Genetic and Biochemical Analyses of *Escherichia coli* Strains Having a Mutation in the Structural Gene (*poxB*) for Pyruvate Oxidase

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Mutants of *Escherichia coli* K-12 deficient in pyruvate oxidase were isolated from an *aceEF* (pyruvate dehydrogenase-deficient) strain by selection for a complete absence of growth on medium lacking acetate. Extracts of two of the mutants were shown to contain normal levels of pyruvate oxidase antigen, although the enzymatic activities of the extracts were reduced or absent. The *poxB* locus was mapped by using closely linked transposon insertions to min 18.7 of the *E. coli* linkage map between the *cmlA* and *aroA* loci, a location far removed from that of the regulatory gene, *poxA*.

The pyruvate oxidase of *Escherichia coli* catalyzes the decarboxylation of pyruvate to acetate. The enzyme has been purified to homogeneity (22), and its enzymology has been extensively studied (for review, see references 7 and 17). The enzyme is a homotetrameric protein having thiamine pyrophosphate and flavin adenine dinucleotide cofactors (18). Pyruvate oxidase is activated by extremely low concentrations of lipids and has a high-affinity lipid-binding site (20, 21). These novel lipid activation properties make pyruvate oxidase an unusually good system for the study of lipid-protein interactions.

In a previous paper (4), we reported the isolation of an *E. coli* strain with a mutation (*poxA*) producing a low level of a functionally normal pyruvate oxidase. We now report the isolation and characterization of strains with mutations in the structural gene (termed *poxB*) for pyruvate oxidase.

MATERIALS AND METHODS

Bacterial strains, media, and genetic techniques. All bacteria were derivatives of *E. coli* K-12 and are listed in Table 1. The genetic techniques for construction of *ΔaceEF*, *pps*, and *pfl* strains were described previously (4). The methods for preparation of random Tn10 insertions into the bacterial chromosome and for screening mutants on an indicator medium were done as previously described (4).

The media and chemicals used were those described previously (4). [³⁵S]methionine and PCS scintillation fluid were the products of Amersham Corp., Arlington Heights, Ill. Na¹²⁵I was purchased from New England Nuclear Corp., Boston, Mass. Protein A-Sepharose (7 mg of protein A per g of gel) was a product of Sigma Chemical Co., St. Louis, Mo.

Mutagenesis and screening of mutants. The *poxB*

mutants were isolated from strain YYC90 after mutagenesis with *N*-methyl-*N*-nitroso-nitrosoguanidine by the following modification of our previous methods (4). Before screening for pyruvate oxidase mutants by using microtiter plates, an ampicillin enrichment step was introduced. First, the mutagenized bacteria were grown overnight in minimal medium supplemented with glucose and acetate. The culture was diluted in the same medium and again grown overnight. The cells were collected, washed, and suspended in minimal medium plus succinate, but lacking acetate, at a density of 10⁷ cells per ml. Ampicillin (20 μg/ml) was added every 2 days, and the culture was shaken slowly at 33°C. After 6 days of ampicillin treatment <1% of the original inoculum survived. Survivors were plated, and single colonies were isolated and screened on microtiter plates as described previously (4).

Enzyme analysis. The methods for extract preparation and the spectrophotometric enzyme assay with ferricyanide as electron acceptor were described previously (4). All extracts (unless otherwise indicated) were prepared from stationary-phase cultures grown in rich medium.

Assay of cross-reacting material. Antiserum raised against purified pyruvate oxidase was kindly provided by R. Krantz and C. Grabau. The antibody was purified through the ammonium sulfate step of Livingston (14). Samples of the cell free extracts of various strains were preincubated with various dilutions of the antibody for 20 min at room temperature, and then either an extract of a wild strain or highly purified pyruvate oxidase from *E. coli* W (provided by R. Gennis) was added. After incubation at room temperature for 20 min the substrate and cofactors were added, and the enzymatic activity was measured spectrophotometrically. When purified pyruvate oxidase was used, 1% (final concentration) Triton X-100 was included in the assay mixture.

[³⁵S]Methionine labeling and gel electrophoresis. Cultures (5 ml) were grown to log phase in glucose-minimal medium supplemented with amino acids (lacking methionine), and then 43 μCi of [³⁵S]methio-

TABLE 1. Bacterial strains

Strain	Relevant markers	Source ^a or reference
CY265	HfrC, $\Delta(aroP-aceEF)$	(4)
CY314	λ c1857 lysogen of CY265	(4)
CY52	<i>pyrD34</i>	
KL290	<i>serC13</i> , <i>serS14</i> , <i>gal-39</i>	CGSC4341
AB478	<i>aroA</i>	
RE103	<i>cmlA</i>	CGSC4698 (19)
X478	<i>thi-1</i> , <i>proC32</i> , <i>purE42</i> , <i>leu-6</i> , <i>metE70</i> , <i>trpE38</i> , <i>lysA23</i> , <i>rpsL109</i> ,	K. Low
Hfr strains, (Hfr6, KL16, KL96, KL99, B7)	Hfr (Fig. 2)	K. Low (15, 16)
F106/KL181	F106 <i>pyrD</i> ⁺ / <i>pyrD34</i> <i>his-68</i> <i>trp45</i> <i>recA1</i>	
YYC7	<i>poxA1</i> of CY314	(4)
YYC63	<i>pps-4</i> , <i>pfl-1</i> of CY265	(4)
YYC90	<i>rpsL</i> of CY314	
YYC115	<i>zje-1::Tn10</i> , <i>poxA</i> ⁺ , <i>rpsL</i> of YYC7	(4)
YYC117	<i>poxB1</i> of YYC90	This work
YYC120	<i>poxB2</i> of YYC90	This work
YYC124	<i>poxB3</i> of YYC90	This work
YYC162	<i>pps-4</i> , <i>pfl-1</i> of YYC117	This work
YYC163	<i>pps-4</i> , <i>pfl-1</i> of YYC120	This work
YYC164	<i>pps-4</i> , <i>pfl-1</i> of YYC124	This work
YYC172	<i>zje-1::Tn10</i> of YYC7	P1 (YYC115) $\times$ YYC7
YYC173	$\Delta(aroP-aceEF)$ of X478	(4)
YYC178	<i>Tn10</i> , <i>poxB</i> ⁺ of YYC162	This work
YYC180	<i>zbj::Tn10</i> , <i>poxB</i> ⁺ of YYC164	This work
YYC187	<i>zbi::Tn10</i> , <i>poxB</i> ⁺ of YYC162	This work
YYC188	<i>zbi::Tn10</i> of YYC162	P1(YYC187) $\times$ YYC162
YYC190	<i>zbi::Tn10</i> of KL290	P1(YYC187) $\times$ KL290
YYC191	<i>zbi::Tn10</i> , <i>pfl-1</i> of AB478	P1(YYC187) $\times$ AB478
YYC192	<i>zbi::Tn10</i> , <i>pfl-1</i> of AB478	P1(YYC180) $\times$ AB478
YYC193	<i>zbi::Tn10</i> , <i>poxB1</i> of YYC173	P1(YYC188) $\times$ YYC173
YYC194	<i>zbi::Tn10</i> of RE103	P1(YYC187) $\times$ RE103
YYC195	<i>zbi::Tn10</i> of RE103	P1(YYC180) $\times$ RE103

^a Strains designated CGSC were obtained from the Coli Genetic Stock Center, Yale University, through the kindness of the curator B. Bachmann.

nine (1,010 Ci/mmol) and cold methionine (0.5 μ g/ml) were added. Labeling was carried out for 90 min at 30°C. The cells were collected and washed twice with 0.01% methionine, and extracts were prepared (4). The efficiency of [³⁵S]methionine incorporation was obtained by counting the protein precipitated by trichloroacetic acid. Equal amounts of radioactivity from each extract were pretreated twice with preimmune serum antibody before use. For immunoprecipitation, each sample was treated with either preimmune antibody or antipyruvate oxidase antibody (about 1 μ g of antibody per μ g of crude extract) overnight at 4°C. Protein A-Sepharose (1 μ l of packed beads per 25 μ g of immunoglobulin G) was then added to precipitate the antigen-antibody complexes. After 20 min at 37°C, the beads were collected by centrifugation and washed, and the radioactive protein was released by boiling in sodium dodecyl sulfate sample buffer and analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12). The gel was stained with Coomassie blue, dried, and autoradiographed.

Radioimmunoassay. The purified pyruvate oxidase was iodinated with Na¹²⁵I by using chloramine T (10). The radioimmunoassay method to determine the amount of pyruvate oxidase antigen was that of Felber

(5). Radioactive antigen-antibody complexes were precipitated by protein A-Sepharose. The radioactivity of the washed Sepharose beads was determined by liquid scintillation counting in PCS scintillation fluid (Amersham).

RESULTS

Isolation of *poxB* mutants. The isolation of mutants in the structural gene for pyruvate oxidase was greatly facilitated by the growth behavior of the *poxA* strain isolated previously (4). Strains of *E. coli* bearing a deletion of the *aceEF* (pyruvate dehydrogenase) loci are unable to grow in media lacking acetate. However, we noticed that in the absence of acetate, those strains formed very small colonies after prolonged (6 to 8 days) incubation. In contrast, the *poxA* Δ *aceEF* strain did not grow on succinate minimal medium even after 2 weeks of incubation. This result suggested that pyruvate oxidase produced the acetate that allowed the very feeble growth of the Δ *aceEF* strains. Based on this reasoning, we isolated pyruvate oxidase mutants

TABLE 2. Pyruvate oxidase activities of various strains

Strains	Genotype	Activity ^a (U/mg of protein)
CY314	Wild type	138
YYC90	Wild type	122
YYC117	<i>poxB1</i>	<1
YYC120	<i>poxB2</i>	<1
YYC124	<i>poxB3</i>	25

^a A unit of activity is 1 nmol of pyruvate decarboxylated per min (4).

by growth at low cell density of mutagenized cultures of $\Delta aceEF$ strains in succinate-minimal medium lacking acetate. Nongrowing cells were then selected by ampicillin selection. The use of succinate as the sole carbon source and the low cell density prevented the activation of pyruvate formate lyase, an enzyme capable of satisfying the acetate requirement of $\Delta aceEF$ strains under anaerobic conditions (8, 9). Cells that survived the selection were screened for pyruvate oxidase activity by using the microtiter plate assay and [1-¹⁴C]pyruvate as previously described (4). Three mutant strains, YYC117, YYC120, and YYC124, were isolated after screening fewer than 100 colonies (compared with 1,000 previously screened to obtain the single *poxA* mutant). Extracts of strains YYC117 and YYC120 had no detectable pyruvate oxidase activity, whereas strain YYC124 had about 20% of the wild-type activity (Table 2). Unlike *poxA* strains, these three strains grew at a normal rate on acetate-containing media and attained a normal cell density. The lack of pyruvate oxidase activity in these mutants was not due to inhibitors present in the extracts, since the addition of a mutant extract to an extract of the wild-type strain gave no inhibition (data not shown). Genetic analysis of these three mutants (see below) indicated they are due to lesions in a new genetic locus which we designate *poxB*.

Biochemical characterization of *poxB* mutants. We have characterized two of the three *poxB* strains by immunological means. Both strain YYC124 (*poxB3*) and strain YYC117 (*poxB1*) produced material that reacted with antibody raised against homogeneous *E. coli* pyruvate oxidase. Strain YYC124 (*poxB3*) produced a normal level of antigenically active material, but had only 15 to 20% of the normal oxidase activity (Table 2). The presence of pyruvate oxidase antigen was demonstrated by cross-reacting material titration (data not shown) and radioimmunoassay. Radioimmunoassay of extracts of strains CY314 and YYC124 gave levels of 5.4 and 7.4 μ g of oxidase antigen per mg of extract protein. Immunoprecipitation of

[³⁵S]methionine-labeled extracts of strain YYC124 gave a protein band with the molecular weight of the oxidase (Fig. 1).

The characterization of the *poxB1* strain YYC117 was less straightforward. Although all extracts of this strain were devoid of enzyme activity, the levels of antigen present depended on the conditions of cell growth. Extracts of stationary-phase cells grown on broth medium had no measurable antigen either by cross-reacting material titration or by radioimmunoassay, whereas extracts of log-phase cells grown on glucose-minimal medium contained normal levels of an antigen with the molecular weight of the

FIG. 1. Autoradiogram of sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of [³⁵S]-labeled immunoprecipitates. Labeling with [³⁵S]methionine, immunoprecipitation, and electrophoresis were as described in the text. The even-numbered samples were treated with anti-pyruvate oxidase antiserum, whereas the parallel odd-numbered samples were treated with preimmune antiserum. Lanes: 1 and 2, strain CY314 (wild type); 3 and 4, strain YYC117 (*poxB1*); 5 and 6, strain YYC124 (*poxB3*). The standards were bovine serum albumin (BSA), ovalbumin (OVA), carbonic anhydrase (CBA), and *E. coli* W pyruvate oxidase (POX). The gel was overexposed to facilitate reproduction; upon exposure in the linear range of the film no bands were visible in the odd-numbered (preimmune serum) lanes.

oxidase (Fig. 1). Both the growth medium and the growth phase seemed to determine the stability of the pyruvate oxidase antigen synthesized by strain YYC117. These parameters may act by alteration of the intracellular electrolyte levels (11).

Genetic characterization of *poxB* mutants. We first tested strains YYC117, YYC120, and YYC124 to see whether they were *poxA* mutants. Strains YYC117, YYC120, and YYC124 were transduced with P1 phage grown on strain YYC115, a *pox*⁺ strain having a Tn10 insertion very near the *poxA* locus (4). Tetracycline-resistant (Tet^r) transductants were isolated and screened by the microtiter plate assay (the feeble growth of $\Delta aceEF$ strains supported by the oxidase is not a feasible method for scoring colonies). None of the 90 Tet^r transductants from the various crosses were *pox*⁺, indicating that the mutational lesions in these strains were not within the *poxA* locus.

To test whether these three mutants are lesions within the same locus or whether they represent mutations in differing genetic loci, we mapped each of the three mutations. Since the *poxB* strains lack a usable growth phenotype, the lesions were mapped by isolating Tn10 insertions close to the gene and then mapping the insertions in relation to the *poxB* gene and the genetic map (4). The isolation of the Tn10 insertions was done by scoring colonies for oxidase activity by using a pyruvate tetrazolium redox indicator medium in strains with lesions in the *aceEF* (pyruvate dehydrogenase), *pfl* (pyruvate formate lyase), and *pps* (phosphoenolpyruvate synthase) loci. First, derivatives of the *poxB* *aceEF* strains YYC117, YYC120, and YYC124 containing lesions in the *pps* and *pfl* genes were constructed by transduction (4). These derivatives, strains YYC162, YYC163, and YYC164, respectively (Table 1), were then transduced with a P1 phage stock grown on a pool of random Tn10 insertions into the chromosome of strain YYC63. Tet^r transductants isolated from each cross were then washed from the plates, diluted, and screened on the indicator medium for Tet^r *pox*⁺ colonies (4).

Several of these recombinants were assayed for oxidase activity; all had levels similar to that of the comparable *pox*⁺ strain. The Tn10 insertions of these strains showed various linkages to *poxB*, the most closely linked being 69% co-transduced with *poxB*. Moreover, those insertions isolated by linkage to one of the *poxB* alleles showed an essentially identical linkage to the other two alleles, indicating that the low oxidase levels of these strains are due to lesions at the same genetic locus. A more direct test of close linkage between the *poxB3* and *poxB1* alleles was done by using the *zbi*::Tn10 insertion

as a selected outside marker. When a P1 stock grown on strain YYC188 (*poxB1*, *zbi*::Tn10) was used to transduce strain YYC164 (*poxB3*) to Tet^r, only 4% of the Tet^r recombinants were *pox*⁺. When the alleles were reversed between the donor and recipient strains, no *pox*⁺ Tet^r recombinants were formed (100 Tet^r colonies were scored in each cross). These data indicate that the two *poxB* alleles are sufficiently closely linked to be within the same gene (23) and suggests that the order of the alleles is *zbi*::Tn10 *poxB3* *poxB1*.

Location of the *poxB* gene. To locate a *poxB*-linked Tn10 insertion on the genetic map, an HfrC strain (CY265) and strain KL16 were transduced with P1 phage grown on strain YYC178, and Tet^r transductants of the two Hfr strains were selected. These strains were then mated for 5 h with the F⁻ strain X478 (which has markers spaced around the chromosome), and Tet^r, streptomycin-resistant recombinants were screened for each of the auxotrophic markers of the strain. The Tn10 insertion of the donor strains was most closely linked to the *trp* and *purE* markers, ~60% to each marker, suggesting that the Tn10 insertion lay between these two markers. To further localize this Tn10 insertion, a number of Hfr strains having points of origin in this region were used (Fig. 2). Each Hfr strain was first transduced to Tet^r with P1 phage grown on strain YYC178 and then briefly (30 min) mated with the F⁻ strain X478, and Tet^r recombinants were selected. Only the derivatives of HfrsB7, KL96, and Hfr6 gave Tet^r recombinants, indicating that Tn10 was inserted into the min 12 to 21 region of the genetic map (Fig. 2).

P1 transduction was then used to more closely locate the Tn10 insertions of strains YYC178, YYC180, and YYC187. Strains carrying readily scorable markers in this region were transduced with P1 stocks grown on strains YYC178, YYC180, and YYC187, and Tet^r transductants were selected. Neither Tn10 insertion was co-transduced with the *gal* (<1%) or *pyrD* (<0.5%) loci, but all three Tn10 insertions were cotransduced with the *serC*, *serS*, *pfl*, *aroA*, and *cmlA* loci (Table 3, Fig. 2) (*serC* and *serS* were always coinherited).

The Tn10 insertions of strains YYC180 and YYC187 were then used to locate the *poxB* gene. The Tn10 insertion of strain YYC180 was co-transduced with *cmlA* and *aroA* at 22 and 21%, respectively, suggesting that the insertion lay between *cmlA* and *aroA*. This was tested by three-factor crosses with phage P1 (crosses 2 and 4 in Table 4), which indicated the order *cmlA* *poxB* *zbi*::Tn10 *aroA*. Similarly, the three-factor crosses of the Tn10 insertion of strain YYC187 with *aroA* and *poxB* and with *cmlA* and *poxB* (Table 4) were only consistent with the

FIG. 2. Relevant portion of the *E. coli* genetic map. The linkages given are cotransduction frequencies (percentages) taken from experiments similar to those of Table 3 and other experiments. The map is a modification of that of Bachmann and Low (1, 2), except for the location of *poxA* (4).

order *cmlA zbi::Tn10 poxB aroA*. Since the *pfl* gene is close to *poxB* and the other markers of interest, we included a *pfl* lesion in strains YYC191 and YYC192 to preclude false readings of the indicator plates due to *pfl*⁺ recombinants. Likewise, all recombinants in crosses 3 and 4 of Table 4 were scored for *pfl*. The *poxB* locus maps at min 18.7 of the *E. coli* genetic map (Fig. 2). It should be noted that transfer of F106 (16) by conjugation from a *recA* donor into strain YYC193 (*poxB*1) resulted in a low level of oxidase activity in the recipient strain, indicating that F106 carries the *poxB* gene, a result consistent with our mapping and the recent report (6) that the chromosomal segment carried by this F' extends almost to min 18 of the genetic map.

Mutant strains containing lesions in both *poxA* and *poxB*. *poxA*1 strains contain about 15 to 20% of the normal enzyme activity and a similar level of oxidase antigen (1.4 μ g of oxidase antigen per mg of extract protein by radioimmunoassay) and thus appear to make a reduced amount of a structurally normal enzyme (4). To test the effect of the *poxA*1 mutation on strains carrying

a *poxB* lesion, the *poxA* lesion was transferred into the *poxB*3 strain YYC124 by transduction with P1 phage grown on strain YYC172, and Tet^r colonies were selected. Extracts of four Tet^r transductants were assayed for pyruvate oxidase activity. Extracts of three of these strains had no pyruvate oxidase activity, whereas the fourth had an activity similar to that of strain YYC124. Furthermore, we found that the extracts lacking pyruvate oxidase activity contained no detectable cross-reacting material. We conclude that *poxA* affects the production of pyruvate oxidase encoded by the *poxB* locus.

DISCUSSION

The more interesting questions concerning the pyruvate oxidase of *E. coli* are its role in metabolism and the physiological relevance of the striking activation of the enzyme by lipid observed in vitro. We hope by isolation of various lesions within the *poxB* locus to investigate these questions.

The isolation of strains carrying lesions in the structural gene for pyruvate oxidase depended

TABLE 3. Cotransductional mapping of the *poxB* locus

P1 donor and relevant markers	Recipient	Marker selected	No. of colonies scored	Cotransduction frequency (%) ^a
YYC187 <i>zbi::Tn10</i>	YYC162 <i>poxB1</i>	Tet ^r	100	69
YYC187 <i>zbi::Tn10</i>	YYC117 <i>poxB1</i>	Tet ^r	44	73
YYC187 <i>zbi::Tn10 pfl</i>	YYC117 ^b	Tet ^r	44	2
YYC188 <i>zbi::Tn10 poxB1</i>	CY314	Tet ^r	88	61
YYC187 <i>zbi::Tn10</i>	KL290 <i>serC serS</i>	Tet ^r	100	13
YYC187 <i>zbi::Tn10</i>	AB478 <i>aroA</i>	Tet ^r	100	5
YYC187 <i>zbi::Tn10</i>	AB478 <i>aroA</i>	<i>aroA</i> ⁺	100	4
YYC187 <i>zbi::Tn10</i>	RE103 <i>cmlA</i>	Tet ^r	192	72
YYC180 <i>zbi::Tn10</i>	YYC164 <i>poxB3</i>	Tet ^r	96	45
YYC180 <i>zbi::Tn10</i>	YYC162 <i>poxB1</i>	Tet ^r	48	43
YYC180 <i>zbi::Tn10</i>	YYC163 <i>poxB2</i>	Tet ^r	48	64
YYC180 <i>zbi::Tn10</i>	YYC117 <i>poxB1</i>	Tet ^r	44	52
YYC180 <i>zbi::Tn10 pfl</i>	YYC117	Tet ^r	44	16
YYC180 <i>zbi::Tn10</i>	KL290 <i>serC serS</i>	Tet ^r	200	21
YYC180 <i>zbi::Tn10</i>	AB478 <i>aroA</i>	Tet ^r	200	21
YYC180 <i>zbi::Tn10 pfl</i>	AB478	Tet ^r	200	19
YYC180 <i>zbi::Tn10</i>	AB478 <i>aroA</i>	<i>aroA</i> ⁺	200	14
YYC180 <i>zbi::Tn10</i>	RE103 <i>cmlA</i>	Tet ^r	189	22
YYC187 <i>pfl</i>	AB478 <i>aroA</i>	<i>aroA</i> ⁺	200	81
YYC190 <i>serC serS</i>	AB478 <i>aroA</i>	<i>aroA</i> ⁺	200	87
YYC180 <i>pfl</i>	AB478 <i>aroA</i>	<i>aroA</i> ⁺	200	63

^a The inheritance of the *pox*⁺ allele of the donor was scored by indicator plates using method 1 (4) or by the ¹⁴CO₂ microtiter dish assay (4). Similar results were obtained with two additional *Tn10* insertions that were 50% (strain YYC178) and 9% cotransduced with all three *poxB* alleles.

^b In crosses where no recipient marker is given, the unselected marker is the marker carried by the donor in addition to *Tn10*.

on the growth properties of strains carrying a deletion of the pyruvate dehydrogenase locus ($\Delta aceEF$). These strains were found to grow (albeit extremely slowly) in aerobic culture on media lacking acetate. Strains that also contained a lesion in the regulatory gene *poxA* were

completely unable to grow under these conditions, presumably due to the lack of acetate production by pyruvate oxidase. Based on this hypothesis, we performed prolonged ampicillin selections of mutagenized cultures of $\Delta aceEF$ strains and readily isolated several different

TABLE 4. Ordering of *zbi::Tn10* and *zbi::Tn10* in relation to *poxB*, *aroA*, and *cmlA*^a

Cross	Donor	Recipient	Marker selected	No. scored	Class	Frequency (%)
1	YYC191 <i>zbi::Tn10 aroA pfl</i>	YYC162 <i>poxB1</i>	Tet ^r	87	<i>poxB</i> ⁺ <i>aroA</i> ⁺	65
					<i>poxB</i> ⁺ <i>aroA</i>	5
					<i>poxB</i> <i>aroA</i> ⁺	30
					<i>poxB</i> <i>aroA</i>	0
2	YYC192 <i>zbi::Tn10 aroA pfl</i>	YYC162 <i>poxB1</i>	Tet ^r	90	<i>poxB</i> ⁺ <i>aroA</i> ⁺	41
					<i>poxB</i> ⁺ <i>aroA</i>	2
					<i>poxB</i> <i>aroA</i> ⁺	44
					<i>poxB</i> <i>aroA</i>	13
3	YYC194 <i>zbi::Tn10 cmlA</i>	YYC162 <i>poxB1</i>	Tet ^r	93	<i>poxB</i> ⁺ <i>cmlA</i>	52
					<i>poxB</i> ⁺ <i>cmlA</i> ⁺	8
					<i>poxB</i> <i>cmlA</i>	30
					<i>poxB</i> <i>cmlA</i> ⁺	10
4	YYC195 <i>zbi::Tn10 cmlA</i>	YYC162 <i>poxB1</i>	Tet ^r	66	<i>poxB</i> ⁺ <i>cmlA</i>	23
					<i>poxB</i> ⁺ <i>cmlA</i> ⁺	18
					<i>poxB</i> <i>cmlA</i>	4
					<i>poxB</i> <i>cmlA</i> ⁺	55

^a The inheritance of the *pox*⁺ allele of the donor was scored by triphenyltetrazolium chloride-containing medium by using method 2 described previously (4).

poxB strains. It should also be noted that LeMaster and Cronan (13) detected endogenous acetate production by $\Delta aceEF$ strains, a result consistent with pyruvate oxidase action. Preliminary results indicate that most of this endogenous production is blocked in *poxB* strains (LeMaster, Chang, and Cronan, unpublished data).

It seems clear that the *poxB* gene is the structural gene for pyruvate oxidase, whereas the *poxA* locus seems to regulate *poxB*. The *poxA* mutants produce reduced amounts of an oxidase with normal enzymatic activity and grow more slowly than isogenic *pox*⁺ strains. Further evidence that the *poxB* gene encodes the oxidase comes from in vitro molecular cloning of the gene into a cosmid vector (C. Grabau and Cronan, unpublished data). Strains carrying various *poxB*⁺ plasmids overproduce the oxidase 1- to 10-fold. These plasmids complement the *poxB1* allele in a *recA* strain, indicating that *poxB*⁺ is dominant over *poxB1*.

It remains unclear why the growth of *E. coli* on the acetate produced by pyruvate oxidase is so feeble. The activity of the oxidase in crude extracts is comparable to that of pyruvate dehydrogenase, and thus an amount of acetate equivalent to the amount of acetyl coenzyme A produced by the dehydrogenase should be available. Why then do dehydrogenase mutants require acetate for aerobic growth? The most likely possibility is that the conversion of the oxidase-produced acetate to acetyl coenzyme A is very inefficient. *E. coli* has two systems for activating acetate to acetyl coenzyme A, the acetate kinase-phosphotransacetylase system and acetyl coenzyme A synthetase (3). The former system has a high activity, but an extremely low affinity for acetate (the Michaelis constant of the acetate kinase for acetate is 0.3 M, one of the highest *K_m* on record), whereas the acetyl coenzyme A synthetase activity has a high affinity for acetate, but is much less active than the kinase-phosphotransacetylase pathway (3). The role of these pathways in utilization of the acetate produced by pyruvate oxidase is under investigation.

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