JOURNAL OF BACTERIOLOGY, Dec. 1994, p. 7223–7232
0021-9193/94/$04.00+0

Vol. 176, No. 23

Molecular Cloning of the *Escherichia coli* B L-Fucose-D-Arabinose Gene Cluster

ERIC A. ELSINGHORST* AND ROBERT P. MORTLOCK
Section of Microbiology, Cornell University, Ithaca, New York 14853

Received 10 June 1994/Accepted 16 September 1994

To metabolize the uncommon pentose D-arabinose, enteric bacteria often recruit the enzymes of the L-fucose pathway by a regulatory mutation. However, *Escherichia coli* B can grow on D-arabinose without the requirement of a mutation, using some of the L-fucose enzymes and a D-ribulokinase that is distinct from the L-fuculokinase of the L-fucose pathway. To study this naturally occurring D-arabinose pathway, we cloned and partially characterized the *E. coli* B L-fucose-D-arabinose gene cluster and compared it with the L-fucose gene cluster of *E. coli* K-12. The order of the *fucA*, *-P*, *-I*, and *-K* genes was the same in the two *E. coli* strains. However, the *E. coli* B gene cluster contained a 5.2-kb segment located between the *fucA* and *fucP* genes that was not present in *E. coli* K-12. This segment carried the *darK* gene, which encodes the D-ribulokinase needed for growth on D-arabinose by *E. coli* B. The *darK* gene was not homologous with any of the L-fucose genes or with chromosomal DNA from other D-arabinose-utilizing bacteria. D-Ribulokinase and L-fuculokinase were purified to apparent homogeneity and partially characterized. The molecular weights, substrate specificities, and kinetic parameters of these two enzymes were very dissimilar, which together with DNA hybridization analysis, suggested that these enzymes are not related. D-Arabinose metabolism by *E. coli* B appears to be the result of acquisitive evolution, but the source of the *darK* gene has not been determined.

Bacteria possess a broad metabolic potential and can use this potential for growth on uncommon substrates by gaining new enzyme activities and pathways through mutation. Establishing the metabolic capacity for growth on the uncommon pentose D-arabinose often involves recruitment of the L-fucose pathway enzymes through a regulatory mutation. L-Fucose is a naturally occurring methyl pentose that is metabolized in enteric bacteria by the inducible enzymes L-fucose permease, L-fucose isomerase, L-fuculokinase (EC 2.7.1.51), and L-fuculose-1-phosphate aldolase (EC 4.1.2.17) to form L-lactaldehyde and dihydroxyacetone phosphate (Fig. 1). Aerobically, L-lactaldehyde is oxidized to L-lactate by an NAD-linked dehydrogenase (EC 1.2.1.22) (45). Anaerobically, L-1,2-propanediol oxidoreductase (EC 1.1.1.77) reduces L-lactaldehyde to L-1,2-propanediol, which is excreted from the cells (16). The L-fucose structural genes of *Escherichia coli* K-12 are organized as a regulon of at least two operons: the *fucPIK* operon encodes the permease, isomerase, and kinase, respectively; and the *fucAO* operon encodes the aldolase and oxidoreductase, respectively (13). The *E. coli* K-12 L-fucose regulon appears to be under positive control (14, 15), with L-fuculose-1-phosphate as the apparent inducer (5).

Although D-arabinose is not usually metabolized by enteric bacteria, mutants that can use this novel pentose as a sole carbon and energy source can be isolated (4, 29, 30, 39). In D-arabinose-positive mutants of *E. coli* K-12, D-ribulose-1-phosphate is recognized as an alternate inducer of the L-fucose regulon (5), and metabolism of D-arabinose occurs via the L-fucose enzymes, yielding dihydroxyacetone phosphate and L-glycoaldehyde (29). D-Arabinose-positive mutants of *Klebsiella pneumoniae* PRLR3 constitutively synthesize the L-fucose enzymes and metabolize D-arabinose via the L-fucose and ribitol pathways in the following manner (6, 39): D-arabinose is transported by L-fucose permease and isomerized to D-ribulose by L-fucose isomerase. D-Ribulose is an intermediate in the ribitol pathway and the apparent inducer of the ribitol catabolic enzymes, which include a D-ribulokinase (EC 2.7.1.47) that phosphorylates D-ribulose at the C-5 position. Further metabolism occurs through the pentose phosphate pathway.

Experiments studying the evolution of an efficient D-arabinose pathway indicate that strains capable of phosphorylating D-ribulose at the C-5 position have a significant competitive advantage over strains that phosphorylate D-ribulose at the C-1 position (4). Additionally, it was shown that continued selection for improved growth on D-arabinose could result in the loss of L-fuculose-1-phosphate aldolase activity (4). Interestingly, *E. coli* B contains a naturally occurring D-arabinose pathway and can metabolize this pentose without the requirement of a mutation. Although *E. coli* B does not contain a ribitol pathway (40), it does possess a D-ribulokinase that phosphorylates D-ribulose at the C-5 position. This D-ribulokinase activity is the product of the *darK* gene, which has been mapped by P1 transduction to within the *E. coli* B L-fucose gene cluster (24). Additionally, *E. coli* B cannot use L-fucose because of the lack of L-fuculose-1-phosphate aldolase activity, but aldolase-positive revertants can grow on L-fucose as a sole carbon and energy source (24). Therefore, the course taken during the evolution of the *E. coli* B D-arabinose pathway appears to follow the steps predicted experimentally.

We are interested in studying this naturally occurring D-arabinose catabolic pathway, with particular interest in the D-ribulokinase activity encoded by the *darK* gene. In this report, we describe the isolation of the genes coding for the *E. coli* B L-fucose-D-arabinose pathway enzymes, including the *darK* gene. Additionally, we purified and partially characterized the *E. coli* B D-ribulokinase. These experiments suggest that the *E. coli* B L-fucose-D-arabinose pathway may have evolved from an *E. coli* K-12-like ancestor by the acquisition of the *darK* gene from an unknown source.

* Corresponding author. Present address: Department of Microbiology, 7042 Haworth Hall, University of Kansas, Lawrence, KS 66045-2106. Phone: (913) 864-4311. Fax: (913) 864-5294.

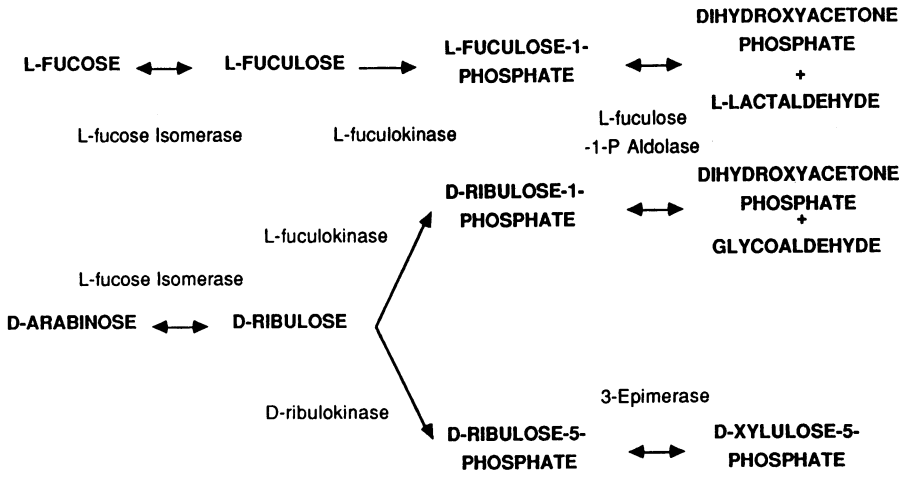

FIG. 1. Metabolic pathways for L-fucose and D-arabinose.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, plasmids, and culture conditions. The bacterial strains, bacteriophage, and plasmids used in this study are listed in Table 1. Except for WA837, all *E. coli* B strains were derived from the wild-type strain EM2000. *recA56* derivatives of *E. coli* B strains were constructed by the two-step procedure described by Csonka and Clark (18).

Cultures were grown in LB broth (36) or minimal salts medium (38) with aeration. Solid media contained 1.5% agar. *E. coli* cultures were routinely grown at 37°C; *Klebsiella* and *Erwinia* strains were grown at 30°C. Antibiotics were added to media to the following final concentrations: ampicillin, 200 μg/ml; kanamycin, 50 μg/ml; and tetracycline, 15 μg/ml. Isopropyl-β-D-thiogalactoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactosidé (X-Gal) were added to solid media at final concentrations of 1 and 0.12 mM, respectively, as indicated. *E. coli* strains were made competent by $CaCl_2$ treatment as described by Maniatis et al. (34).

Construction of libraries. Chromosomal DNA from strain EM2022 was partially digested with *Sau*3A1 to give an average fragment size of 10 to 14 kb and then ligated into *Bam*HI-cut, alkaline phosphatase-treated pBR322. To select for complementation of the *darK* gene, the ligation mixture was used to transform *E. coli* B strain EM5524 (*fucK28 darK110* Fuc$^-$ Dar$^-$). Transformed cells were washed with minimal salts solution and spread on 0.5% D-arabinose minimal salts plates containing ampicillin.

A second library was constructed in lambda vector λ2001. EM2022 chromosomal DNA was digested with *Xho*I, treated with alkaline phosphatase, and then ligated into *Xho*I- and *Eco*RI-digested λ2001. Ligation mixtures were packaged in vitro (41) and used to infect *E. coli* Q359. Approximately 22,000 independent plaques were screened by plaque hybridization (34) for sequences homologous to ^{32}P-labeled pfuc16. Hybridizing plaques were purified by four rounds of plaque hybridization.

DNA hybridizations. Purified plasmid DNA was labeled with [^{32}P]dCTP (Amersham Corp.) by using a nick translation kit (Bethesda Research Laboratories). DNA was electrophoresed through agarose gels and then transferred to nitrocellulose (Schleicher & Schuell, Inc.) by the method of Southern (44). Filters were prehybridized for 4 h at 37°C in a hybridization buffer containing 30 mM Tris (pH 7.5), 1 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 10× Denhardt solution (21), and 50 μg of denatured salmon sperm DNA (Sigma Chemical Co.) per ml. After prehybridization, 1 × 10^7 to 3 × 10^7 cpm of denatured probe DNA was added to the prehybridization solution, and incubation was continued until 2 × $C_o t_{1/2}$ to 3 × $C_o t_{1/2}$ was achieved (34). Filters were washed in a 0.1 × SSC–0.1% SDS buffer at 55°C. During homology studies between the *darK* gene and genomic DNA, the filters were washed in a 2× SSC–0.1% SDS buffer at 30°C. This less stringent condition allowed for 35% base pair mismatch (35).

Tn*5* mutagenesis. Strain EM3001 containing the plasmid to be mutagenized was infected with the Tn*5* vector λNK467 by the procedure of deBruijn and Lupski (20). Plasmids from kanamycin-resistant colonies were purified and used to transform the indicated *E. coli* B strains to ampicillin and kanamycin resistance. Inactivation of L-fucose-D-arabinose genes was determined by enzyme assay or by the inability to complement chromosomal mutations. The position of Tn*5* insertions was determined by *Eco*RI, *Sal*I, *Pst*I, and *Hin*dIII restriction mapping.

In vitro transcription-translation analysis. Plasmid-encoded proteins synthesized in vitro were radiolabeled with L-[4,5-^{3}H]leucine, using a prokaryotic DNA-directed translation kit (Amersham Corp). Cesium chloride-ethidium bromide equilibrium gradient-purified plasmids (34) were used as templates in each analysis. Each 12.5-μl reaction mixture contained 30 μCi of [^{3}H]leucine and 1.25 μg of the indicated plasmid. Samples (8 μl) from each reaction mixture were electrophoresed under denaturing conditions through SDS–7.5% polyacrylamide gels by the method of Laemmli (27). The gels were processed for fluorography by using En^{3}Hance (New England Nuclear Corp.).

Enzyme assays. The growth of cultures for enzyme specific activity measurements and the preparation of cell extracts by sonication and centrifugation have been previously described (24). Data shown in the tables are from single extracts or preparations and are representative of values obtained from replicate extracts or preparations. L-Fucose isomerase activity was measured by determining the rate of NADH oxidation in a coupled assay system using purified ribitol dehydrogenase (39). L-Fuculose-1-phosphate aldolase activity was determined by measuring the formation of dihydroxyacetone phosphate in

TABLE 1. Bacteria, bacteriophage, and plasmids used in this study

Bacterial strain, bacteriophage, or plasmid	Description[a]	Reference or source
Bacterial strains		
Escherichia coli B		
EM2000	Wild-type *E. coli* B (*fucA1*)	24
EM2022	*araB rhaD rha-4 fucA*$^+$ derivative of EM2000	24
EM2402	*fucI6* derivative of EM2022	24
EM2524	*fucK28 darK110* derivative of EM2022	24
EM2543	*fucI203 fucK28* derivative of EM2022	24
EM3001	*recA56* derivative of WA837	This study
EM5000	*recA56* derivative of EM2000 (*fucA1*)	This study
EM5524	*recA56* derivative of EM2524 (*fucK28 darK110*)	This study
EM5543	*recA56* derivative of EM2543 (*fucI203 fucK28*)	This study
WA837	*met-100 hsdR11 gal-151* λ^s	47
E. coli K-12		
Q359	*hsdR*$_K^-$ hsdM$_K^+$ *supE* P2	25
JM109	*recA1* Δ*lac-pro endA1 gyrA96 thi-1 hsdR17 supE44 relA1* F′*traD36 proAB*$^+$ *lacI*^{q}*ZΔM15*	48
C600	F$^-$ *thi-1 thr-1 leuB6 lacY1 tonA21 supE44* λ^-	3
JM1000	Fuc$^+$ Dar$^-$ *pro rif*	4
Klebsiella pneumoniae PRLR3	Fuc$^+$ Dar$^-$ Ura$^-$	39
Erwinia chrysanthemi LM8200	*Fuc*$^-$ *Dar*$^+$ *Rtl*$^-$ *pro-1*	28
Bacteriophages		
P1cam	P1::Tn*9 clr-100*	43
λNK467	λ*b*221 *rex*::Tn*5* *c*I857 *O*am29 *P*am80	20
λ2001	λ cloning vector	26
λ19A2	*E. coli* B *fucI* and *fucK* on an 18.2-kbp *Xho*I insert in λ 2001	This study
λdAR	λ carrying the ribitol and D-arabitol catabolic loci	42
Plasmids		
pBR322	Plasmid cloning vector	7
pHG165	pBR322 copy number derivative of pUC8	46
pfuc16	*E. coli* K-12 *fucA*, *fucO*, *fucP*, *fucI*, and part of *fucK* on a 7.6-kbp insert in pBR322	15
p21-193	*araB* on a 6.5-kbp *Pst*I insert in pBR322	N. Lee
pEM101	*E. coli* B *darK* and *fucP* on a 13.1-kbp insert in pBR322	This study
pEM201	*E. coli* B *darK* and *fucA* on an 11.4-kbp insert in pBR322	This study
pEM231	Subclone of pEM201 in pBR322	This study
pEM1911	Subclone of λ19A2 in pHG165	This study

[a] *rha-4*, loss of L-rhamnose isomerase and L-rhamnulokinase activities; *fucK28*, lack of L-fuculokinase activity (*fucK*); *darK110*, lack of D-ribulokinase activity (*darK*); *fucP*, L-fucose permease; *fucI203*, lack of L-fucose isomerase activity (*fucI*); *fucA1*, lack of L-fuculose-1-phosphate aldolase activity (*fucA*); Fuc$^-$, L-fucose negative; Dar$^+$, D-arabinose positive; Rt1$^+$, ribitol positive; Srl, sorbitol; Ura$^-$, uracil requirement.

a coupled assay with α-glycerol phosphate dehydrogenase (EC 1.1.1.8) and NADH (29). L-Fucose permease activity was measured by determining the rate of L-[1-^{14}C]fucose uptake (24). Kinase activities were measured by determining the rate of NADH oxidation in a coupled assay system employing pyruvate kinase and lactate dehydrogenase (1), with dithiothreitol replacing glutathione in the reaction mixtures. In kinase assays, the sugar substrates were added to a final concentration of 6.6 mM. For the determination of kinetic parameters, the activity of a fixed concentration of each purified enzyme was measured at various substrate concentrations. For each substrate concentration, activity was calculated after the reaction had reached a steady state. The linked kinase assay could be used for the determination of kinetic parameters since the overall reaction is thermodynamically irreversible because of the very large positive equilibrium constants and low K_m values of the linking enzymes. In determining substrate specificities of the kinases, the final concentration of substrate was increased to 100 mM.

Protein purifications. All steps in the protein purifications were performed at 4°C or in ice water baths unless otherwise indicated. Protein concentrations were determined by the method of Bradford (10). The buffer routinely used in the purifications was TME buffer (50 mM Tris-HCl [pH 7.6] at 4°C, 8 mM magnesium acetate, 1 mM EDTA, 10 mM 2-mercaptoethanol) or TMGE buffer (50 mM Tris-HCl, 8 mM magnesium chloride, 0.5 mM EDTA, 1 mM 2-mercaptoethanol, 5% glycerol). The pH of TMGE buffer was adjusted to 7.4 or 8.2, at room temperature, as indicated.

D-Ribulokinase and L-fuculokinase were purified from strains EM2543 and EM2402, respectively. Each strain was grown at 37°C with aeration to late exponential phase in 10 liters of minimal salts medium containing 1.0% casein hydrolysate and 0.2% D-arabinose. Cells were harvested by centrifugation, washed in TME buffer, and then broken by sonication. Nucleic acids were removed by precipitation with protamine sulfate (30). Each extract was brought to 30% saturation of ammonium sulfate (1.60 M), with the resulting precipitate removed by centrifugation. The subsequent supernatants were brought to 50% saturation of ammonium sulfate (2.67 M) and then recentrifuged. The supernatants were discarded, and each precipitate was slowly resuspended in 55 ml of TME buffer and then dialyzed against three 1-liter volumes of TME buffer. After dialysis, each extract was loaded onto a DEAE-cellulose column (3.7 by 22 cm) previously equilibrated with TME buffer. Each column was washed with 2 column volumes of TME buffer containing 100 mM NaCl and then eluted with a step gradient from 100 to 200 mM NaCl, in 5 mM NaCl

increments of 100 ml each, at a flow rate of 3 ml/min. Fractions were collected and assayed for kinase activity.

D-Ribulokinase-active DEAE-cellulose fractions were pooled, and proteins were adsorbed with 9 g of hydroxylapatite that had been rehydrated in TME buffer. The hydroxylapatite slurry was poured into a column, allowed to settle, and then packed at a constant flow rate of 12 ml/h until it reached a stable volume (1.8 by 8.8 cm; 22.4-ml bed volume). Proteins were eluted with a 125-ml linear gradient of 0 to 20 mM potassium phosphate (pH 7.6) in TME buffer at a flow rate of 12 ml/h. D-Ribulokinase-active fractions were pooled and concentrated to 0.25 ml by vacuum dialysis (Bio-Molecular Dynamics) against 1 liter of TME buffer. The concentrate was further purified by high-pressure liquid chromatography (HPLC) using a TSK DEAE-5PW anion-exchange column (7.5 by 75 mm; Bio-Rad Laboratories). Proteins were eluted with TMGE buffer (pH 7.4) containing a 0 to 200 mM NaCl linear gradient at a flow rate of 1 ml/min over 60 min. D-Ribulokinase-active fractions were pooled, concentrated by vacuum dialysis, and then passed through the TSK DEAE-5PW column under the conditions described above but with TMGE buffer at pH 8.2. D-Ribulokinase-active fractions were pooled and concentrated by vacuum dialysis. The concentrate was injected onto a Zorbax GF-250 gel filtration column (9.4 mm by 25 cm; Dupont) and eluted with TMGE buffer (pH 7.4) containing 200 mM NaCl at a flow rate of 0.5 ml/min. D-Ribulokinase-active fractions were pooled and stored at −20°C in TME buffer containing 20% glycerol.

L-Fuculokinase-active DEAE-cellulose fractions were pooled, and proteins were adsorbed with 5.8 g of hydroxylapatite that had been rehydrated in TME buffer. The slurry was poured, settled, and packed as described above. The packed bed (1.5 by 8.0 cm, 14.4-ml bed volume) was eluted with a 70-ml linear gradient of 0 to 20 mM KPO_4 (pH 7.6) in TME buffer at a flow rate of 6 ml/h. L-Fuculokinase-active fractions were pooled and concentrated to 0.25 ml by vacuum dialysis against 1 liter of TME buffer. The concentrate was loaded into a preparative electrophoresis unit (Bethesda Research Laboratories) with a 4-cm 5% native polyacrylamide gel and a 1.5-cm stacking gel. Electrophoresis and elution conditions were as previously described (22) except that TME buffer was used for elution. L-Fuculokinase-active fractions were pooled, concentrated by vacuum dialysis, and stored at −20°C in TME buffer containing 20% glycerol.

Electrophoresis and molecular weight determinations. Subunit molecular weight was determined by electrophoresis under denaturing conditions by the method of Laemmli (27). Electrophoresis under nondenaturing conditions and determination of native molecular weight were performed as described by Davis (19) and Bryan (11), using slab gels. $MgCl_2$ (2 mM) was added to stacking and separating gels and lower tank buffers used during nondenaturing electrophoresis. For the determination of native molecular weights, purified kinases were electrophoresed in 6, 7, 8, 9, and 10% native polyacrylamide gels. Gels were stained for protein with Coomassie blue.

Reagents and chemicals. L-[1-^{14}C]fucose was from New England Nuclear Corp. Enzyme-grade ultrapure ammonium sulfate was from Schwarz-Mann. HPLC-grade water and magnesium acetate were purchased from J. T. Baker Chemical Co. Hydroxylapatite (Bio-Gel HTP hydroxylapatite, DNA grade) was obtained from Bio-Rad Laboratories.

L-Ribulose was prepared by the biological isomerization of L-arabinose by strain EM2524. EM2524 was grown to late exponential phase in minimal salts medium containing 0.5% casein hydrolysate and 0.5% L-arabinose. Cells were harvested and washed by centrifugation, resuspended in an equal volume of 250 mM potassium borate buffer (pH 7.6) containing 0.5% L-arabinose, and incubated at 37°C with aeration for 24 h. The cells were removed by centrifugation, and the supernatant was filtered through a 0.45-μm-pore-size membrane filter. The L-ribulose was purified according to the L-fuculose purification procedure of Bartkus and Mortlock (5). Thin-layer chromatography in 5:7:1 butanol-ethanol-water showed the L-ribulose to be free of residual L-arabinose.

L-Fuculose was prepared from L-fucose by using whole cells as previously described (5). D-Ribulose was prepared from ribitol as previously described (40), and contaminating ribitol was removed by passage through a column of Dowex 50W-X4 (200/400 mesh) resin in the Ca^{2+} form (2). D- and L-xylulose were previously prepared (23).

RESULTS

Isolation of the *darK*, *fucP*, and *fucA* genes. In a first approach to cloning the *E. coli* B L-fucose-D-arabinose gene cluster, a library was constructed in pBR322 by using a *Sau*3A1 partial digest of chromosomal DNA from strain EM2022 (a *fucA*$^+$ revertant). The library was screened for recombinants that could complement the chromosomal *darK* (D-ribulokinase) mutation in strain EM5524, and two clones, pEM101 and pEM201, were isolated. These plasmids contained inserts of approximately 13.1 and 11.4 kb, respectively, and had identical restriction sites over a region of about 6.3 kb (Fig. 2).

Plasmid pEM101 directed the constitutive synthesis of D-ribulokinase and L-fucose permease activities but could not complement L-fucose isomerase-, L-fuculokinase-, or L-fuculose-1-phosphate aldolase-negative mutations. D-Ribulokinase specific activities greater than the constitutive level could be induced from pEM101 by the addition of L-fucose or D-arabinose to the growth medium (Table 2). Since an L-fucose permease mutant was not available, it was not determined if L-fucose or D-arabinose could induce higher levels of permease activity from pEM101.

Plasmid pEM201 directed the constitutive synthesis of D-ribulokinase and L-fuculose-1-phosphate aldolase activities but could not complement L-fuculokinase- or L-fucose isomerase-negative mutations and did not appear to direct the synthesis of L-fucose permease (Table 2). L-Fucose or D-arabinose did not induce D-ribulokinase specific activities greater than the constitutive level from pEM201. However, addition of L-fucose slightly increased L-fuculose-1-phosphate aldolase activity above the constitutive level (Table 2).

Isolation of the *fucI* and *fucK* genes. Plasmids pEM101 and pEM201 could not complement *fucI* or *fucK* mutations. Other attempts to isolate these genes by complementation of *fucI* or *fucK* mutations by using plasmid-based libraries constructed with partial or complete digests of *E. coli* B DNA were unsuccessful. Therefore, in another approach to isolate the *fucI* and *fucK* genes, EM2022 DNA was digested completely with *Xho*I and then cloned into the bacteriophage λ cloning vector, λ2001. Recombinant phage were screened for *E. coli* B *fuc* gene sequences by plaque hybridizations with ^{32}P-labeled pfuc16. Three hybridizing plaques were isolated, and each contained an identical 18.2-kb insert. One of these phage, λ19A2, was selected for subsequent experiments. Based on a similarity in the positions of some restriction sites between one end of the λ19A2 insert and the *E. coli* K-12 *fuc* genes (15) (Fig. 2), a 5.8-kb *Bam*HI fragment was subcloned into pHG165 to generate plasmid pEM1911.

Plasmid pEM1911 complemented the *fucI* and *fucK* mutations of strain EM5543 and constitutively synthesized L-fucose isomerase and L-fuculokinase. Addition of IPTG to the growth

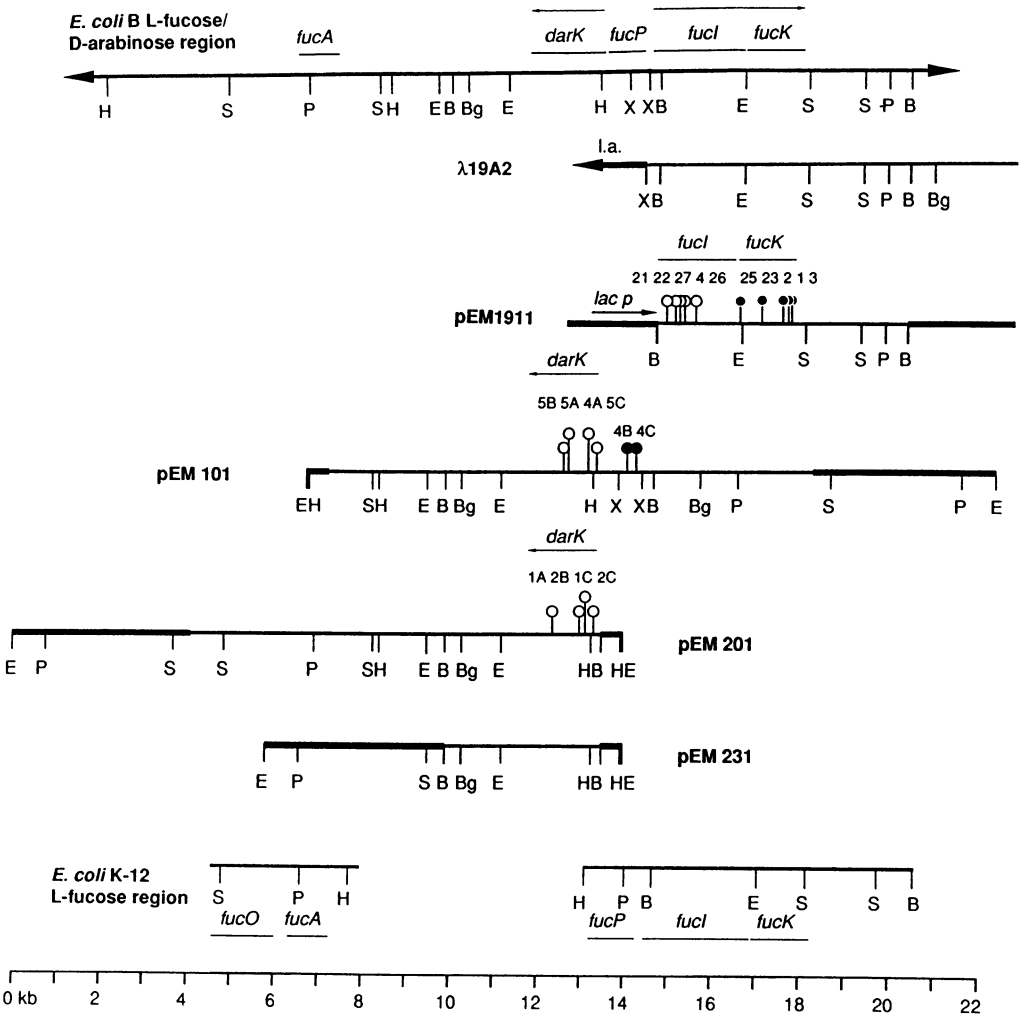

FIG. 2. Restriction maps of L-fucose-D-arabinose clones. The thick lines represent vector sequences, and the thin lines represent insert sequences. For λ19A2, the part of the phage containing *fuc* sequences and the junction with the left arm (l.a.) of the vector is shown. The symbols above the plasmid maps indicate the positions of Tn*5* insertions, which are identified by number. For pEM101 and pEM201, the open circles represent D-ribulokinase-negative insertions, and the closed circles represent L-fucose permease-negative insertions. For pEM1911, the open circles represent insertions lacking both L-fucose isomerase and L-fuculokinase activity, and the closed circles represent insertions lacking only L-fuculokinase activity. In pEM1911, *lac p* indicates the direction of transcription from the *lac* promoter. The composite *E. coli* B L-fucose-D-arabinose region is shown at the top, with the positions and directions of transcription of the *fucI*, *fucK*, and *darK* genes indicated. The *E. coli* K-12 L-fucose region (15) is show at the bottom and has been separated into two segments for comparison with the *E. coli* B region. Restriction enzymes: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sal*I; X, *Xho*I.

medium induced these activities further, but L-fucose could not induce them above the levels found in casein hydrolysate-grown cells (Table 2). IPTG-induced pEM1911 directed the synthesis of L-fuculokinase at a level that was 5-fold higher than that for L-fucose-induced EM2022 yet synthesized L-fucose isomerase at a specific activity that was 10-fold less than that for induced EM2022 (Table 2). The positions of Tn*5* insertions that abolished isomerase activity indicated that the *fucI* gene is near the end of the pEM1911 insert (see below). The lower specific activity of the isomerase than of the kinase might be due to the loss of some N-terminal amino acids of the isomerase during subcloning and/or to a fusion of the isomerase gene to the *lacZ* fragment in pHG165. Such alterations might reduce the activity of the isomerase, and therefore, specific activity measurements would not accurately reflect the transcription of the gene.

Transposon mutagenesis. To localize the *darK* gene, plasmids pEM101 and pEM201 were mutagenized with transposon Tn*5* and then used to transform strain EM5524 (*fucK28 darK110*). Transformants were scored for the ability to grow on D-arabinose minimal medium. Four independent Tn*5* insertions resulting in a D-arabinose-negative phenotype were identified in each plasmid. These insertions mapped to the same area within the region of identity between pEM101 and pEM201 (Fig. 2). The pEM101::Tn*5* insertion mutants were still capable of synthesizing L-fucose permease, and the pEM201::Tn*5* mutants could still complement the *fucA* mutation of EM5000.

The inability of pEM201 and pEM1911 to direct the synthesis of L-fucose permease suggested that the *fucP* gene was located in a 1.2-kb segment of pEM101 that was not shared by either of the former plasmids. To localize the *fucP* gene, the pEM101::Tn*5* pool was screened for mutants that did not synthesize L-fucose permease activity in casein hydrolysate-

TABLE 2. Enzyme specific activities encoded by plasmids

Strain or plasmid	Growth substrate[a]	Sp act[b]				
		L-Fucose permease	D-Ribulokinase	L-Fuculose-1-P aldolase	L-Fucose isomerase	L-Fuculokinase
Strain EM2022 (Fuc$^+$ Dar$^+$)	CH	<0.1	<5	<5	<5	<5
	CH + L-fucose	6.52	222	83	460	120
	CH + D-arabinose	5.25	304	64	400	100
Plasmids						
pEM101	CH	10.72[c]	1,060[d]	ND[e,f]	ND[g]	<5[h]
	CH + L-fucose	ND	1955	ND	ND	<5
	CH + D-arabinose	ND	2150	ND	ND	<5
pEM201	CH	<0.1[c]	144[d]	507[e]	ND[g]	<5[h]
	CH + L-fucose	ND	153	676	ND	<5
	CH + D-arabinose	ND	140	ND	ND	<5
pEM1911	CH	<0.1[c]	207[d]	ND[e]	18[g]	302[i]
	CH + L-fucose	ND	204	ND	17	296
	CH + IPTG	ND	471	ND	40	672

[a] All cultures were grown in minimal salts broth containing 0.5% casein hydrolysate (CH). Where indicated, L-fucose or D-arabinose was added to a final concentration of 0.5%, and IPTG was added to a final concentration of 1 mM.

[b] Expressed in the following units: L-fucose permease, nanomoles of L-[1-^{14}C]fucose transported per minute per milligram of cell dry weight; L-fucose isomerase, nanomoles of L-fucose isomerized per minute per milligram of protein; L-fuculokinase, nanomoles of L-fuculose phosphorylated per minute per milligram of protein; D-ribulokinase, nanomoles of D-ribulose phosphorylated per minute per milligram of protein; aldolase, nanomoles of dihydroxyacetone phosphate formed per minute per milligram of protein.

[c] L-Fucose permease activity was determined in uninduced strain EM5524, which has <0.1 U of permease per mg of cell dry weight when grown on casein hydrolysate.

[d] D-Ribulokinase activity was determined in strain EM5524 (*fucK28 darK110*; <5 U/mg of protein).

[e] L-Fuculose-1-phosphate aldolase activity was determined in strain EM5000 (*fucA1*; <5 U/mg of protein). Plasmids pEM101 and pET1911 could not complement the aldolase-negative mutation of this strain.

[f] ND, not determined.

[g] L-Fucose isomerase activity was measured in strain EM5543 (*fucI203 fucK28*; <5 U/mg of protein). Plasmids pEM101 and pEM201 could not complement the isomerase mutation in this strain.

[h] L-Fuculokinase activities of plasmids pET101 and pET102 were determined in strain EM5524 (*fucK28 darK110*; <5 U/mg of protein).

[i] L-Fuculokinase activity of plasmid pET1911 was determined in strain EM5543 (*fucI203 fucK28*; <5 U/mg of protein).

grown EM5524. Two independent permease-negative insertion mutants were isolated. These plasmids could still complement the *darK* mutation of EM5524. The Tn*5* insertions mapped within the region of pEM101 that was not shared by either pEM201 or pEM1911 (Fig. 2).

To localize the *fucI* and *fucK* genes, plasmid pEM1911 was mutagenized with Tn*5* and used to transform strain EM5543 (*fucI203 fucK28*). Transformants were scored for the ability to grow on L-fucose minimal medium, and IPTG-induced levels of L-fucose isomerase and L-fuculokinase were measured in L-fucose-negative insertion mutants. All insertions that abolished L-fucose isomerase activity also abolished L-fuculokinase activity. Several L-fucose-negative mutants that lacked kinase activity but still retained isomerase activity were isolated (Fig. 2). This polarity suggests that the *fucI* and *fucK* genes are part of an operon, with the *fucI* gene transcribed before the *fucK* gene.

The position of the *fucA* gene was identified by Tn*5* mutagenesis of additional clones not described here. These insertions mapped to a region carried by pEM201 not shared with pEM101 and allowed for the placement of the *fucA* gene as indicated in Fig. 2. This placement was in agreement with the *E. coli* K-12 map (12, 15).

In vitro transcription-translation analysis of pEM101 and pEM201. The polypeptides encoded by pEM101, pEM201, and their *darK*::Tn*5* insertion mutants were identified by in vitro transcription-translation. Plasmid pEM101 directed the synthesis of three polypeptides with apparent molecular weights of 59,500, 44,000, and 36,000, in addition to the precursor of β-lactamase from pBR322 (30,500; Fig. 3). Plasmid pEM201 directed the synthesis of five novel polypeptides with apparent molecular weights of 59,500, 44,000, 40,000, 36,000, and 26,000, in addition to β-lactamase (Fig. 3). The *darK*::Tn*5*

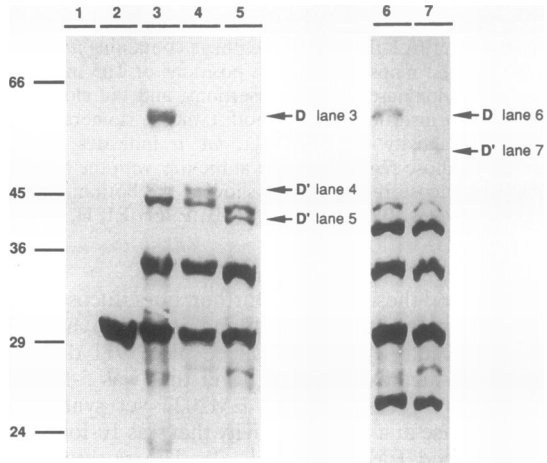

FIG. 3. In vitro transcription-translation analysis of pEM101 and pEM201. Shown is a fluorograph of an SDS–7.5% polyacrylamide gel showing polypeptides encoded by pBR322::Tn*5* (lane 2), pEM101 (lane 3), pEM101::Tn*5*-5B (lane 4), pEM101::Tn*5*-5A (lane 5), pEM201 (lane 6), and pEM201::Tn*5*-1A (lane 7). Lane 1 is a no-DNA control for the in vitro reaction, and lane 2 is a control for the polypeptides synthesized by pBR322 and Tn*5*. Gene products of interest are indicated by arrows: D is the *darK* gene product (D-ribulokinase) in lanes 3 and 6, and D′ indicates a truncated form of D-ribulokinase due to Tn*5* insertion. The migration of protein standards is indicated in kilodaltons to the left.

insertion mutants did not produce the 59.5-kDa peptide. However, three insertion mutants, pEM101::Tn*5*-5A and -5B and pEM201::Tn*5*-1A, produced truncated peptides of 42.5, 45.0, and 51.0 kDa, respectively (Fig. 3). These results indicated that the 59.5-kDa polypeptide was responsible for the D-ribulokinase activity. Transposon Tn*5* contains stop codons in all three reading frames near the ends of its inverted repeats. Therefore, the size of the truncated proteins produced by the insertion mutants, together with the points of Tn*5* insertion, identified the position and direction of transcription of the *darK* gene (Fig. 2). The apparent molecular weight for D-ribulokinase was in agreement with purification studies that showed this protein to have a subunit molecular weight of 60,000 (see below).

The *E. coli* B gene cluster contains sequences not shared with *E. coli* K-12. Taken together, the three plasmids, pEM101, pEM201, and pET1911 contained the *fucPIKA* and *darK* L-fucose-D-arabinose gene cluster from *E. coli* B. Although isolated on three plasmids, the inserts represented a contiguous region of the chromosome. The contiguous nature of the inserts contained in plasmids pEM101 and pEM201 was made apparent by the region of restriction site identity. The relationship between the inserts of pEM101 and pEM1911 was not apparent because of the divergent restriction sites between these plasmids. However, DNA hybridization experiments have shown that these plasmids have homologous sequences in the region of pEM1911 containing the *fucI* gene (data not shown). This region of homology did not extend through the entire *fucI* gene, a result that was in agreement with the inability of pEM101 to direct the synthesis of isomerase activity. This result suggests that DNA scrambling had occurred during the formation of pEM101 and that the insert downstream of the *fucI* homologous region was not part of the L-fucose-D-arabinose gene cluster. The region upstream of the *fucI* homologous region was not scrambled, as shown by the restriction site identity between plasmids pEM101 and pEM201. The homology between pEM101 and pEM1911 allowed for the alignment of the *E. coli* B *fuc* gene sequences contained in these plasmids (Fig. 2). The validity of this alignment was strengthened by the presence of common *Xho*I and *Bam*HI sites in pEM101 and λ19A2 (Fig. 2).

An alignment of the restriction sites found in the *E. coli* K-12 L-fucose gene cluster (15) with those of the *E. coli* B L-fucose-D-arabinose gene cluster required the K-12 restriction map to be separated (Fig. 2). The *E. coli* B L-fucose-D-arabinose gene cluster contained a 5.2-kb region that was not shared with the K-12 L-fucose genes. This segment was located between the *fucP* and *fucA* genes and contained the *darK* gene (Fig. 2). Hybridization experiments showed that this 5.2-kb sequence was not homologous to plasmids containing the K-12 L-fucose genes. Between the *darK* and *fucA* genes was an approximately 3.5-kb segment with no known function. This segment may direct the synthesis of at least one polypeptide; pEM101 and pEM201 each directs the synthesis of a polypeptide with a molecular mass of about 36 kDa, and qualitatively, it is produced to the same extent by both plasmids (Fig. 3). In consideration of this similarity, the 36-kDa peptide may be encoded within the 3.5-kb segment.

A 3.6-kb *Bam*HI fragment carrying the *darK* gene was subcloned from pEM201 to generate plasmid pEM231 (Fig. 2). This plasmid was used as a hybridization probe to determine if the *darK* gene could be found in other organisms capable of growth on D-arabinose. Chromosomal DNA was isolated from *E. coli* K-12, *Klebsiella pneumoniae* PRLR3, and *Erwinia chrysanthemi* LM8200, digested completely with *Bam*HI, and probed with ^{32}P-labeled pEM231 under conditions that would

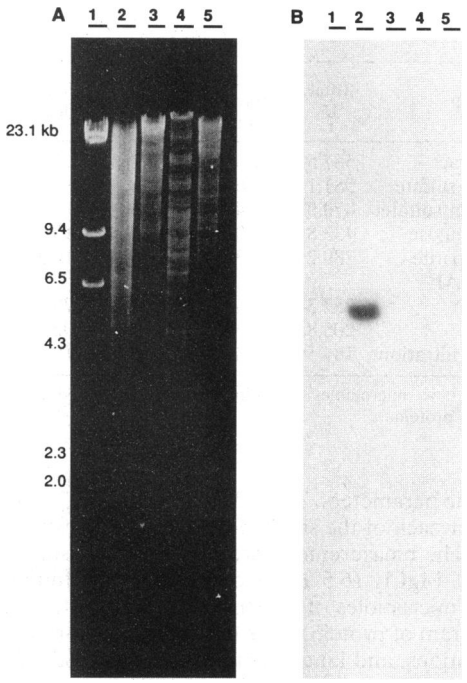

FIG. 4. Hybridization of the *darK* gene to enteric bacteria. Genomic DNAs were completely digested with *Bam*HI, electrophoresed through a 0.5% gel, transferred to nitrocellulose, and then probed with ^{32}P-labeled pEM231 (see Fig. 2). Lane 1, λ *Hin*dIII DNA standards; lane 2, *E. coli* B; lane 3, *E. coli* K-12; lane 4, *K. pneumoniae* PRLR3; lane 5, *Erwinia chrysanthemi* LM8200. (A) Electrophoretic pattern prior to transfer; (B) autoradiograph of probed filter.

allow 35% base pair mismatch. *Erwinia chrysanthemi* LM8200 is phenotypically similar to wild-type *E. coli* B; it can grow on D-arabinose without the requirement of a mutation, but cannot grow on L-fucose (28). Only *E. coli* B chromosomal DNA showed homology to pEM231 (Fig. 4). A single band was observed, indicating that only a single copy of this sequence could be found in the *E. coli* B chromosome.

The *darK* gene also was examined for homology to genes coding for other pentulokinases. Plasmid p21-193 carries the *araB* gene coding for the L-ribulokinase of the L-arabinose pathway. The lambda phage λdAR carries the *rtlK* gene coding for the D-ribulokinase of the ribitol pathway. Under low stringency, pEM231 did not hybridize with p21-193 or λdAR (data not shown).

D-Ribulokinase and L-fuculokinase. We were interested in characterizing the D-ribulokinase encoded by the *darK* gene and comparing it with the L-fuculokinase encoded by the *fucK* gene. We therefore purified and partially characterized these two enzymes. Summaries of the D-ribulokinase and L-fuculokinase purifications are shown in Tables 3 and 4, respectively. The purification achieved at some of the steps is shown in Fig. 5. Both enzymes were homogeneous, as judged by SDS-polyacrylamide gel electrophoresis (Fig. 5) and native polyacrylamide gels. The native and subunit molecular weights of D-ribulokinase were estimated to be 124,000 and 60,000, respectively. The native and subunit molecular weights of L-fuculokinase were estimated to be 82,000 and 45,000, respectively. Each purified enzyme produced a single band in both native and SDS-polyacrylamide gels and therefore appeared to be a dimer composed of a single subunit type.

The purified proteins were studied for substrate specificities

TABLE 3. Purification of D-ribulokinase from *E. coli* B strain EM2543

Step	Total U	Total protein (μg)	Sp act[a]	Fold purification	% Recovery
Crude extract	587.5	941.6	0.62	1	100
Protamine sulfate	581.1	908.6	0.64	1	99
Ammonium sulfate	470.0	416.4	1.13	1.8	80
DEAE-cellulose	433.8	70.0	6.20	10.0	74
Hydroxylapatite	289.2	44.3	6.53	10.5	49
HPLC-DEAE					
pH 7.4	257.3	1.48	173.9	279	44
pH 8.2	205.8	0.656	313.7	506	35
HPLC-gel filtration	164.5	0.371	443.4	715	28

[a] Expressed as micromoles of D-ribulose phosphorylated per minute per milligram of protein.

and kinetic parameters. Table 5 lists the K_m, V_{max}, and relative activity for each of the sugars found to be a substrate for each enzyme. The measurements were made at constant ATP (3.3 mM) and $MgCl_2$ (6.5 mM) concentrations. Initial rates of reaction (micromoles of substrate phosphorylated per minute per milligram of protein) were determined at various substrate concentrations, and kinetic parameters were estimated by the direct linear plot method of Cornish-Bowden and Eisenthal (17). In addition to the sugars indicated in Table 5, others were tested for the ability to act as a substrate. No activity was found on 100 mM D-xylulose, xylitol, L-arabitol, D-mannitol, D-sorbitol, L-arabinose, D-arabinose, D-xylose, D-lyxose, L-lyxose, L-fucose, D-glucose, D-mannose, L-sorbose, D-fructose, glycerol, or 2-deoxy-D-ribose for either of the enzymes.

DISCUSSION

E. coli B can metabolize D-arabinose without mutation, and its L-fucose-D-arabinose regulon appears to be evolved toward D-arabinose metabolism and away from L-fucose metabolism. Since physiological evidence has suggested that the *E. coli* B regulon evolved from a K-12-like ancestor (24), the differences observed between the two regulons should indicate the steps taken by nature in the evolution of a true D-arabinose pathway. The proposed organization of the *E. coli* B L-fucose-D-arabinose gene cluster is summarized in Fig. 2. The positions and lengths of the genes were determined from cloning, Tn*5* mutagenesis, and protein purifications as described in these experiments and from published subunit molecular weights of L-fucose enzymes (9). The *E. coli* B L-fucose-D-arabinose regulon appears to be composed of at least three operons. One

TABLE 4. Purification of L-fuculokinase from *E. coli* B strain EM2402

Step	Total U	Total protein (μg)	Sp act[a]	Fold purification	% Recovery
Crude extract	422.0	1,767.0	0.24	1	100
Protamine sulfate	274.6	1,668.7	0.16	1	65
Ammonium sulfate	213.0	349.7	0.61	2.5	50
DEAE-cellulose	142.4	23.5	6.06	25.3	34
Hydroxylapatite	138.2	3.16	43.2	180	33
Preparative electrophoresis	15.7	0.098	160.2	668	3.7

[a] Expressed as micromoles of L-fuculose phosphorylated per minute per milligram of protein.

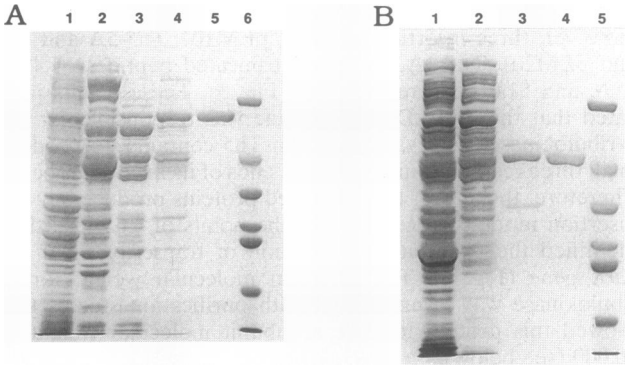

FIG. 5. Purification of kinases. Samples taken from steps in the purification of D-ribulokinase and L-fuculokinase were separated on SDS–7.5% polyacrylamide gels and stained with Coomassie blue. (A) D-Ribulokinase purification steps. Lane 1, crude extract (100 μg); lane 2, DEAE-cellulose eluate (100 μg); lane 3, hydroxylapatite eluate (50 μg); lane 4, HPLC-DEAE pH 7.4 eluate (50 μg); lane 5, HPLC-gel filtration eluate (10 μg); lane 6, molecular weight standards (bovine albumin [66,000], egg albumin [45,000], glyceraldehyde-3-phosphate dehydrogenase [36,000], carbonic anhydrase [29,000], trypsinogen [20,100], and trypsin inhibitor [20,100]). (B) L-Fuculokinase purification steps. Lane 1, crude extract (100 μg); lane 2, DEAE-cellulose eluate (100 μg); lane 3, hydroxylapatite eluate (25 μg); lane 4, preparative electrophoresis (10 μg); lane 5, molecular weight standards as in panel A.

operon contains the *fucI* and *fucK* genes and is transcribed from left to right. A second operon contains the *darK* gene and is transcribed from right to left. A third operon contains the *fucA* gene. The directions of *fucA* and *fucP* transcription were not determined, but these genes do not appear to be part of an operon with the *darK* gene since transposon insertions in *darK* do not affect aldolase activity, and transposon insertions in *fucP* do not affect D-ribulokinase activity. In *E. coli* K-12, the *fucP* gene is part of an operon with the *fucI* and *fucK* genes, and this could also be the case in *E. coli* B. The position of the *fucO* gene in *E. coli* B has not been determined.

The relative order of the *E. coli* B *fucP*, *-I*, *-K*, and *-A* genes was the same as that in *E. coli* K-12 (12, 15, 49) and verified the *E. coli* B gene order as previously determined in two- and three-factor transductional crosses (24). The major difference between the K-12 and B regulons was the presence of the *darK*

TABLE 5. Substrate specificities and kinetic parameters of pentulokinases

Enzyme	Substrate	K_m (mM)	V_{max}[a]	% Relative activity[b]
D-Ribulokinase	D-Ribulose	1.8	434.6	100
	D-Ribose	0.26	71.4	34.6
	Ribitol	65	279.8	12.1
	L-Ribulose	76	351.3	11.3
	D-Arabitol	53	23.8	10.3
L-Fuculokinase	L-Fuculose	0.16	178.3	100
	D-Ribulose	5.3	90.4	73.2
	L-Xylulose	ND[c]	ND	5.4

[a] Expressed as micromoles of substrate phosphorylated per minute per milligram of protein.

[b] In determining relative activity, the final concentration of all substrates was 100 mM.

[c] ND, not determined. L-Fuculokinase was not saturated at the highest L-xylulose concentration available (100 mM); therefore, the kinetic parameters were not determined for that substrate.

gene in *E. coli* B. The *darK* gene was found within a 5.2-kb DNA segment located between the *fucA* and *fucP* genes. Other than D-ribulokinase, this segment did not encode any known enzymatic activities. The *darK* segment was not found in *E. coli* K-12 or in other D-arabinose-positive organisms examined. Acquisition of the *darK* gene represents a major step in the evolution of a true D-arabinose pathway in *E. coli* B, since the D-ribulokinase activity that it encodes provides a route for efficient metabolism of D-arabinose.

Additional steps taken in the evolution of a true D-arabinose pathway may have involved regulatory mutations. The *E. coli* K-12 L-fucose regulon appears to be under positive control (14, 15), with L-fuculose-1-phosphate as the apparent inducer (5). In contrast, the apparent inducers of the *E. coli* B regulon are L-fucose and D-arabinose (24). The *fucR* gene, which encodes the positive regulatory protein of the K-12 regulon, is located just downstream from the *fucK* gene (14, 15). Mutations in *E. coli* B that result in the constitutive synthesis of the *fucP*, *fucI*, *fucK*, *fucA*, and *darK* genes also map downstream of the *fucK* gene and may be in a regulatory protein (24). Plasmids pEM101 and pEM201 would not contain this putative regulator, and the endogenous promoters contained in these clones would titrate the chromosomally encoded regulatory protein. Under this condition, the constitutive synthesis of D-ribulokinase, L-fucose permease, and L-fuculose-1-phosphate aldolase directed by pEM101 and pEM201 would be indicative of a negative control mechanism. The observed increase in specific activity when the clones are grown in the presence of inducer supports this hypothesis. If *E. coli* B did evolve from a K-12-like ancestor, then mutations must have occurred to account for these changes in regulation. Additionally, the *darK* gene responds to L-fucose regulation signals. After insertion of the *darK* gene, mutations may have been required to establish this level of control.

We are interested in the origins of the *E. coli* B D-ribulokinase. Given the dissimilarities in molecular weight, kinetic parameters, substrate specificities, and mode of action of D-ribulokinase and L-fuculokinase, it appears unlikely that these proteins are related. This conclusion is supported by the lack of DNA hybridization between the *darK* and *fucK* genes. The molecular weight and kinetic parameters of the *E. coli* B D-ribulokinase suggest that this protein may be related to the L-ribulokinase of the L-arabinose pathway or the D-ribulokinase of the ribitol pathway. Each of these enzymes is a dimer of 60-kDa subunits, phosphorylates D-ribulose at the C-5 position, and can provide an alternate route for D-arabinose metabolism in *E. coli* (8, 31–33, 37). However, our low-stringency hybridization experiments showed that the *darK* gene is not homologous with either the *araB* or *rtlK* gene, encoding L-ribulokinase or D-ribulokinase, respectively. Analysis of the *darK* DNA sequence may reveal a similarity between these genes or their protein products.

The experimentally evolved D-arabinose metabolic pathways identified in ribitol-positive *E. coli* K-12 are very similar to the naturally evolved D-arabinose pathway found in *E. coli* B. This similarity indicates that the experimental approach to understanding evolutionary mechanisms provides an accurate prediction of the steps taken by nature during the development of a pathway for a novel compound. Further molecular analysis of the *E. coli* B L-fucose-D-arabinose regulon will provide a greater understanding of the events shaping the evolution of this naturally occurring D-arabinose pathway.

ACKNOWLEDGMENTS

We thank E. C. C. Lin and Y.-M. Chen for interesting discussions and for plasmid pfuc16. We thank Albey Reiner for λdAR and Nancy Lee for plasmid p21-193. We thank Joanne Bartkus, Bianca Brahamsha, Reed Doten, Valley Stewart, and Mike Weiner for helpful discussions.

REFERENCES

1. **Anderson, R. L., and W. A. Wood.** 1962. Purification and properties of L-xylulokinase. J. Biol. Chem. **237**:1029–1033.
2. **Angyal, S. J., G. S. Bethell, and R. J. Beveridge.** 1979. The separation of sugars and of polyols on cation-exchange resins in the calcium form. Carbohydr. Res. **73**:9–18.
3. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
4. **Bartkus, J. M., and R. P. Mortlock.** 1986. Construction of an improved D-arabinose pathway in *Escherichia coli* K-12. J. Bacteriol. **165**:704–709.
5. **Bartkus, J. M., and R. P. Mortlock.** 1986. Isolation of a mutation resulting in constitutive synthesis of L-fucose catabolic enzymes. J. Bacteriol. **165**:710–714.
6. **Bisson, T. M., E. J. Oliver, and R. P. Mortlock.** 1968. Regulation of pentitol metabolism by *Aerobacter aerogenes*. II. Induction of the ribitol pathway. J. Bacteriol. **95**:932–936.
7. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
8. **Boulter, J., B. Gielow, M. McFarland, and N. Lee.** 1974. Metabolism of D-arabinose by *Escherichia coli* B/r. J. Bacteriol. **117**:920–923.
9. **Boulter, J. R., and W. O. Gielow.** 1973. Properties of D-arabinose isomerase purified from two strains of *Escherichia coli*. J. Bacteriol. **113**:687–696.
10. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
11. **Bryan, J. K.** 1977. Molecular weights of protein multimers from polyacrylamide gel electrophoresis. Anal. Biochem. **78**:513–519.
12. **Chakrabarti, T., Y.-M. Chen, and E. C. C. Lin.** 1984. Clustering of genes for L-fucose dissimilation by *Escherichia coli*. J. Bacteriol. **157**:984–986.
13. **Chen, Y.-M., Z. Lu, and E. C. C. Lin.** 1989. Constitutive activation of the *fucAO* operon and silencing of the divergently transcribed *fucPIK* operon by an IS*5* element in *Escherichia coli* mutants selected for growth on L-1,2-propanediol. J. Bacteriol. **171**:6097–6105.
14. **Chen, Y.-M., J. F. Tobin, Y. Zhu, R. F. Schleif, and E. C. C. Lin.** 1987. Cross-induction of the L-fucose system by L-rhamnose in *Escherichia coli*. J. Bacteriol. **169**:3712–3719.
15. **Chen, Y.-M., Y. Zhu, and E. C. C. Lin.** 1987. The organization of the *fuc* regulon specifying L-fucose dissimilation in *Escherichia coli* as determined by gene cloning. Mol. Gen. Genet. **210**:331–337.
16. **Cocks, G. T., J. Aguilar, and E. C. C. Lin.** 1974. Evolution of L-1,2-propanediol catabolism in *Escherichia coli* by recruitment of enzymes for L-fucose and L-lactate metabolism. J. Bacteriol. **118**:83–88.
17. **Cornish-Bowden, A., and R. Eisenthal.** 1974. Statistical considerations in the estimation of enzyme kinetic parameters by the direct linear plot and other methods. Biochem. J. **139**:721–730.
18. **Csonka, L. N., and A. J. Clark.** 1980. Construction of an Hfr strain useful for transferring *recA* mutations between *Escherichia coli* strains. J. Bacteriol. **143**:529–530.
19. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. **121**:404–427.
20. **deBruijn, F. J., and J. R. Lupski.** 1984. The use of transposon Tn5 mutagenesis in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids—a review. Gene **27**:131–149.
21. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementing DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
22. **Doten, R. C., and R. P. Mortlock.** 1984. Directed evolution of a second xylitol catabolic pathway in *Klebsiella pneumoniae*. J. Bacteriol. **159**:730–735.

23. **Doten, R. C., and R. P. Mortlock.** 1985. Production of D- and L-xylulose by mutants of *Klebsiella pneumoniae* and *Erwinia uredovora*. Appl. Environ. Microbiol. **49:**158–162.
24. **Elsinghorst, E. A., and R. P. Mortlock.** 1989. D-Arabinose metabolism in *Escherichia coli* B: induction and cotransductional mapping of the L-fucose-D-arabinose pathway enzymes. J. Bacteriol. **170:**5423–5432.
25. **Karn, J., S. Brenner, L. Barnett, and G. Cesareni.** 1980. Novel bacteriophage λ cloning vectors. Proc. Natl. Acad. Sci. USA **77:**5172–5176.
26. **Karn, J., H. W. D. Matthes, M. J. Gait, and S. Brenner.** 1984. A new selective phage cloning vector, λ2001, with sites for *Xba*I, *Bam*HI, *Hin*dIII, *Eco*RI, *Sst*I, and *Xho*I. Gene **32:**217–224.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
28. **LaQuier, F. W.** 1986. M. S. thesis. Cornell University, Ithaca, N.Y.
29. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: a new pathway in *Escherichia coli*. J. Bacteriol. **106:**90–96.
30. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: origin of a D-ribulokinase activity in *Escherichia coli*. J. Bacteriol. **106:**82–89.
31. **LeBlanc, D. J., and R. P. Mortlock.** 1972. The metabolism of D-arabinose: alternate kinases for the phosphorylation of D-ribulose in *Escherichia coli* and *Aerobacter aerogenes*. Arch. Biochem. Biophys. **150:**774–781.
32. **Lee, N., and I. Bendet.** 1967. Crystalline L-ribulokinase from *Escherichia coli*. J. Biol. Chem. **242:**2043–2050.
33. **Lin, H.-C., S.-P. Lei, and G. Wilcox.** 1985. The *araBAD* operon of *Salmonella typhimurium* LT2. I. Nucleotide sequence of *araB* and primary structure of its product, ribulokinase. Gene **34:**111–122.
34. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Meinkoth, J., and G. Wahl.** 1984. Hybridization of nucleic acids immobilized on solid supports. Anal. Biochem. **138:**267–284.
36. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **Neuberger, M. S., B. S. Hartley, and J. E. Walker.** 1981. Purification and properties of D-ribulokinase and D-xylulokinase from *Klebsiella aerogenes*. Biochem. J. **193:**513–524.
38. **Oliver, E. J., T. M. Bisson, D. J. LeBlanc, and R. P. Mortlock.** 1969. D-Ribulose production by a mutant of *Aerobacter aerogenes*. Anal. Biochem. **27:**300–305.
39. **Oliver, E. J., and R. P. Mortlock.** 1971. Growth of *Aerobacter aerogenes* on D-arabinose: origin of enzyme activities. J. Bacteriol. **108:**287–292.
40. **Reiner, A. M.** 1975. Genes for ribitol and D-arabitol catabolism in *Escherichia coli*: their loci in C strains and absence in K-12 and B strains. J. Bacteriol. **123:**530–536.
41. **Rosenberg, S. M., M. M. Stahl, I. Kobayashi, and F. W. Stahl.** 1985. Improved in vitro packaging of coliphage lambda DNA: a one-strain system free from endogeneous phage. Gene **38:**165–175.
42. **Scangos, G. A., and A. M. Reiner.** 1978. Ribitol and D-arabitol metabolism in *Escherichia coli*. J. Bacteriol. **134:**492–500.
43. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–507.
45. **Sridhara, S., and T. T. Wu.** 1969. Purification and properties of lactaldehyde dehydrogenase in *Escherichia coli*. J. Biol. Chem. **244:**5233–5238.
46. **Stewart, G. S. A. B., S. Lubinsky-Mink, C. G. Jackson, A. Cazzel, and J. Kuhn.** 1986. pHG165: a pBR322 copy number derivative of pUC8 for cloning and expression. Plasmid **15:**172–181.
47. **Wood, W. B.** 1966. Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16:**118–133.
48. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
49. **Zhe, L., and E. C. C. Lin.** 1989. The nucleotide sequence of *Escherichia coli* genes for L-fucose dissimilation. Nucleic Acids Res. **17:**4883–4884.