0273-2289/90/2425—0899$02.60

Depolymerization and Chemical Modification of Lignite Coal by *Pseudomonas cepacia* Strain DLC-07

RAJINDER K. GUPTA, LEE A. DEOBALD, AND DON L. CRAWFORD*

Department of Bacteriology and Biochemistry, Institute for Molecular and Agricultural Genetic Engineering (IMAGE), University of Idaho, Moscow, ID 83843

ABSTRACT

Coal metabolizing *Pseudomonas cepacia* DLC-07 was isolated from soil and shown to use soluble lignite coal as a carbon/energy source. *Pseudomonas* modified coal residues were characterized by FT-IR, HPLC, elemental analysis, and ^{13}C-NMR. *P. cepacia* DLC-07 depolymerized lignite, as shown by HPLC. FT-IR analysis showed fewer carbonyl and carboxyl groups, decreased etheric oxygen, and fewer aromatic and conjugated carbon–carbon double bonds in modified, compared to control coal. ^{13}C-NMR indicated a decrease in carbonyls and heteroaromatic carbons containing N, O, and S, a decrease in unsubstituted aromatic carbons, and an increase in long chain methylenes in treated coal. Elemental analysis supported the spectral data concerning metabolism of the coal polymer. *P. cepacia* cometabolized coal model compounds and utilized *p*-hydroxy substituted benzoic or cinnamic acids or aldehydes as sole carbon and energy sources.

Index Entries: *Pseudomonas;* coal; lignite; solubilization; depolymerization.

INTRODUCTION

Several groups have reported the degradation or solubilization of coals by fungi (*3,8,13,14,23,25,26*), bacteria (*9,13*), and actinomycetes (*10,24*). Some data indicates that enzymatic mechanisms are involved in

*Author to whom all correspondence and reprint requests should be addressed.

the biotransformation of coal (*4,5,21,27,28*). The most convincing evidence is that presented by Wondrack et al. (*28*), who showed that the lignin peroxidase of the fungus *Phanerochaete chrysosporium* will partially depolymerize water soluble subbituminous and lignite coal polymers. Evidence is more conclusive that microbially prouced alkaline materials can be involved in coal solubilization (*10,19,24,29*). In this case, the products are water soluble polymers associated with inorganic salts (*10,17,18*). Eventually, it may be shown that both enzyme and base-catalyzed reactions play roles in the process.

In the present paper, we report the isolation and characterization of a lignite coal-depolymerizing bacterium, *Pseudomonas cepacia* DLC-07. This strain will also metabolize low molecular weight coal substructure model compounds. It will also grow in an acidic minimal medium containing soluble lignite coal polymer as a sole source of carbon and energy. As a result of metabolism by *P. cepacia* DLC-07, lignite is partially depolymerized and altered significantly in chemical structure.

METHODS

Coal Substrates

The coal used as a substrate for enrichments was weathered Cuba Alabama lignite (*10*). This coal was solubilized in 0.1*N* NaOH, and then the solution was neutralized with 0.1*N* HCl to pH 7.1 before use. A second polymeric coal substrate soluble at pH 5.5 was prepared from 200 g of Vermont lignite (*10*). The coal was soaked in water for 6 h and then treated with 20% HNO_3 for 6 h. This coal was washed, air-dried, powdered, and dissolved in 1 L 1*N* NaOH. The resulting solution was centrifuged to remove undissolved particles, and the supernatant was adjusted to pH 7.0 with dilute HCl. The precipitated coal was centrifuged, washed, dried, and powdered (yield: 100 g). This coal dissolved readily at pHs above 7. The supernatant from the precipitation was acidified to pH 5.5. The resulting precipitate was recovered by centrifugation, washed (pH 5.5), dried, and powdered (yield: 20 g). The remaining coal in solution was acid precipitated at pH 1.5. The precipitate was recovered by centrifugation, washed (pH 1.5), dried (70°C; 10 h), and powdered (yield: 20 g). This latter coal polymer dissolves in solutions of pH 5.5 or higher and was used as the substrate for metabolic studies with *P. cepacia* DLC-07.

Isolation of *Pseudomonas cepacia* DLC-07

The bacterium was isolated from Gascoyne soil (North Dakota) by enrichment. The enrichment medium contained a mineral salts solution of the following composition in g/L: NH_2SO_4, 2.0; KH_2PO_4, 2.0; $MgSO_4$, 0.5; and $CaCl_2$, 0.1. The medium also contained 0.1 g/L yeast extract (Difco,

Detroit, MI) and 2.0 g/L of soluble Alabama lignite coal. Before autoclaving, the pH was adjusted to 5.5. Soil (1 g) was added to 100 mL of the medium in a 250 mL flask. Shaking incubation (250 rpm) was for 2 wk at 30°C. Then, 100 μL of the enrichment was transferred to 100 mL of fresh medium, and incubation was continued. After two more such biweekly transfers (except for use of a loopful of inoculum in the final transfer), pure cultures were isolated by streaking onto Sabouraud Maltose Agar (SMA) plates. Pure colonies were streaked onto agar plates of soluble Vermont lignite coal (2 g/L)-mineral salts medium (pH 5.5), and only organisms that showed considerable growth were selected for evaluation. *P. cepacia* DLC-07, one of the isolates, was maintained on Sabouraud Dextrose Agar (SDA) slants (at 4°C).

Growth of *Pseudomonas cepacia* DLC-07 on Coal

The growth medium contained mineral salts solution (as above) and soluble Vermont lignite coal (2.0 g/L dry wt). The presolubilized coal was dissolved by stirring, and the medium pH was adjusted to 5.5–5.6 with dilute KOH. Three 1 L flasks, each containing 500 mL coal medium, were sterilized by autoclaving. One flask of sterile medium served as the control. Ten milliliters of a 2-d-old *P. cepacia* DLC-07 seed culture from Sabouraud dextrose broth was used to inoculate the second flask. The third flask was inoculated with a loopful of DLC-07 cells from an SDA slant. Flasks were incubated with shaking (250 rpm) at 30°C. After 2 wk, the seed culture inoculated flask was harvested by centrifugation (1000*g*, 10 min). The supernatant was acidified to pH 2.0 with dilute HCL. Precipitated coal was recovered by centrifugation, washed (pH 2.0), and dried at 70°C for a few hours before being stored under vacuum. The other inoculated flask was incubated for 4 mo. Prior to harvest, a serial dilution plate count was carried out on SDA (2.9×10^6 colonies/mL). The contents were centrifuged to remove cell mass, and the supernatant was acidified. Precipitated coal was recovered by centrifugation, washed, dried, and stored as described above. The 4 mo control was harvested similarly.

Determination of *Pseudomonas cepacia* DLC-07 Growth Curve in Coal Medium

One gram of soluble Vermont lignite coal was added to 200 mL distilled water, and the solution was stirred overnight (25°C). A small amount of undissolved coal was then removed by filtration, and the following mineral salts were added: $(NH_4)SO_4$, 0.2 g; KH_2PO_4, 0.2 g; $MgSO_4$, 0.05 g; and $CaCl_2$, 0.01 g. The pH was adjusted to 5.5–5.6. Two 250 mL Erlenmeyer flasks, each containing 100 mL of the medium, were sterilized by autoclaving (20 min). One of the flasks was inoculated with 1.0 mL (approx. 10^6 cells) of a *P. cepacia* DLC-07 cell suspension from an SDA slant. The inoculated flask was shaken for a few minutes, and then the time

zero cell number was determined by serial dilution and plating on SDA. Plate counts were performed every day for 1 wk and then every week for the next 9 wk. Finally, plate counts and medium pH values were recorded at 6, 8, and 10 wk. Culture medium samples were taken weekly, as were samples from an uninoculated control. These were used as the source of microbially-modified coal for determinations of coal molecular weight distribution using HPLC (*see below*).

Coal Molecular Weight Determinations by HPLC

Coal samples from the growth curve study were analyzed for molecular weight distribution on a Hewlett Packard 1090A high performance liquid chromatograph (HPLC) equipped with an HP-1040A diode array detector and a Synchropak GPC-300 column (1000–500,000 mw separation capability) (Synchrom, Inc., Lafayette, IN). The mobile phase consisted of buffer (0.02*M* KH_2PO_4 containing 0.5% Tween 80, pH 7.1) set at a flow rate of 0.25 mL/min. High and low molecular weight protein standards from Pharmacia were used for plotting a standard curve. Samples from the growth curve study (0.5 mL) were centrifuged, and the supernatants were acidified to pH 2. The precipitated coal was centrifuged, and the resulting wet residue was dissolved in 0.5 mL of HPLC solvent. These samples were injected into the HPLC and eluted with continuous monitoring at 254 nm.

Cometabolism of Model Compounds

A loopful of cells from an SDA stock slant of *P. cepacia* DLC-07 was used to inoculate 250 mL Erlenmeyer flasks containing 50 mL Sabouraud dextrose broth. Flasks were incubated at 30°C shaking (250 rpm). After 18–20 h, 5 mg of model compound, dissolved in 100 μL of dimethylformamide (DMF), was added to the flask and to a corresponding uninoculated control. Shaking incubation was continued for 3 d. Samples were then withdrawn from each flask. Cells were removed from the samples by filtration, and then their UV-visible spectra were measured. The recordings were made on a Spectronic 2000 recording spectrophotometer (Bausch and Lomb, Rochester, NY).

Metabolism of Model Compounds in Minimal Medium

Sterile solutions of model compounds (1 mg/10 μL in DMF) were added to test tubes containing 5 mL of sterile mineral salts (pH 5.5–5.6), and 100 μL of washed *P. cepacia* DLC-07 cell suspension in mineral salts was added. An uninoculated tube served as the control. During shaking incubation at 30°C, samples were taken periodically for measurement of culture turbidity (600 nm) and UV-visible adsorption spectra. Cell growth

and removal of compound from the medium were considered indicative of use of a compound as a sole source of carbon and energy.

Plate Assay for Model Compound Utilization

A *P. cepacia* DLC-07 cell suspension (100 μL) in mineral salts was spread over the surface of a mineral salts–Noble agar (2.0% w/v: Difco) plate and allowed to dry for 1 h. Then, a sterile filter paper disk previously dipped into DMF solution containing model compound (5 mg/100 μL), was placed on the agar surface. These plates were incubated at 30°C for 3 d. Growth of the bacterium on the disk indicated positive utilization of the compound. Controls received disks dipped in DMF containing no compound.

Elemental Analysis

Elemental analyses of 1–3 mg samples of coals and coal-derived products for percent carbon, hydrogen, oxygen, sulfur, and ash were performed by Desert Analytics, Inc. (Tuscon, AZ), using standard procedures *10*).

Infrared Spectroscopy

FT-IR spectra of coal samples were recorded using Digilab Qualimatic spectrometer. KBr pellets, which had been well-dried and stored under vacuum, were used for all analyses. Spectra were obtained using 2 mg coal and 150 KBr. Scans were signal averaged to obtain a primary spectrum in wave numbers from 4000 to 400 cm^{-1}.

NMR Spectroscopy

The solid state ^{13}C cross-polarization magic angle spinning (CP/MAS) NMR was measured at 70 MHz for ^{13}C on an IBM NR-300 NMR spectrometer with a 0.35 cm^3 vol doty probe (Doty Scientific, Inc., Columbia, SC). The pulse width of 90° was 6 μs. A repetition time of 3 s, 5 kHz spinning rate, and 12,000 scan time were selected.

RESULTS AND DISCUSSION

A Gram negative, aerobic rod-shaped bacterial isolate that grew well on the minimal coal agar medium was selected for detailed study. This bacterium strain DLC-07, was identified as a strain of *Pseudomonas* on the basis of its biochemical properties, according to Bergey's Manual of Determinative Bacteriology (*1*). A gas chromatographic (GC) analysis of its cellular fatty acids, followed by computer library search, identified the strain as *Pseudomonas cepacia*. The GC fatty acid profile and computer-aided identification was performed by Microcheck, Inc. (Northfield, VT).

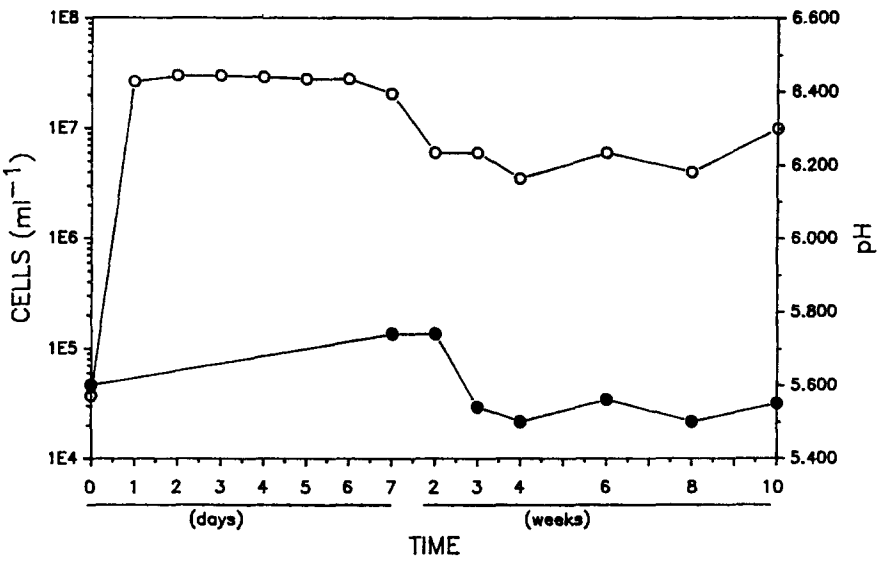

Fig. 1. Growth curve and culture medium pH of a *Pseudomonas cepacia* DLC-07 culture growing in minimal medium containing soluble Vermont lignite coal as a sole source of carbon and energy. Culture medium pH, ●; Cell count/mL, ○.

Coal is heterogenous and contains not only lignin- and other plant-derived materials, but also mineral impurities (*7*). Low rank lignite coals are also rather soluble in alkaline solutions (*19*). To minimize potential problems with abiotic base-catalyzed coal solubilization reactions as a factor to be dealt with in later studies, we chose to isolate a coal-metabolizing culture that grew well on coal at acid pH. Therefore, in order to produce a uniform pH 5.5 water soluble coal substrate low in inorganics, we fractionated nitric acid pretreated Vermont lignite coal for use as a substrate. The pH 5.5 soluble coal fraction readily dissolved in water and was of high molecular weight. In addition, by use of a soluble coal substrate, better spectral and elemental data were obtained, compared to what would have been possible with insoluble, raw coal.

As shown in the growth culture (Fig. 1), *P. cepacia* DLC-07 grew in the minimal medium, which contained coal as the only source of carbon and energy. A cell density of 10^7 to 10^8 cells/mL was reached after 1 d of incubation, and a population of more than 10^6 cells/mL was maintained for as long as 10 wk. This indicates indirectly, but strongly, that the bacterium utilized polymeric coal as a source of carbon and energy, particularly because the purified substrate was essentially free of low molecular weight impurities (Fig. 2).

The HPLC data (Fig. 2) shows that, during growth on the coal, *P. cepacia* DLC-07 partially depolymerized the lignite substrate. During elution of samples, the pH of the mobile phase was maintained slightly basic to prevent any clogging of the column. In addition, acid precipitated coal

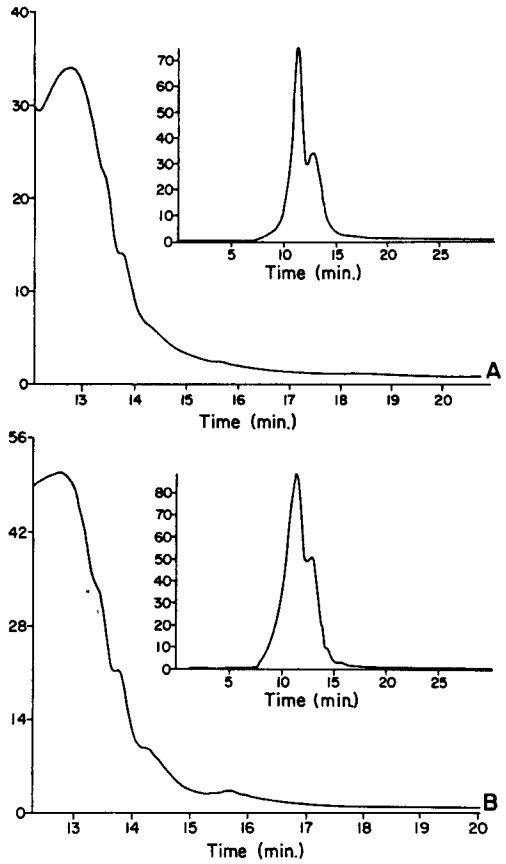

Fig. 2. HPLC elution profiles of control and 6 w *Pseudomonas cepacia* DLC-07 treated Vermont lignite coals. (**A**), Control; (**B**), 6 w treated coal. Absorbence values on the *Y* axis are in terms of milliabsorbence units. The insets show the complete HPLC chromatograms (0–25 min), whereas the larger figures show a blow-up of the chromatograms for retention times 12–20 min.

products were not allowed to dry prior to injection into the HPLC in order to minimize any reassociation of depolymerized coal components. The starting coal exhibited sharp peaks with retention times (RT) of 11.36 and 12.80 min, and a shoulder peak at 13.80 min. Control samples were never different from the starting coal. Although the HPLC profiles of 2-, 3-, 4-, and 6-wk-old samples from the inoculated culture (only control and 6 wk chromatograms shown) showed these same peaks, there was a steady increase in the relative amount of the 12.80 and 13.80 min peaks, and shoulder peaks appeared at RT = 14.25 and RT = 15.65 min. The approximate molecular weight values for the peaks were calculated from a standard curve (Fig. 3), plotted as log molecular weight vs retention time using protein standards. This curve had a coefficient of linearity of 0.96143. The molecular weights for the different retention times were calculated using the equation $\log_{10}$ (mol wt) = (7.1031 − 0.1827 × RT). The

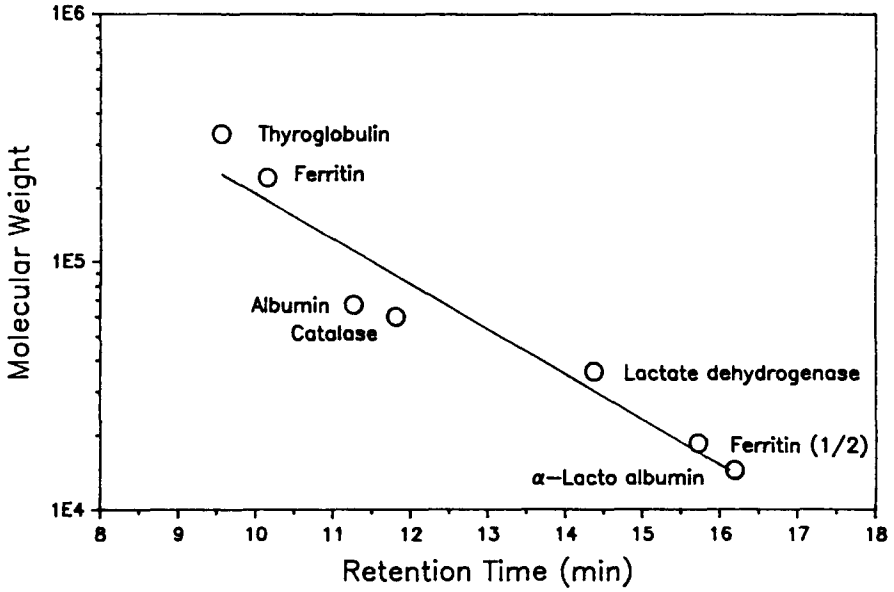

Fig. 3. Standard curve of protein molecular weights vs retention time on the Synchropak GPC-300 HPLC column.

Table 1
Elemental Analysis of Control and *Pseudomonas cepacia* DLC-07-Treated Vermont Lignite Coal Samples

	Elemental composition, % dry weight basis					
Coal sample	C	H	N	O	S	Ash
Control	49.4	4.3	4.9	33.8	0.8	3.6
Treated (15 d)	51.8	4.3	4.4	34.1	1.0	3.4
Treated (120 d)	52.8	4.0	3.0	31.2	0.9	3.2

peaks at 11.36, 12.80, 13.80, 14.25, and 15.65 min had calculated mol wts of 106,568, 58,149, 38,180, 31,595, and 17,533, respectively. Thus, though much of the high molecular weight peak of the starting coal remained after 6 wk incubation, *P. cepacia* DLC-07 clearly partially depolymerized the coal. Although the exact molecular weight values observed by HPLC cannot be guaranteed, because of possible column-coal polymer interactions, the relative changes in molecular weight distributions over time for the inoculated culture are a clear indication that microbially-catalyzed depolymerization reactions were occurring.

In the experiment where the coal medium was inoculated with 10 mL broth seed culture or a loopful of cell suspension, we performed elemental analyses on samples harvested at different time intervals (Table 1). There was little change in elemental composition of microbially-treated

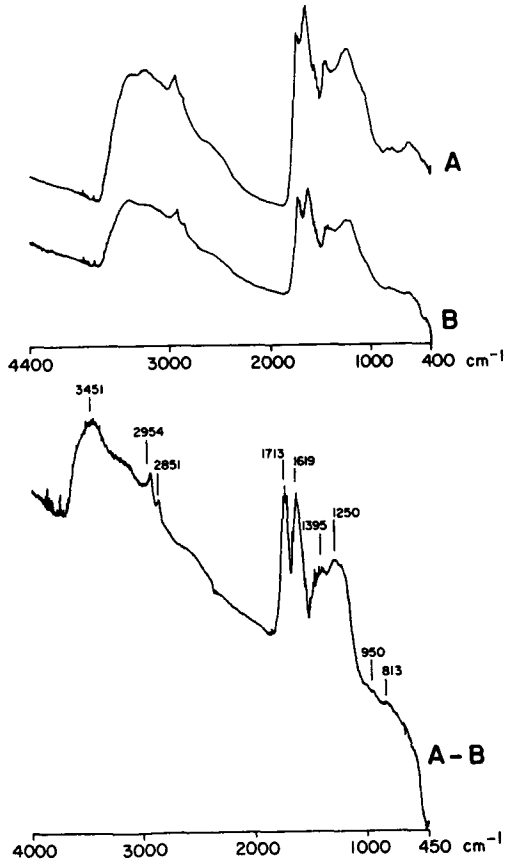

Fig. 4. FT-IR spectra of control coal vs a 120 d treated coal sample. (**A**), Control; (**B**), 120 d treated coal; (**A – B**), Control minus 120 d difference spectrum.

coal samples, compared to the control after 15 d incubation. The biggest change was the approximate 10% decrease in coal nitrogen content. After 120 d, differences were more obvious and included a 5% increase in relative carbon content, a 39% reduction in nitrogen content, and an 8% decrease in oxygen content. The hydrogen content was reduced by approximately 7%, whereas the sulfur content appeared to increase by about 12%. The most significant effect was the reduction in the nitrogen content.

IR spectroscopy supported the conclusions drawn from the elemental analyses (Fig. 4). The difference spectrum for control coal vs samples from 120-d-old inoculated cultures showed very intense bands at 1713, 1619, 1395, and 1250 cm^{-1}, indicating a decrease of carbonyl content (aldehyde, ketone, and carboxyl groups), aromatic and conjugated C–C bonds, and etheric oxygen of the type Ph–$OCOCH_3$ or Ph–CH_2–OCH_3 (*6,7*).

Solid state ^{13}C NMR spectroscopy (see ref. *15* and *22* for discussions of NMR spectra of coals and lignins) also provided valuable information on the changes that occurred during bacterial activity on the coal. Figure 5

Fig. 5. ^{13}C-(CP/MAS) NMR spectra of control and 120 d *Pseudomonas cepacia* DLC-07 treated coal samples. **A**, Control coal; **B**, 120 d treated coal.

shows the spectra of control and 120 d microbially-treated samples. The band at 165–185 ppm is greatly decreased in the treated coal, indicating a decrease in carbonyl carbons and carbons of heteroaromatic rings containing N, O, and S. Also, a decrease in unsubstituted aromatic carbons in the treated coal is indicated by the lack of a shoulder around 100–110 ppm. Flattening of the region 65–90 ppm also indicates a loss of carbons (alpha and beta) of the β-O-4 lignin struture. Interestingly, carbons of the $-OCH_3$ type did not appear to be appreciably decreased. There was a large increase in signals in the treated coal, representing long chain methylenes and hydroaromatic rings, which is indicated by the increase and sharpening of the area between 28.5 and 34.5 ppm. Overall, these ^{13}C NMR results show that coal metabolism by *P. cepacia* DLC-07 resulted in a net decrease in carbon atoms representing carbonyl, heteroaromatic, heterocyclic, and β-O-4 lignin-line structures. These results provide added support for the elemental and IR data.

The results strongly support the conclusion that *P. cepacia* DLC-07 chemically modifies and depolymerizes lignite coal. We also grew this strain on a number of model compounds (Table 2). The organism cometabolized numerous compounds, including heterocyclic, heteroaromatic, and lignin model compounds. Whereas uninoculated controls showed no changes in UV-visible spectra over time, when *P. cepacia* cometabolized a substrate, the spectrum was either significantly altered, reduced in intensity, or removed from the medium (Table 2). In fact, strain DLC-07 grew on *p*-hydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, *p*-coumaric acid, and caffeic acid as sole carbon sources in a minimal medium. Thus, *P. cepacia* DLC-07 is versatile in its ability to utilize and/or modify aromatic compounds with structures like those in coals and lignins.

The catabolic versatility of *Pseudomonas* species is well known (*20*), and *P. cepacia* is one of the most versatile (*12*) Odier et al. (*16*) studied the

Table 2
Cometabolism of Coal and Lignin Substructure Model Compounds by *Pseudomonas cepacia* DLC-07 as Determined by Monitoring Ultraviolet/Visible Spectra in Culture Medium Samples for 3 d[a]

Compound	Cometabolism	Compound	Cometabolism
Toulene	+	2,2′-Dihydroxybiphenyl	+ + +
Xylene	+ +	4,4′-Dihydroxybiphenyl	−
Naphthalene	+[b]	Bibenzyl	+ +
Anthracene	+	Benzoic acid	+ +
Phenanthracene	+	*p*-Hydroxybenzoic acid	+
Acenaphthene	+[b]	2,4-Dihydroxybenzoic acid	+ +
Fluorene	+[b]	*p*-Hydroxyphenylacetic acid	+
9-Fluorenone	+	*p*-Hydroxypropanoic acid	+
Phenol	+	Vanillic acid	+ +
Resorcinol	+	Vanillyl alcohol	+ +
Catechol	+ + +	Vanillin	+ + +
Guaicol	+	3,4-Dihydroxybenzaldehyde	+ + +
Veratrol	+[b]	Vanillylacetone	+ + +
p-Cresol	+	Syringaldehyde	+ + +
2-Napthol	+ +	Syringic Acid	+
Biphenyl	+	Cinnamic Acid	+
Trans-stilbene	−	*p*-Coumaric Acid	+ + +
Ferulic acid	+ + +	Caffeic Acid	+ + +
Diphenic acid	+ +	Guaiacylglycerol ether	+
Lignin model 1[c]	+ + +	2-Hydroxy-1, 4-naphthoquinone	+ + +[b]
Lignin model 2[d]	+ +	4-Methylumbelliferone	+ + +
Lignin model 3[e]	+ + +	5,7-Dihydroxyflavone	+
Dibenzofuran	+ + +[b]	Indole	+ + +
Thianapthene	+	Carbazole	+ +
Dibenzothiophene	+ + +[b]	8-Hydroxyquinoline	+ +

+, Some changes in spectrum of compound observed; + +, Major changes in spectrum of compound observed; + + +, Total removal of spectrum of compound observed; and −, No change in spectrum.

[a]Chemicals were obtained from commercial sources or synthesized. Cultures were grown in 250 mL flask cultures of Sabouraud Dextrose Broth supplemented with 5 mg of specific chemical, which was added after 18–20 h incubation (*see* Materials and Methods section).

[b]Seven day incubation period; all others were 3 d.

[c]1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-3-hydroxypropan-1-one.

[d]1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propane-1, 3-diol.

[e]1-(4-Hydroxy-3-methoxyphenyl)-2-(4-methylumbelliferone ether)-ethanol.

catabolism of lignin and lignin model compounds by *P. cepacia* strain 122 and concluded that it carried out Cα–Cβ cleavage during the degradation of arylglycerol-B-aryl ethers. *P. cepacia* AC100 was reported to degrade the herbicide 2,4,5-T and other halohydrocarbons (*2,11*). Thus, it is not surprising that *P. cepacia* might also attack structures within coal.

Strain DLC-07 appears to depolymerize water soluble lignite without further oxidizing it. Thus, the fuel value of the coal is not adversely affected. We must now determine if the organism will depolymerize base-solubilized coal that has not been pretreated with nitric acid. An unexpected, but fortuitous result was the observed 39% reduction in nitrogen

content of the soluble lignite after treatment with *P. cepacia*. Follow-up studies are needed to determine the nature of the nitrogen that is removed by microbial action.

ACKNOWLEDGMENTS

This research was supported by grant DE-FG22-88PC88919 from the US Department of Energy-Pittsburg Energy Technology Center, by a subcontract from EG&G Idaho, Inc., funded by Department of Energy Contract DE-AC07-76IDO1570, and by the Idaho Agricultural Experiment Station (#89512). We thank Margaret LaMarche for excellent technical assistance during this research.

REFERENCES

1. Buchanon, R. E. and Gibbon, N. E. (1974), *Bergey's Manual of Determinative Bacteriology,* Williams and Wilkins, New York, p. 232.
2. Chatterjee, D. K., Kilbane, J. J., and Chakrabarty, A. M. (1982), *Appl. Environ. Microbiol.* **44,** 514.
3. Cohen, M. S. and Gabriele, P. D. (1982), *Appl. Environ. Microbiol.* **44,** 23.
4. Cohen, M. S., Bowers, W. C., Aronson, H., and Gray, E. T., Jr. (1987), *Appl. Environ. Microbiol.* **53,** 2840.
5. Cohen, M. S., Aronson, H., Feldman, K., and Gray, E. T., Jr. (1989), *Proc. Thirteenth Ann. EPRI Conf. on Fuel Science and Conversion,* EPRI, Palo Alto, CA, p. 9.3.
6. Dryden, I. G. C. (1963), *Chemistry of Coal Utilization,* suppl. vol. (Lowry, H. H., ed.), Wiley, New York, p. 232.
7. Evans, D. G. and Hooper, R. J. (1981), *Coal Structure* (Gorbaty, M. L. and Ouchi, K., eds.), Amer. Chem. Soc., Washington, DC, p. 195.
8. Faison, B. D. and Lewis, S. N. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 743.
9. Fakoussa, R. M. and Truper, H. G. (1983), *Kolloquim in der Bergbau-Forschung Gmblt.* (Rehm, H. J., ed.), Steinkohlenbergauverein, Essen, FRG, p. 41.
10. Gupta, R. K., Spiker, J. K., and Crawford, D. L. (1988), *Can. J. Microbiol.* **34,** 667.
11. Kilbane, J. J., Chatterjee, D. K., Karns, J. S., Kellogg, S. T., and Chakrabarty, A. M. (1982), *Appl. Environ. Microbiol.* **44,** 72.
12. Lessie, T. G. and Gaffney, T. (1986), *The Bacteria,* vol X (Sokatch, J. R., ed.), Academic, New York, p. 39.
13. Maka, A., Srivastava, V. J., Kilbone, J. J., II, and Akin, C. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 715.
14. Moolick, R. T., Linden, J. C., and Karim, M. N. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 731.
15. Nimz, H. D., Robert, O. F., and Neur, M. (1981), *Holzforschung* **35,** 16.
16. Odier, E. and Rolando, C. (1985), *Biochemie* **67,** 191.
17. Quigley, D. R., Breckenridge, C. R., Dugan, P. R., and Ward, B. (1988), *Amer. Chem. Soc. Div. Fuel Chem. Reprints* **33,** 580.

18. Quigley, D. R., Wey, J. E., Breckenridge, C. R., and Stoner, D. L. (1988), *Resourc. Conserv. Recycl.* **1,** 163.
19. Quigley, D. R., Ward, B., Crawford, D. L., Hatcher, H. J., and Dugan, P. R. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 753.
20. Palleroni, N. J. (1986), *The Bacteria,* vol. X (Sokatch, J. R., ed.), Academic, New York, p. 10.
21. Pyne, J. W., Stewert, D. L., Frederickson, J., and Wilson, B. W. (1987), *Appl. Environ. Microbiol.* **53,** 2844.
22. Rosenberger, H., Scheler, G., and Kunstner, E. (1988), *Fuel* **67,** 508.
23. Scott, C. D., Strandberg, G. W., and Lewis, S. N. (1986), *Biotechnol. Prog.* **2,** 131.
24. Strandberg, G. W. and Lewis, S. N. (1987), *J. Ind. Microbiol.* **1,** 371.
25. Ward, B. (1985), *Syst. Appl. Microbiol.* **6,** 236.
26. Wilson, B. W., Bean, R. M., Franz, J. A., Thomas, B. L., Cohen, M. S., Aronson, H., and Gray, E. T., Jr. (1987), *Energy Fuels* **1,** 80.
27. Wilson, B. W., Frederickson, J., Stewert, D. L., Thomas, B. L., Campbell, J. A., and Bean, R. M. (1989), *Proc. Thirteenth Ann. Conf. on Fuel Science and Conversion,* EPRI. Palo Alto, CA, p. 3.3.
28. Wondrack, L., Szanto, M., and Wood, W. A. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 765.
29. Yeh, G. J. C., Ward, B., Quigley, D. R., Crawford, D. L., and Meuzalaar, H. L. C. (1988), *Amer. Chem. Soc. Div. Fuel Chem. Reprints* **33,** 612.