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MICROBIAL REMOVAL OF ORGANIC SULFUR FROM COAL (BACTERIAL DEGRADATION OF SULFUR-CONTAINING HETEROCYCLIC COMPOUNDS)

B. Klubek **D. Clark**

FINAL REPORT
March 1, - December 31, 1987

Southern Illinois University at Carbondale
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by

Brian Klubek
Department of Plant and Soil Science

David Clark
Department of Microbiology

Southern Illinois University at Carbondale
Carbondale, Illinois 62901

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DISCLAIMER

ABSTRACT

The presence of substantial levels of sulfur in coal is a major source of air pollution, and considerable efforts are being made to devise a cost-effective way of removing it. One promising method is microbial desulfurization. Almost all of the inorganic sulfur can be removed from coal by the bacteria *Thiobacillus* or *Sulfolobus*, which convert sulfide to sulfate but leave the organic sulfur untouched. If strains of bacteria are developed which remove organic sulfur from coal and are used in conjunction with inorganic sulfur-oxidizing bacteria, the result should be an effective desulfurization method. We are using two approaches to develop bacteria which remove organic sulfur. One method is to mutate a laboratory species, *Escherichia coli*, an organism which is genetically well understood and whose pathways for the metabolism of sulfur-containing amino acids have been extensively investigated. Such thiophene degraders can be genetically analyzed, and the genes involved can be cloned in order to amplify their products. The second approach is the development of naturally occurring bacteria capable of thiophene degradation. Enrichment culture techniques, mutagenesis of current isolated strains, and mixed culture studies with crushed coal comprise an alternative approach in our study. The degradation rates of our model-thiophene compounds and the preliminary testing of our isolates with coal will index the efficiency of our strains in coal desulfurization. Ultimately, the genes responsible for thiophene degradation by our isolated strains will be transferred to our *E. coli* strain, creating a single organism capable of degrading a broad spectrum of thiophene compounds.

INTRODUCTION

Utilization of domestic coal within the U.S. has increased dramatically since 1970. Coal production is expected to double by the year 1990, with Appalachian and Midwest fields as the largest single sources (Dugan and Apel 1978). One problem associated with the utilization of such coal is the emission of SO_2 during its direct combustion. Physical and chemical methods of coal desulfurization are either expensive or result in a loss of fine coal particles (Meyers 1977, Wheelock 1977, Elliot 1978). The sulfur content of Appalachian and midwestern coal varies from 3.0 to 5.5% (Dugan and Apel 1978). This sulfur is a mixture of inorganic sulfur (mostly pyrites) and organic sulfur (thiols, sulfides, disulfides, and thiophene groups) (Wheelock 1977, Chandra et al. 1979).

Microbial desulfurization of coal before combustion should cost less and be more energy efficient than high-temperature chemical processes (Dugan and Apel 1978, Detz and Barvinchak 1979). The removal of sulfur compounds from coal via microbial catalysis can also be accomplished without any loss of fine coal particles. The large deposits of high-sulfur coal east of the Mississippi River and the presence of eastern markets provide economic incentives for the removal of sulfur from coal.

Both *Thiobacillus* and *Sulfolobus* are capable of converting the inorganic sulfur in coal into soluble sulfate which can be washed out. Our intention is to complement this by developing bacteria capable of degrading organic sulfur into sulfate or sulfide.

METHODS

Isolation of Thiophene-Degrading Bacteria from Soil

Soil samples (0 to 15 cm deep) were collected from various locations in southern Illinois. These samples were then sieved to remove coarse fragments and used

for the isolation of thiophene-degrading bacteria employing enrichment culture techniques (Klubek and Clark 1985, 1986). Subsamples from these enrichment cultures were taken over time and transferred to a minimal salts medium for the isolation of bacteria capable of degrading dibenzothiophene (DBT), thiophene acetic acid (TAA), or thiophene carboxylic acid (TCA). In later experiments, tetrahydrothiophene (THT), 2-methylthiophene (2-MT), 3-methylthiophene (3-MT), thiophenemethanol (TMOH), thiophenemethylamine (TMA), dibenzothiophene sulfone (DBTS), benzene sulfonic acid (BSA), cystine (CYI), and 3-formyl-2-hydroxybenzothiophene were used as alternative substrates for growth. Those isolates which showed the best growth were chosen for further strain characterization and preliminary experiments with coal.

Characterization of Isolates for the Desulfurization of Model Compounds

To assess the desulfurization potential of our isolated strains specific for their respective model compound, sulfate-sulfur was determined after a 15-day incubation period using the methodologies described by Klubek and Clark (1986,1987). Sulfate was determined from the partitioned water fraction employing the procedure described by Bardsley and Lancaster (1965). The amount of remaining substrate (model compound) and possible intermediates of metabolism are currently being determined by HPLC analysis.

Preliminary Experiments for Coal Desulfurization

Five g of crushed Illinois No. 5 coal ($\leqq$ 250 µm particle size) was added to 50 mL of a mineral salts medium. Additional treatments included the addition of 0.05% succinate, 0.05% yeast extract, or an 0.05% solution of a model compound. A non-inoculated control was also included for each tested strain. All of the treatments were replicated three times and incubated for 5 days at 30°C in a reciprocal shaker. Following incubation, each treatment was filtered and the collected coal was then

air-dried for total S analysis. The collected filtrate was saved for the determination of sulfate-sulfur as previously described.

Genetic Procedures

The detailed procedures used for mutations, mapping, and genetic analysis of thiophene degrading strains of *E. coli* have been published (Abdulrashid and Clark 1987). More recent work has included three point crosses to order genes. These were performed by transduction using the previously published methods.

Cloning of the *E. coli* thiophene degrading genes has used methods detailed in Maniatis et al. (1982) and in the recombinant DNA volumes of the Methods in Enzymology series. In brief, the procedures used were as follows. Plasmid DNA was isolated from plasmid-bearing cells after amplification with chloramphenicol to improve the yield. Cells were lysed by lysozme, tris, and EDTA, and the plasmid DNA was purified by cesium chloride gradient ultra-centrifugation. Chromosomal DNA was purified by the phenol/isoamyl alcohol extraction procedure followed by ethanol precipitation. Restriction and ligation reactions were carried out under standard conditions in buffers specified by the manufacturers of the enzymes used. Plasmids constructed were transformed into recipient cells by either the standard calcium chloride procedure or the newer rubidium/DMSO method.

Enzyme Assays

Sulfite reductase was assayed using sulfite and NADPH as substrates (deVito and Dreyfuss 1964) and rhodanese by the cyanide/thiosulfate method (Leiminger and Westly 1968).

RESULTS

Characterization of Isolates on the Desulfurization of Model Compounds

Table 1 summarizes the final characterization of our strains that degrade dibenzothiophene (DBT). Selection of these strains for future experiments on strain improvement was based on the ability of each organism to form sulfate from DBT. Strains B_{wt}, 89N, and 89T were selected based upon the net change in sulfate-sulfur as determined by the Least Significant Difference (LSD) test and the percent desulfurization of DBT. From these strains, further selection for strain improvement by mutation will be based upon the detection of plasmids if they occur in these organisms.

Table 1

Final Characterization of Selected DBT-Decomposing Microorganisms*

Strain	$SO_4^=$-S Concentration (ppm)			% Desulfurization
	Initial	Final	Net Change*	
89B	9.25	8.03	-1.23	0
B_{wt}	5.50	9.18	3.70	7.4
89N	8.75	11.8	3.18	6.4
89R	8.25	9.2	0.95	1.9
89S	6.0	7.45	1.45	2.9
89T	6.5	8.48	1.98	4.0

*Statistical significance: strain LSD = 2.18, significant at $P<0.05$.

We are completing similar experiments with dibenzothiophene sulfone, benzene sulfonic acid, thiophene carboxylic acid, and cystine. The results for DBTS

are summarized in Table 2, where desulfurization rates of 64.4 to 91.3% were determined after a 15-day incubation period. Statistical analysis of the net change in $SO_4^=$-S will allow us to choose the best strains for mutation and enhanced desulfurization activity. The desulfurization of benzene sulfonic acid, thiophene carboxylic acid, and cystine will also be statistically analyzed to identify the best strains for improved desulfurization activity. We are also determining the intermediate products of the metabolism of these model compounds and the amount of remaining substrate (model compound) by HPLC-UV diode array chromatography.

Table 2

Final Characterization of Selected DBTS-Decomposing Microorganisms*

Strain	$SO_4^=$-S Concentration (ppm)			% Desulfurization
	Initial	Final	Net Change*	
2	13.5	78.9	64.5	86.0
4	14.5	70.5	56.0	74.7
18	18.0	66.3	48.3	64.4
19B	15.5	84.0	68.5	91.3
22B	19.0	78.0	59.0	78.7
26	7.0	65.8	58.8	78.7

*75 ppm S as DBTS.

Preliminary Experiments for Coal Desulfurization

We have been evaluating the potential of some of our natural isolates and *E. coli* strains to remove organic sulfur from Illinois No. 5 coal ($\leqq$ 250 μm particle size). Table 3 summarizes these results. Coal samples that were not pretreated with nitric acid showed a 0 to 3.6% rate in desulfurization. However, pretreatment of the coal

Table 3

Characterization of Some Isolated Strains for the Desulfurization Potential of Illinois No. 5 Coal*

Strain/Treatment	Final $SO_4^=$-S Concentration (ppm)		Total S in Coal		% Desulfurization of Coal	
	Non-Acid Wash	Acid Wash	Non-Acid Wash	Acid Wash	Non-Acid Wash	Acid Wash
2MT-5						
Non-Inoculated Control	14.5		1.83			
Inoculated	15.7		1.81		1.1	
Inoculated + 0.05% Succinate	17.5		1.79		2.2	
Inoculated + 0.05% Yeast Extract	16.8		1.82		0.5	
THT-1						
Non-Inoculated Control	14.4		1.93			
Inoculated	20.2		1.95		0	
Inoculated + 0.05% Succinate	4.7		1.91		1.0	
Inoculated + 0.05% Yeast Extract	19.9		1.91		1.0	
2MT-8						
Non-Inoculated Control	15.5		1.87			
Inoculated	17.2		1.86		0.5	
Inoculated + 0.05% Succinate	18.6		1.89		0	
Inoculated + 0.05% Yeast Extract	17.7		1.90		0	
DC625						
Non-Inoculated Control	21.1	24.6	1.83	1.41		
Inoculated	24.3	27.5	1.81	1.40	1.1	0.7
Inoculated + 0.05% Succinate	14.8	28.8	1.82	1.41	0.5	0
Inoculated + 0.05% Yeast Extract	18.3	29.7	1.84	1.38	0	2.1
NAR 41						
Non-Inoculated Control	19.7	24.6	1.81	1.43		
Inoculated	22.0	25.1	1.84	1.40	0	2.1
Inoculated + 0.05% Succinate	17.2	29.7	1.79	1.40	1.1	2.1
Inoculated + 0.05% Yeast Extract	20.6	32.3	1.80	1.40	0.5	1.4
NAR 30						
Non-Inoculated Control	21.1	24.6	1.87	1.42		
Inoculated	20.5	16.2	1.85	1.40	1.1	1.4
Inoculated + 0.05% Succinate	19.2	17.2	1.85	1.40	1.1	1.4
Inoculated + 0.05% Yeast Extract	21.7	17.8	1.86	1.39	0.5	2.1

*Five g of Illinois No. 5 coal (60 mesh) was added to 50 mL of growth medium.

Table 3

Characterization of Some Isolated Strains for the Desulfurization Potential of Illinois No. 5 Coal*

Strain/Treatment	Final $SO_4^=$-S Concentration (ppm)		Total S in Coal		% Desulfurization of Coal	
	Non-Acid Wash	Acid Wash	Non-Acid Wash	Acid Wash	Non-Acid Wash	Acid Wash
CY-66						
Non-Inoculated Control	21.1		1.93			
Inoculated	21.2		1.89		2.1	
Inoculated + 0.05% Succinate	23.2		1.87		3.1	
Inoculated + 0.05% Yeast Extract	26.2		1.86		3.6	
BS-51						
Non-Inoculated Control	11.5		1.85			
Inoculated	16.0		1.84		0.5	
Inoculated + 0.05% Succinate	13.3		1.83		1.1	
Inoculated + 0.05% Yeast Extract	16.1		1.84		0.5	
TRP-11						
Non-Inoculated Control	11.5		1.85			
Inoculated	17.4		1.85		0	
Inoculated + 0.05% Succinate	12.0		1.82		1.6	
Inoculated + 0.05% Yeast Extract	13.8		1.82		1.6	
DBTS-18						
Non-Inoculated Control	11.5	24.6	1.85			
Inoculated	9.0	23.1	1.84		0.5	3.5
Inoculated + 0.05% Dibenzothiophene	21.4	37.8	1.86		0	0
Inoculated + 0.05% Yeast Extract	1.9	35.0	1.83	1.46	1.1	0
89-Q						
Non-Inoculated Control	15.7	24.6	1.91	1.41		
Inoculated	14.7	24.4	1.90	1.37	0.5	2.8
Inoculated + 0.05% Dibenzothiophene	12.5	29.6	1.95	1.32	0	6.4
Inoculated + 0.05% Yeast Extract	17.4	26.9	1.90	1.35	0.5	4.2
B_{wt}						
Non-Inoculated Control		24.6		1.28		
Inoculated		21.8		1.31		0
Inoculated + 0.05% Succinate		22.3		1.30		0
Inoculated + 0.05% Yeast Extract		20.7		1.28		0

*Five g of Illinois No. 5 coal (60 mesh) was added to 50 mL of growth medium.

Table 3

Characterization of Some Isolated Strains for the Desulfurization Potential of Illinois No. 5 Coal*

Strain/Treatment	Final $SO_4^=$-S Concentration (ppm)		Total S in Coal		% Desulfurization of Coal	
	Non-Acid Wash	Acid Wash	Non-Acid Wash	Acid Wash	Non-Acid Wash	Acid Wash
BS-56						
Non-Inoculated Control		24.6		1.28		
Inoculated		23.9		1.24		3.1
Inoculated + 0.05% Succinate		24.5		1.28		0
Inoculated + 0.05% Yeast Extract		19.2		1.29		0
DBTS-4						
Control		24.6		1.42		
Inoculated		31.2		1.37		3.5
Inoculated + 0.05% Succinate		34.0		1.43		0
Inoculated + 0.05% Yeast Extract		39.9		1.46		0
CYI-64						
Control		24.6		1.42		
Inoculated		19.6		1.41		0.7
Inoculated + 0.05% Succinate		19.7		1.38		2.8
Inoculated + 0.05% Yeast Extract		21.7		1.39		2.1
84-B2						
Control		24.6		1.42		
Inoculated		23.6		1.32		7.04
Inoculated + 0.05% Succinate		23.5		1.36		4.2
Inoculated + 0.05% Yeast Extract		20.0		1.33		6.33

*Five g of Illinois No. 5 coal (60 mesh) was added to 50 mL of growth medium.

with nitric acid showed a broader range in the desulfurization of this coal (0 to 7.04%), indicating that pyritic sulfur and possibly heavy metals associated with the non-acid washed coal may inhibit the growth of the applied inoculum. Increases in sulfate-sulfur were also observed from those coal samples without pretreatment and corresponded to the determined desulfurization rates. H_2S may have been formed but was not determined. This may be especially true for strain 84-B2 where the final concentration of sulfate-sulfur did not correspond to the reduction in the S content of the coal. Nevertheless, the best two strains from these coal desulfurization studies were 89-Q and 84-B2.

Cloning of *E. coli thd* genes

We started by cloning the first of the three *thd* genes of the thiophene degrading mutant NAR30, i.e., *thd A*.

This work has involved several stages:

1. Isolation and purification of the multicopy vector pUC19.
2. Linearization of the vector plasmid at the multiple cloning site by using restriction endonucleases Pst1, Kpn1, Sa11, and Sma1.
3. Extraction and digestion of chromosomal DNA from a derivative of NAR30 carrying tetracycline resistance close to the *thdA* gene by using the same restriction endonucleases.
4. Chromosomal DNA fragments were ligated to cloning vector.

Digestion and ligation of DNA fragments to the plasmid pUC19 were confirmed by running agarose gel electrophoresis. The ligated DNA was transformed back into strain DC625, which is a wild-type *E. coli* and the parent of NAR30. Transformants were selected on plates containing ampicillin and 0.1% furfuryl alcohol and also on plates containing ampicillin and tetracycline. We originally isolated 3

clones, 2 of them from Pst1 digestion and one from Kpn1. All 3 clones are resistant to ampicillin and oxidize furfuryl alcohol. But all these clones seemed to have a tendency to lose the plasmid quickly. If the cloned plasmids are too big and also high in copy number, they get lost quickly. The presence of plasmids was confirmed in all these clones by doing mini plasmid preparations running gels and comparing with plasmid pUC19. Later experiments gave several stable isolates. These were obtained mostly by using SmaI and a couple with Kpn1. The plasmids in the stable isolates were substantially smaller. The inserted fragment of DNA, which carries the *thd A* gene was estimated to be approximately two kilobases by agarose gel electrophoresis. None of these plasmids contained the tetracycline gene located next to *thd A* and they were isolated by the selection procedure involving ampicillin (which selects for the vector pUC19) and the ability to use furfuryl alcohol.

The *thdA* mutation

NAR30 was made in three stages and shown to have three novel mutations (*thdA*, *thdC*, and *thdD*). However, we did not know how the gene location corresponded with the order of selection. Thus, the first mutant, NAR10 could slowly oxidize furans due to gaining one of the three *thd* mutations. We demonstrated that NAR10 in fact contains the *thdA* mutation by transducing NAR10 with P1 grown on SG20253 which contains Tn10 near *thdA*. We observed cotransduction between the furan oxidizing ability of NAR10 and Tn10. Thus *thdA* was the mutation selected first in our previous construction of NAR30. We were then able to transduce the *thdA* mutation of NAR10 back into the wild type, DC625, and created a new strain which possessed the same degradative properties as the original NAR10. In addition, we found that strain DC625 contains a plasmid that carries the cloned *thdA* gene from NAR30 and behaves much like NAR10. Thus increasing the copy number of the *thdA* mutation has little effect, at least in the absence of *thdC* and *D*.

Sulfur Transferase

For historical reasons, the enzyme which transfers sulfur between suitable molecules is called rhodanese. The reaction normally used to assay this enzyme is the transfer of sulfur from thiosulfate to cyanide to give sulfite and thiocyanate ("rhodanide" in German):

$$S_2O_3^{-2} + CN^- \rightarrow CNS^- + SO_3^{-2}$$

In the cell, rhodanese transfers sulfur between a variety of donors and acceptors. Previous work, mostly in animal cells, suggests that two or more enzymes share this function, though how the various sulfur transfer reactions are allocated among the two isozymes is unknown. Until recently, there have been no mutations affecting rhodanese activity in any organism and it has therefore been difficult to know precisely the necessary functions of this enzyme. We have isolated mutants affecting rhodanese levels by using selenium, an element which acts in biological systems as a toxic analog of sulfur.

Parental strain W1485 is killed by 1 mM selenite. Resistant mutants were selected on minimal agar with glucose as carbon source and incorporating 1 or 2 mM selenite. Many of these mutants were bright red when grown in the presence of selenite. This is due to the reduction of selenite (SeO_3^{2-}) to elemental selenium (Se°), which forms red amorphous crystals in the bacterial colonies. These mutants proved to require sulfite ($NaHSO_3$) for growth, but could not use the sulfur-containing amino acid cysteine. Genetic mapping indicates a location in the 27 to 28 min region of the *E. coli* chromosome (Table 4).

We assayed these strains and their parent, W1485, for rhodanese. The data (Table 5) showed greatly increased rhodanese levels in the selenium-resistant mutants (WL256 to 259) when these were grown in rich broth (RB). When grown in

Table 4

Mapping of Selenium Resistant Mutants

P1 Donor	Location of Tn (min)	% Cotransduction
RW11 *fadR*::Tn*10*	26	60
EE1 *chlC*::Tn*5*	27	90
EE201 *chlC*::Tn*5*	27	85
DC300 *zch*::Tn*10*	27	50
RK4913 *zch*::Tn*10*	27	40
NK6022 *trp*::Tn*10*	28	30

Cotransduction frequencies are averages for the three selenium resistant mutants WL256, 258, and 259. In all cases transductants were selected for resistance to tetracycline (Tn*10*) or kanamycin (Tn*5*) and then tested for selenium resistance.

Table 5

Rhodanese Assays

Strain	Rhodanese Activity (Units/Protein)		
	RB	MM	Ratio (RB/MM)
W1845	0.035	0.013	2.7
WL256	0.23	0.011	20.4
WL257	0.213	0.008	28.4
WL258	0.376	0.002	170.5
WL259	0.195	0.015	12.8
DC272	0.024	0.020	1.2
DD105	0.004	0.005	0.8

RB = grown in rich broth, MM = grown in minimal medium.

minimal medium with glucose as carbon source (MM), the parent W1485 and the mutants all showed low rhodanese activity (approximately 0.01). By good fortune, the Se-resistant mutation is close to the gene *adh*. Deletions of *adh* and nearby genes have been made previously and we examined these. Some strains carrying extensive deletions also require bisulfite, but when assayed for rhodanese, DD105, a deletion mutant, had reduced rhodanese levels in all media tested compared to parental strain DC272 (Table 5).

Our present theory is that there are indeed two rhodanese enzymes, one always present in lower amounts and unaffected by our mutations. A second enzyme is induced in rich medium, is present in larger amounts, is increased in Se-resistant mutants, and is abolished by deletions in the 27 to 28 min region of the chromosome. We have added a variety of organic sulfur compounds to the minimal medium, but none caused significant induction (data not shown). Thus, the specific nature of the inducer is still to be discovered.

We have also assayed rhodanese in the thiophene-degrading mutants NAR10, NAR20, and NAR30. However, all gave results equivalent to their parent DC625 (a close relative of DC272). We will be moving our rhodanese mutations into the thiophene degraders in the near future in order to test for any effect on thiophene metabolism.

We also assayed sulfite reductase as a representative enzyme of the sulfur reduction pathway in *E. coli*. The Se-resistant mutants showed an approximately two-fold increase relative to their parent W1485.

DISCUSSION

We are completing our evaluation of the natural isolates for their potential to desulfurize their model compounds. Degradation products from strains specific for dibenzothiophene, thiophene carboxylic acid, dibenzothiophene sulfone, benzene

sulfonic acid, and cystine are currently being determined. These strains, in addition to strains B_{wt}, 89N, and 89T, will be evaluated for the presence of plasmids and mutated for enhanced activity. Those strains indicating enhanced activity will be characterized for their potential to desulfurize their specific model compound. These strains will also be used as new sources of genetic material to be cloned into our improved strain of *E. coli*.

Our next approach is to evaluate our natural isolates and strains of *E. coli* developed on other coals, since Illinois No. 5 coal is primarily pyritic. Since a broader range in desulfurization rates was observed with the acid pretreatment, the data suggest that better success in the microbial desulfurization of organic S in coal may be achieved with coals that are characterized predominately by organic S. Illinois No. 6 is such a coal, and one that is characterized by simple thiophenes. We will use this coal to further assess our strains' ability to remove organic S from coal.

Additional studies with coal will also include the grinding of Illinois No. 6 coal to a particle size of $\leqq 10$ μm. The size of coal particles may be a more critical factor in the removal of organic S rather than the 20- or 60-mesh (250 μm) size required for the microbial removal of pyritic S from coal. We are also initiating studies of coal toxicity on our isolated and developed strains. Cultures will be added to coal slurry and incubated over time to assess their viability in the presence of coal and to assess their ability to grow on culture media treated with a coal extract. These experiments will provide us with information on the sensitivity of our strains to the presence of heavy metals in the coal or toxic organic compounds associated with coal. The data will serve as a base to select for resistant strains to both of these toxicities.

We have started the cloning of the thiophene degrading genes of our *E. coli* multiple mutant NAR30. We obtained a variety of isolates of which many were unstable and at first we thought that multiple copies of the *thdA* gene might be

detrimental. However, further work gave smaller, stable plasmids which carry *thdA* and these hybrid plasmids are not lost by the host cells. Presumably the original problems were due to some neighboring gene. In any case, we now have *thdA* cloned into the multicopy vector pUC19. In the future we hope to clone *thdC* and *thdD* also.

Apart from those involving amino acids, reactions transferring sulfur in cells are poorly understood. We made mutants with increased sulfur transferase activity by selecting for resistance to the toxic sulfur analog, selenium. Future work will assess the effect of sulfur transferase mutations on thiophene degradation.

SUMMARY

We have isolated several bacterial strains that have the ability to utilize dibenzothiophene, dibenzothiophene sulfone, benzene sulfonic acid, or cystine as a sole carbon source for growth. The desulfurization potentials varied from 0 to 91.3% as H_2S or sulfate depending on which strain was tested. We are currently determining the amount of substrate (model compound) that has remained after 15 days of incubation. Desulfurization studies with some of our isolated strains and mutants of *E. coli* on Illinois No. 5 coal showed low rates of sulfur removal (0 to 7%). These results suggest the possibility of coal toxicity on our tested strains. We were able to clone the *thdA* gene for thiophene degradation from *E. coli*. This gene is carried on a 2kb fragment of DNA, in our stable plasmid constructs. Strains carrying solely the clone *thdA* show enhanced furan oxidation but cannot degrade thiophenes; presumably *thdC* and *thdD* functions are required for full activity. Mutants with altered sulfur transferase levels have been isolated and mapped at 27 to 28 min on the *E. coli* chromosome. They are resistant to selenium toxicity and require sulfite for growth.

PUBLICATIONS, PAPERS AND PRESENTATIONS

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