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Isolation and Genetic Analysis of Mutations Allowing the Degradation of Furans and Thiophenes by *Escherichia coli*

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Successive mutations of *Escherichia coli* yielded a strain that was able to degrade a variety of heterocyclic oxygen- and sulfur-containing ring compounds. In particular, this strain could use both furan-2-carboxylic acid and thiophene-2-carboxylic acid as sole carbon and energy sources. Nitrogen-containing heterocyclic compounds were not degraded. This mutant was isolated by selecting first for oxidation of furan derivatives and then for thiophene degradation. Genetic analysis revealed that mutations in three novel genes, *thdA* (12 min), *thdC* (92 min), and *thdD* (98 min), were required for thiophene degradation. In addition, constitutivity at both of the previously characterized *fadR* and *atoC* loci was required for efficient thiophene breakdown. The pathway of furan and thiophene degradation remains obscure, but the inability of our mutants to degrade 5-nitro- or 5-bromo-substituted furan derivatives suggests that hydroxylation at position 5 may be involved. Thiophene derivatives were toxic when they were present at concentrations of 0.1% or greater; however, addition of trace amounts of phenylalanine plus tyrosine greatly reduced this effect.

Although compounds containing thiophene rings are rarely found in living organisms, they form a substantial part of the organic sulfur fraction of fossil deposits, such as crude oil and coal (14, 16). Since sulfur dioxide emission from the burning of high-sulfur midwestern United States coals is largely responsible for the production of acid rain, it is very important to develop bacteria that are capable of efficiently removing the sulfur from coal before combustion (10, 14, 16). Strains of *Thiobacillus* and *Sulfolobus* have been shown to effectively remove inorganic sulfur (i.e., iron sulfides) from coal (12–14); however, removal of organic sulfur remains a problem. Since around 60 to 70% of the organic sulfur found in typical high-sulfur Illinois coals consists of simple (mononuclear) thiophene derivatives (1), we decided to construct an organism capable of degrading the heterocyclic thiophene ring as a first step toward the production of an efficient organic sulfur-scavenging bacterium. Representative structures are shown in Fig. 1.

Although degradation of aromatic compounds is generally regarded as characteristic of *Pseudomonas* and related genera, it has recently been shown that *Escherichia coli* can degrade phenylacetic acid and related compounds (4, 8). Therefore, we decided that, since *E. coli* has been extensively characterized at the genetic level, it would be a sensible idea to isolate mutants of *E. coli* capable of degrading aromatic sulfur compounds, such as thiophenes. Our approach was to start with a wild-type *E. coli* strain that was capable of degrading phenylacetate and to successively select derivatives that were able to degrade furan derivatives and then the corresponding thiophene analogs. We were eventually able to isolate a mutant, strain NAR30, that was capable of degrading both thiophene and furan derivatives.

This strain was isolated after three rounds of selection and proved to carry three novel mutations required for thiophene and furan degradation. These mutations were in previously unidentified genes and were designated *thdA*, *thdC*, and *thD* (*thd* stands for thiophene degradation). The map locations of these three novel mutations were determined by using a new mapping procedure involving the use of a set of Hfr strains with Tn*10* insertions as proximally transferred markers (24). In addition to these novel genes, thiophene degradation required constitutive mutations in both the *fadR* (19) and *atoC* (20) genes.

MATERIALS AND METHODS

Bacterial strains and media. All of the strains used were *E. coli* K-12 strains; these organisms are listed in Table 1. Rich broth contained (per liter) 10 g of tryptone, 5 g of NaCl, and 1 g of yeast extract. The minimal medium used was M9 medium (15). Fatty acids and aromatic compounds were added to concentrations of 0.1% (wt/vol) when they were used as carbon sources, whereas sugars, succinate, glycerol, etc. were used at concentrations of 0.4%. Fatty acids were dissolved by adding the detergent Brij 58 (4 g/g of fatty acid) and an equimolar amount of KOH. Most amino acids were provided to auxotrophic strains at concentrations of 50

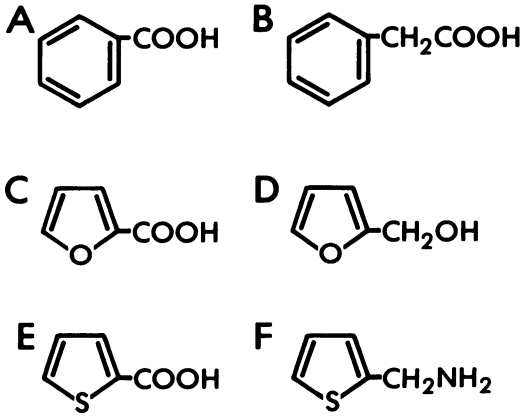

FIG. 1. Structures of aromatic substrates. (A) Benzoic acid. (B) Phenylacetic acid. (C) 2-Furoic acid. (D) Furfuryl alcohol. (E) Thiophene-2-carboxylic acid. (F) Thiophene-2-methylamine.

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TABLE 1. Bacterial strains

Strain	Relevant markers	Source
DC625	*nalA atoC fadR adhC mel-1 supF*	D. Clark
NAR10	First-stage thiophene-degrading mutant of DC625	See text
NAR20	Second-stage thiophene-degrading mutant of DC625	See text
NAR30	Third-stage thiophene-degrading mutant of DC625	See text
NAR33	Spontaneous streptomycin-resistant derivative of NAR30	See text
NAR40	Fourth-stage thiophene-degrading mutant of DC625	See text
W1485	Wild type, λ^-	B. Bachman
BW5659	HfrKL98 *zdh*::Tn*10 mglP1 xyl-7 lacY1*	B. Wanner
BW5660	HfrPK19 *srlC300*::Tn*10* Δ(*gpt-lac*)*205 supE44 thi-1*	B. Wanner
BW6156	HfrP4X *zje*::Tn*10 relA1 spoT1 metB1*	B. Wanner
BW6159	HfrKL14 *ilv*::Tn*10 relA1 spoT1 thi-1*	B. Wanner
BW6160	HfrBroda8 *zdh*::Tn*10 relA1 spoT1 metB1* λ^-	B. Wanner
BW6163	HfrKL16 *zed-977*::Tn*10 relA1 spoT1 thi-1*	B. Wanner
BW6164	HfrRa-2 *thr-43*::Tn*10 supE42 mal-28* (λ^r) *sfa-4*	B. Wanner
BW6165	HfrP801 *argE*::Tn*10* (λ Ind$^-$) *ara-41 lacY1* or *Y40 xyl-7 mtl-7*	B. Wanner
BW6166	HfrJ4(P10) *zhf*::Tn*10 thi-1 malB16* λ^-	B. Wanner
BW6169	HfrAB313 *argA*::Tn*10 leuB6 lacY1 supE44 gal-6 thi-1* λ^-	B. Wanner
BW6175	HfrPK3 *argE*::Tn*10 thr-1 leuB6 azi-15 tonA21 supE44 thi-1* λ^-	B. Wanner
BW7261	HfrC *leu63*::Tn*10 tonA22*Δ(*argF-lac*)*169 ompF627 relA1 spoT1* T2	B. Wanner
BW7620	HfrKL99 *zed-977*::Tn*10 supD lac-42 spoT1 thi-1* λ^-	B. Wanner
BW7622	HfrKL96 *trpB114*::Tn*10* λ^- *relA1 spoT1 thi-1*	B. Wanner
BW7623	HfrBroda7 *purE79*::Tn*10* λ^- *relA1? spoT1*	B. Wanner
NK6051	HfrHayes *purE79*::Tn*10*Δ(*gpt-lac*)*5 relA1 spoT1 thi-1*	N. Kleckner
DC757	*zcf*::Tn*10 atoC12*	D. Clark
DEV22	*mia*::Tn*10*	D. Elseviers
DF1062	*zjh-920*::Tn*10 araD139* Δ(*ara-leu*)*7697*Δ(*lacI-Y*)*74 galEl5 galK16 relA1 rpsL150 hsdR2* λ^-	D. Fraenkel
GR401	*cycA30*::Tn*10 araD139* Δ(*argF-lac*)*205 ptsF25 relA1 rpsL150 deoCl* Δ(*his-gnd*) *rbsR thi araE*? λ^-	I. Booth
LA5651	*zeg-722*::Tn*10 thr-1 leuB6 lacY1 phoA1 rpsL114 malA1 metB1 his gatA mglB55 gyrA*	Y. Nishimura
N3055	*uvrA277*::Tn*10* IN(*rrnD-rrnE*)*1* λ^-	R. G. Lloyd
RK4342	*zbd-601*::Tn*10 pro-3* Δ(*lac*)*6 entA403 supE44* (*glnV44*) *his-218 rpsL109 xyl-5* or *xyl-7 ilvC7 metB1* λ^-	R. Kadner
SG20253	*zba-300*::Tn*10* Δ*lac*	S. Gottesman
SK2257	*zbe-280*::Tn*10 thyA6 rpsL120 deoCl*?	S. Kushner
SK472	*zjj-202*::Tn*10 serB22 relA spoT1 thi-1* λ^-	S. Kushner
SS3242	*zji-2*::Tn*10 lacI22 lacZ his-85 rpsL azi-9 gyrA* λ^- P1^s	S. A. Short
YYC188	*zbc*::Tn*10 pps-4 pfl-1 poxB* Δ(*aceEF-aroP*) *str* λ?	Y. Y. Chang
BJW43	*zjd*::Tn*10 melA hisA323 mal*	B. J. Weiss

mg/liter; cysteine and the aromatic amino acids were used at concentrations of 25 mg/liter. Solid media contained 1.5% agar. The tetrazolium redox indicator plates used were the same as those described by Bochner and Savageau (3), except that M9 salts was used as the buffer and substrates were added at final concentrations of 0.1%.

Mutagenesis. An overnight culture was diluted 1 in 10 into fresh rich broth and was grown at 37°C to mid-exponential phase. Ethyl methanesulfonate was then added to a final concentration of 1.0% (vol/vol). Incubation of the culture at 37°C was continued for 60 min, after which the cells were harvested by centrifugation, washed, and suspended in fresh rich broth. The mutagen-treated culture was grown overnight to allow segregation before use in mutant-screening procedures.

Genetic procedures. A spontaneous streptomycin-resistant derivative of thiophene-degrading mutant NAR30 was selected on rich broth agar containing 100 mg of streptomycin per liter; this derivative was designated NAR33. Strain NAR33 was then crossed with each of a set of Hfr strains which had various origins of transfer; each of the Hfr strains carried a Tn*10* insertion proximal to its origin of transfer (24). Conjugational mating was carried out by standard procedures (15), as described previously (25). Exconjugants were selected on rich broth agar containing streptomycin (100 mg/liter) and tetracycline (10 mg/liter) and then tested on tetrazolium indicator plates containing 0.1% thiophene-2-carboxylic acid. White colonies on these plates indicated a loss of the ability to degrade thiophenes and hence cotransfer of the *thd* gene(s) with the Tn*10* insertion from the Hfr donor. Red colonies indicated retention of the thiophene-degrading ability. The Hfr::Tn*10* set was constructed by B. Wanner (24) and was provided by B. Bachmann of the *E. coli* Genetic Stock Center.

Cotransduction by using bacteriophage P1*vir* was performed substantially as described by Miller (15). Tetracycline-resistant transductants were selected on medium E (23) containing glucose (0.4%), casein hydrolysate (0.1%), and tetracycline (10 mg/liter). The transductants were then tested for degradative ability toward thiophenes as described above.

RESULTS

Preliminary observations indicated that wild-type *E. coli* K-12 was unable to degrade furan or thiophene derivatives but was able to grow very slowly on phenylacetate or benzoate (Fig. 1). As previously noted by Cooper (8), growth of *E. coli* on aromatic substrates is poor at 37°C and better at lower temperatures. We confirmed this and routinely used 33°C for growth with benzene, furan, or thiophene derivatives. We also found that wild-type *E. coli* K-12 could not

TABLE 2. Aromatic compounds as sole carbon sources[a]

Carbon source	Use as carbon source by strain:				
	DC625	NAR10	NAR20	NAR30	NAR40
Benzoate	+	+	+	++	++
Phenylacetate	+	++	++	++	++
3-Hydroxyphenylacetate	−	+/−	+	+	+
Furfuryl alcohol	−	−	+/−	+	+
Furan-2-carboxylate	−	−	+/−	+	+
Ascorbic acid	−	−	++	++	++
Thiophene-2-carboxylate	−	−	+/−	+	+
Thiophene-2-acetate	−	−	−	+	+
Amino-3-thiophene acetate	−	+/−	+	+	+
Thioproline	−	−	−	−	+/−
Homocysteine thiolactone hydrochloride	−	+/−	+	++	++
Picolinic acid	−	−	−	−	−

[a] M9 minimal agar (pH 7.5) containing a carbon source at a concentration of 0.1% was incubated at 33°C for 6 days. Symbols: −, +, and ++, increasing levels of growth.

use either thiophene-2-carboxylate (Fig. 1) or thiophene-2-acetate as a sulfur source.

Isolation of thiophene-degrading mutants. Parental strain DC625 was treated with the mutagen ethyl methanesulfonate and then plated to give single colonies on tetrazolium indicator plates containing the furan derivative furfuryl alcohol. Strain DC625 cannot oxidize furans and is consequently white on these indicator plates. We selected a mutant, NAR10, which was pink on furfuryl alcohol (Fig. 1) indicator medium, indicating moderate oxidation of this substrate. However, NAR10 could not use furfuryl alcohol as a sole carbon source

First-stage mutant NAR10 was further mutagenized, and we selected a second-stage isolate, NAR20, which was red on the furfuryl alcohol indicator medium. Mutant NAR20 was able to grow, albeit slowly, by using several furan derivatives as sole carbon and energy sources (Table 2). However, NAR20 was only pink on thiophene-2-carboxylate indicator plates. Therefore, third and fourth rounds of mutagenesis and selection were performed, and mutants NAR30 and NAR40, each successively redder on thiophene-2-carboxylic acid indicator plates, were isolated.

TABLE 3. Oxidation of aromatic compounds[a]

Carbon source	Oxidation by strain:				
	DC625	NAR10	NAR20	NAR30	NAR40
Benzoate	P	R	R	RR	R
Phenylacetate	P	R	R	R	R
3-Phenylpropionate	P	R	R	R	R
trans-Cinnamate	W	P	P	P	P
3-Hydroxyphenylacetate	W	P	P	P	P
4-Hydroxyphenylacetate	W	P	P	R	R
Thiosalicylate	W	W	W	W	P
Furfuryl alcohol	W	P	R	R	P
Furan-2-carboxylate	W	P	P	P	P
Thiophene-2-carboxylate	W	P	P	R	R
Thiophene-2-acetate	W	W	P	R	R
Thiophene methanol	W	P	P	R	W
Thiophene methylamine	W	P	P	R	P
Amino-3-thiophene acetate	W	P	P	R	R
Homocysteine thiolactone hydrochloride	P	R	R	R	R
Thioproline	W	W	P	R	R

[a] Oxidative ability was assessed on tetrazolium indicator plates containing a substrate at a concentration of 0.1%. W, White (no oxidation); P, pink (slight oxidation); R, red (moderate oxidation); RR, deep red (good oxidation).

TABLE 4. Toxicity of thiophene-2-carboxylate[a]

Strain	Growth with Thiophene-2-carboxylate at a concn of:					
	0.0%	0.05%	0.10%	0.15%	0.20%	0.25%
DC625	+	+/−	−	−	−	−
NAR10	+	+/−	−	−	−	−
NAR20	+	+	+/−	−	−	−
NAR30	+	+	++	+/−	−	−

[a] The plates contained M9 minimal medium and 0.4% succinate as a carbon source. The plates were incubated at 37°C for 3 days; −, +, and ++ indicate increasing levels of growth.

The ability of these successive mutants to oxidize aromatic compounds, as assessed by the tetrazolium indicator plate system, is shown in Table 3. In addition, these mutants and their parent were tested for ability to use a variety of aromatic substrates and related ring compounds as sole carbon and energy sources in M9 minimal medium (Table 2). It is clear that progressive improvement in degradative ability occurred in the series from NAR10 to NAR40; benezenoid compounds were generally more readily oxidized than furans, which in turn were easier to degrade than thiophene derivatives. However, none of the mutants was able to degrade the nitrogen-containing heterocyclic compounds pyrole-2-carboxylate and picolinic acid (i.e., pyridine-2-carboxylate). Mutant NAR10 showed superior oxidation of several compounds compared with strain DC625, but the second mutation leading to NAR20 was required to allow growth on a variety of novel substrates, including furans and ascorbic acid (whose ring form is similiar in structure to furans). Reasonable growth on several thiophene derivatives was found with NAR30, and the additional mutation in NAR40 extended the degradative ability to several other sulfur compounds, such as thioproline and thiosalicylate.

Although we used carboxylic acid derivatives for most of our isolation and screening procedures, it should be pointed out that both thiophene-2-methanol and thiophene-2-methylamine were oxidized by NAR30 to an extent comparable to the level of oxidation of thiophene-2-carboxylate (Table 3). The furan derivatives 5-bromofuran-2-carboxylate and 5-nitrofuran-2-carboxylate were also tested but were not oxidized by any mutant (data not shown). We also tested a few nonaromatic ring compounds (e.g., cyclohexanone and cyclohexane carboxylic acid); however, none of these was oxidized by any mutant (data not shown).

We also tested the possible use of thiophene methylamine (Fig. 1) as a nitrogen source by replacing the ammonium chloride in M9 succinate medium with 2 mM thiophene methylamine. Neither parent strain DC625 nor mutant NAR10 used this compound whereas NAR20, NAR30, and NAR40 grew well with thiophene methylamine as a nitrogen source.

Toxicity of thiophenes. One problem with growth on thiophene derivatives is that in addition to the question of catabolic breakdown, the thiophenes are inherently toxic. Table 4 shows that even when *E. coli* was provided with succinate as a carbon source, addition of thiophene-2-carboxylic acid prevented growth. The inhibitory concentration of thiophene-2-carboxylate was greater for mutants NAR20 and NAR30 than for parental strain DC625 (Table 4). Further investigation, in which thiophene-2-carboxylate was used as the sole carbon source, showed that growth was much better under mildly alkaline conditions (pH 7.5 or 8.0) than at pH 6.5 (the pH of unmodified M9 medium). Further-

TABLE 5. Mapping of thiophene-2-carboxylate genes[a]

Hfr Tn*10* donor	Origin of transfer (min)	Tn*10* location (min)	No. of exconjugants tested	% of cotransfer of Tn*10* with Thd
BW7623	30	12	100	63.0
NK6051	0/100	12	15	73.0
BW7622	44	28	200	74.5
BW6160	18	37	200	51.5
BW5659	44	37	100	0.0
BW7620	22	43	200	80.5
BW6163	61	43	100	33.0
BW5660	43	58	100	7.0
BW6169	85	61	200	13.5
BW6166	92	76	100	0.0
BW6159	61	82	200	13.5
BW6165	4	90	36	27.0
BW6175	79	90	200	94.5
BW6156	3	94	200	3.0
BW6164	88	100	200	63.5
BW7261	13	2	300	66.0

[a] All Hfr donors were crossed with NAR33 (Str^r derivative of NAR30). The exconjugants were selected by using streptomycin plus tetracycline and were tested for thiophene degradation (Thd).

more, at pH 6.5 growth was greatly stimulated by the addition of phenylalanine and tyrosine (25 mg/liter each).

Genetic mapping. Third-stage mutant NAR30 was the first strain to show the ability to degrade a wide range of benzenoid, furan, and thiophene derivatives. Therefore, we picked this mutant for genetic analysis. Initial mapping was conducted by selecting a derivative of NAR30, designated NAR33, which was resistant to streptomycin (100 mg/liter) and crossing it with the Hfr::Tn*10* set. Exconjugants which were resistant to both streptomycin and tetracycline were screened for the ability to oxidize thiophenes (see Materials and Methods for details). The results of this analysis (Table 5) showed that there were three regions of the *E. coli* chromosome which harbored genes involved in thiophene degradation; these were at 5 to 15 min (*thdA*), 30 to 45 min (*thdB*), and 90 to 100 min (*thdC*). More accurate mapping within these regions was carried out by transducing NAR33 (or in some cases NAR30) with P1 phage grown on strains carrying Tn*10* insertions at known locations on the *E. coli* chromosome. Each of the three suspect regions was analyzed by using Tn*10* insertions deployed at approximately 1-min intervals. The *thdA* gene cotransduced at a level of around 90% with Tn*10* insertions at 12.2 min (*purE*::Tn*10* of NK6051) and 13 min (Tn*10* of RK4342), indicating a location for *thdA* at approximately 12.5 min (Table 6). The 90- to 100-min region turned out to contain two *thd* genes (*thdC* at 92 min and *thdD* at 97.5 min) (Table 6).

Investigation of the *thdB* region showed that the mutations required for thiophene degradation were not in novel genes but were in fact the *fadR* and *atoC* regulatory mutations which were present in parental strain DC625 before the selection of thiophene-degrading mutants. (Since these mutations are absent in the Hfr::Tn*10* donor strains, they showed up during the conjugational analysis.) The requirement for *fadR* or *atoC* was formally demonstrated by tranducing NAR33 to *fadR*$^+$ or *atoC*$^+$ by using Tn*10* insertions close to these genes. Phage P1 grown on DC757 (*fadR*$^+$, Tn*10* close to *fadR*) was crossed with NAR33. Of 100 tetracycline-resistant transductants, the 35 *fadR*$^+$ colonies were all unable to oxidize thiophene-2-carboxylic acid, whereas the 65 colonies which retained *fadR* all retained thiophene-degrading ability. A similar experiment in which we used P1 grown on LA5651 (*atoC*$^+$, Tn*10* near *atoC*$^+$) showed that transduction of NAR33 to *atoC*$^+$ resulted in a loss of thiophene degradation. Thus, both *atoC* (48 min) (1, 18) and *fadR* (26 min) (1, 17) were required for thiophene degradation, and the *thdB* mutation is not in a new gene.

DISCUSSION

Starting from an *E. coli* strain which showed poor but detectable degradation of phenylacetate, we isolated mutants that were able to grow on furan and thiophene derivatives after three rounds of selection (see Fig. 1 for structures). Until recently, the ability to degrade aromatic compounds was considered proper for pseudomonads and related organisms, but was thought to be largely absent from facultative anaerobes, such as the enterobacteria. The pioneering work of Cooper and associates showed that some strains of *E. coli* can degrade 3- or 4-hydroxyphenylacetate (8), and later work extended these observations to other derivatives of phenylacetate and phenylpropionate (4). Our parental strain DC625 could slowly degrade both phenylacetate and benzoate; however, it was unable to use 3-hydroxyphenylacetate. Successive selection of mutants gave both an extended range of growth substrates and greater oxidative ability toward previously usable compounds. Our third- and fourth-stage mutants were able to degrade a wide range of both benzenoid and oxygen- or sulfur-containing heterocyclic aromatic compounds, although nitrogen heterocyclic compounds were not broken down. When testing a selection of vitamins and trace metals for possible stimulation of growth on thiophenes, we noticed that NAR20 and succeeding mutants were able to grow with vitamin C (ascorbic acid) as a carbon source. The ring form of ascorbic acid may be viewed either as an hydroxylated furan derivative or as a lactone of ketogulonic acid. Thus, it is not surprising in retrospect that a mutant selected for degradation of furfuryl alcohol is also able to degrade ascorbic acid.

Although unable to grow on thiophenes as sole carbon sources, NAR20 was able to metabolize thiophene methylanine sufficiently to use it as a nitrogen source. Mutants NAR30 and NAR40 could use this compound either as a carbon source or as a nitrogen source.

A pathway for the degradation of furan-2-carboxylate involving initial activation of the carboxyl group with coen-

TABLE 6. Cotransductional mapping of *thd* genes[a]

Tn*10* location (min)	Donor strain	No. of colonies tested	% Cotransduction
thdA locus			
10	SG20253 *zba*::Tn*10*	84	49
12	YYC188 *zbc*::Tn*10*	122	23
12.2	NK6051 *purE*::Tn*10*	100	94
13	RK4342 *zbd*::Tn*10*	100	91
14	SK2257 *zbe*:Tn*10*	100	1
thdC and *thdD* loci			
90	BW6175 *argE*::Tn*10*	100	63
92	N3055 *uvrA*::Tn*10*	100	91
93	BJW43 *zjd*::Tn*10*	150	6
95	DEV22 *mia*::Tn*10*	100	0
96	GR401 *cycA*::Tn*10*	100	21
97	DF1062 *zjh*::Tn*10*	100	92
98	SS3242 *zji*::Tn*10*	100	100
99	SK472 *zji*::Tn*10*	100	43

[a] P1 grown on the donor strains was crossed with NAR33, and tetracycline-resistant transductants were scored for thiophene oxidation on tetrazolium indicator plates.

zyme A has been proposed for a *Pseudomonas* sp. strain by Trudgill (20, 21). Other authors have suggested that this accounts for thiophene-2-carboxylate degradation by the only natural isolate reported to fully degrade thiophenes (9; M. J. Amphlett, Ph.D. thesis, University of Cardiff, Cardiff, Wales, 1968; M. J. Amphlett and A. G. Callely, Biochem. J. **112**:12p, 1969; R. E. Cripps, Ph.D. thesis, University of Warwick, Warwick, England, 1971). We attempted to assay the formation of thiophene-2-carboxyl coenzyme A thioester in extracts of our mutants by using the same assay used by these authors (9; Amphlett and Callely, Biochem J. **112**:12p, 1969), but were unable to find any activity (data not shown). Furthermore, our mutants were able to degrade derivatives such as ascorbic acid and thiophene methylamine, which lack appropriate carboxyl groups for activation. Thus, it seems unlikely that the coenzyme A thioester pathway applies to our *E. coli* mutants. The increased degradation of benzenoid compounds suggests that the pathway proposed for phenylacetate derivatives by Cooper and Skinner (8) or the related scheme for phenylpropionate (4) may apply. These two pathways differ in the sites of hydroxylation and cleavage of the benzene ring, and recent work has shown that mutants unable to degrade phenylacetate (*pac*) can still degrade phenylpropionate (7). However, our mutants have increased ability to degrade both phenylacetate and phenylpropionate derivatives, suggesting at least some common reactions. As far as hydroxylation is concerned, it may be significant that blockage of position 5 of the furan ring prevents degradation by our mutants.

It has been known for a long time that heterocyclic analogs of aromatic amino acids, such as thienylalanine (11) or furylalanine (6), are highly toxic and that the toxicity may be counteracted by supplying the appropriate biological amino acid. We found that thiophenes were indeed toxic, even when they were not specifically designed to mimic biological aromatic amino acids, and that provision of phenylalanine and tyrosine largely overcame these toxic effects.

Genetic analysis indicated that two previously known regulatory mutations, *fadR* and *atoC*, were required and that three novel loci, *thdA* (12.5 min), *thdC* (92 min), and *thdD* (97.5 min) were also involved in the degradation of thiophene-2-carboxylate. Whether genes at other loci are required is presently unknown. The only other mutations known to affect aromatic degradation are those of Cooper et al. (7), which prevent growth on phenylacetate but allow 3-phenylpropionate to be metabolized normally. These *pac* mutations map at 30.4 min (7) and are thus far from any of the *thd* loci.

The involvement of *fadR* and *atoC* may perhaps reflect the need for the *ato* (20) and *fad* (5, 19) systems for the degradation of four- and five-carbon carboxylic acid derivatives (reviewed in reference 6). Although the degradative pathway for furans and thiophenes is still obscure, the ring fission products almost certainly contain a four-carbon segment derived from the heterocyclic ring, together with whatever substituents were present before cleavage.

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