfur model compounds in bitumen. Environ. Sci. Technol. \textbf{32}:421–426.

31. Matsui, T., T. Onaka, Y. Tanaka, T. Tenka, M. Suzuki, and R. Kurane. 2000. Alkylation benzothiophene desulfurization by \textit{Rhodococcus} sp. strain T09. Biosci. Biotechnol. Biochem. \textbf{64}:596–599.

32. Murgich, J., J. A. Abanero, and O. P. Strausz. 1999. Molecular recognition in aggregates formed by asphaltene and resin molecules from the Athabasca oil sand. Energy Fuels \textbf{13}:278–286.

33. Oldfield, C., N. T. Wood, S. C. Gilbert, F. D. Murray, and F. R. Faure. 1998. Desulfurization of benzothiophene and dibenzothiophene by actinomycete organisms belonging to the genus \textit{Rhodococcus}, and related taxa. Antonie Leeuwenhoek \textbf{74}:119–132.

34. Omori, T., Y. Saiki, K. Kasuga, and T. Kodama. 1995. Desulfurization of alkyl and aromatic sulfides and sulfonates by dibenzothiophene-desulfurizing \textit{Rhodococcus} sp. strain SY1. Biosci. Biotechnol. Biochem. \textbf{59}:1195–1198.

35. Pearson, R. J., W. P. Inskeep, J. M. Wraith, S. D. Comfort, and H. M. Gaber. 1996. Observed and simulated solute transport under varying water regimes: I. Bromide and pentavalent bensulphate acid. J. Environ. Qual. \textbf{25}:646–653.

36. Piddington, C. S., B. R. Kovacevich, and J. Rambousek. 1995. Sequence and molecular characterization of a DNA region encoding the dibenzothiophene desulfurization operon of \textit{Rhodococcus} sp. strain IGT8. Appl. Environ. Microbiol. \textbf{61}:468–475.

37. Prashad, M., Y. Lu, H.-Y. Kim, B. Hu, O. Repic, and T. J. Blacklock. 1999. An improved and practical Sharpless oxidation of primary alcohols to the carboxylic acids. Synth. Commun. \textbf{29}:2937–2942.

38. Rhee, S.-K., J. H. Chang, Y. K. Chang, and H. N. Chang. 1998. Desulfurization of dibenzothiophene and diesel oils by a newly isolated \textit{Gordona} strain, CYKSI. Appl. Environ. Microbiol. \textbf{64}:2327–2331.

39. Schlömann, M., E. Schmidt, and H.-J. Knackmuss. 1990. Different types of diene lactone hydrolase in 4-fluorobenzoate-utilizing bacteria. J. Bacteriol. \textbf{172}:5112–5118.

40. Schlömann, M., P. Fischer, E. Schmidt, and H.-J. Knackmuss. 1990. Enzymatic formation, stability, and spontaneous reactions of 4-fluoromalonic acid, a metabolite of the bacterial degradation of 4-fluorobenzoate. J. Bacteriol. \textbf{172}:5119–5129.

41. Trost, B. M., and D. P. Curran. 1981. Chemoselective oxidation of sulfides to sulfones with potassium hydrogen persulfate. Tetrahedron Lett. \textbf{22}:1287–1290.

42. Uotila, J. S., V. H. Kuitunen, T. Saastamoinen, T. Coote, M. M. Hägglom, and M. S. Salkinoja-Salonen. 1992. Characterization of aromatic dehalogena
des of \textit{Mycobacterium fortuitum} CG-2. J. Bacteriol. \textbf{174}:5669–5675.

43. Van Hamme, J. D., E. T. Wong, H. Dettman, M. R. Gray, and M. A. Pickard. 2003. Dibenzyl sulfide metabolism by white-rot fungi. Appl. Environ. Microbiol. \textbf{69}:1320–1324.