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Constitutive Activation of L-Fucose Genes by an Unlinked Mutation in *Escherichia coli*

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Wild-type *Escherichia coli* cannot grow on L-1,2-propanediol; mutants that can do so have increased basal activity of an NAD-linked L-1,2-propanediol oxidoreductase. This enzyme belongs to the L-fucose system and functions normally as L-lactaldehyde reductase during fermentation of the methylpentose. In wild-type cells, the activity of this enzyme is fully induced only anaerobically. Continued aerobic selection for mutants with an improved growth rate on L-1,2-propanediol inevitably leads to full constitutive expression of the oxidoreductase activity. When this occurs, L-fuculose 1-phosphate aldolase concomitantly becomes constitutive, whereas L-fucose permease, L-fucose isomerase, and L-fuculose kinase become noninducible. It is shown in this study that the noninducibility of the three proteins can be changed by two different kinds of suppressor mutations: one mapping external to and the other within the *fuc* gene cluster. Both mutations result in constitutive synthesis of the permease, the isomerase, and the kinase, without affecting synthesis of the oxidoreductase and the aldolase. Since expression of the *fuc* structural genes is activated by a protein specified by the regulator gene *fucR*, and since all the known genes of the *fuc* system are clustered at minute 60.2 of the chromosome, the external gene in which the suppressor mutation can occur probably has an unrelated function in the wild-type strain. The internal suppressor mutation might be either in *fucR* or in the promoter region of the genes encoding the permease, the isomerase, and the kinase, if these genes belong to the same operon.

L-1,2-Propanediol does not serve *Escherichia coli* as a source of carbon and energy. Successive mutants selected for improved aerobic growth rate on this compound show progressively elevated basal levels of an NAD-linked oxidoreductase encoded by a gene of the L-fucose system (3, 9, 20). L-Fucose is dissimilated by the sequential action of L-fucose permease (8), L-fucose isomerase (6), L-fuculose kinase (10), and L-fuculose 1-phosphate aldolase (5) (Fig. 1). The last enzyme catalyzes the formation of dihydroxyacetone phosphate and L-lactaldehyde. Aerobically, an NAD-linked dehydrogenase is highly induced to oxidize L-lactaldehyde to L-lactate (19), which in turn induces the flavin-linked L-lactate dehydrogenase (3). Anaerobically, an NADH-linked oxidoreductase is highly induced to reduce L-lactaldehyde to L-1,2-propanediol, which is excreted into the medium apparently via a cytoplasmic membrane protein and thus becomes irretrievably lost (3, 7). It is the enzyme anaerobically serving the wild-type strain as L-lactaldehyde reductase which is recruited by the mutants to function aerobically as L-1,2-propanediol dehydrogenase. The change of function is accomplished by allowing an active enzyme to be constitutively synthesized under aerobic conditions.

The aerobic basal activity of the oxidoreductase is barely detectable in the wild-type strain. The first mutation that confers the ability to grow aerobically on L-1,2-propanediol is associated with a 10-fold increase in this basal activity (8). Aerobic growth of this mutant in the presence of L-fucose increases the basal activity fourfold, as in the case of the wild-type strain (8). The basal and induced levels of the proteins in the trunk pathway (the permease, isomerase, kinase, and aldolase) are not affected in the initial-stage mutants. However, when the aerobic basal oxidoreductase activity is raised greatly above that seen in anaerobically induced wild-type cells by a further mutation, the inductive effect of L-fucose disappears. Invariably, with the full constitutivity of the oxidoreductase activity, the aldolase also becomes constitutive, while the permease, the kinase, and the isomerase become noninducible (8, 9). The ability to grow on L-fucose is thereupon lost. Strains ECL3 and ECL421 are independently derived mutants with this phenotype (3, 8, 9).

Strain ECL3 gives spontaneous L-fucose-positive pseudorevertants at a frequency of about 5×10^{-7}; these strains synthesize the permease, the isomerase, and the kinase constitutively at levels below the fully induced ones in the wild-type strain (8). Strain ECL421 also gives rise to fucose-positive revertants at a frequency of about 5×10^{-7}. A clone which has been analyzed also synthesizes the permease, the isomerase, and the kinase constitutively at submaximal levels (this study). However, a critical genetic difference between a revertant of strain ECL3 and a revertant of strain ECL421 has been discovered and is the subject of this report.

MATERIALS AND METHODS

Chemicals. L-Lactaldehyde was prepared by the reaction of ninhydrin with D-threonine (23). L-Fuculose 1-phosphate was prepared by a modified (8) enzymatic procedure (10). L-[6-^{3}H]fucose (2 Ci/mmol) was from ICN Pharmaceuticals, Inc., Irvine, Calif. L-Fucose was from Sigma Chemical Co., St. Louis, Mo. Vitamin-free casein hydrolysate (CAA) was from ICN Nutritional Biochemicals, Cleveland, Ohio. MacConkey agar base (without lactose) was from Difco Laboratories, Detroit, Mich. All other chemicals used were commercial products of reagent grade.

Preparation of L-fuculose isomerase. *Klebsiella pneumoniae* W70, (kindly provided by R. P. Mortlock) which requires uracil and lacks L-fuculose 1-phosphate aldolase, L-arabinose kinase, D-arabitol dehydrogenase, and ribitol dehydrogenase, was grown aerobically to stationary phase in 1 liter of mineral medium (21) containing L-fucose (10^{-5} M), uracil (5 mg/liter) and CAA (5 g/liter). The incubation was

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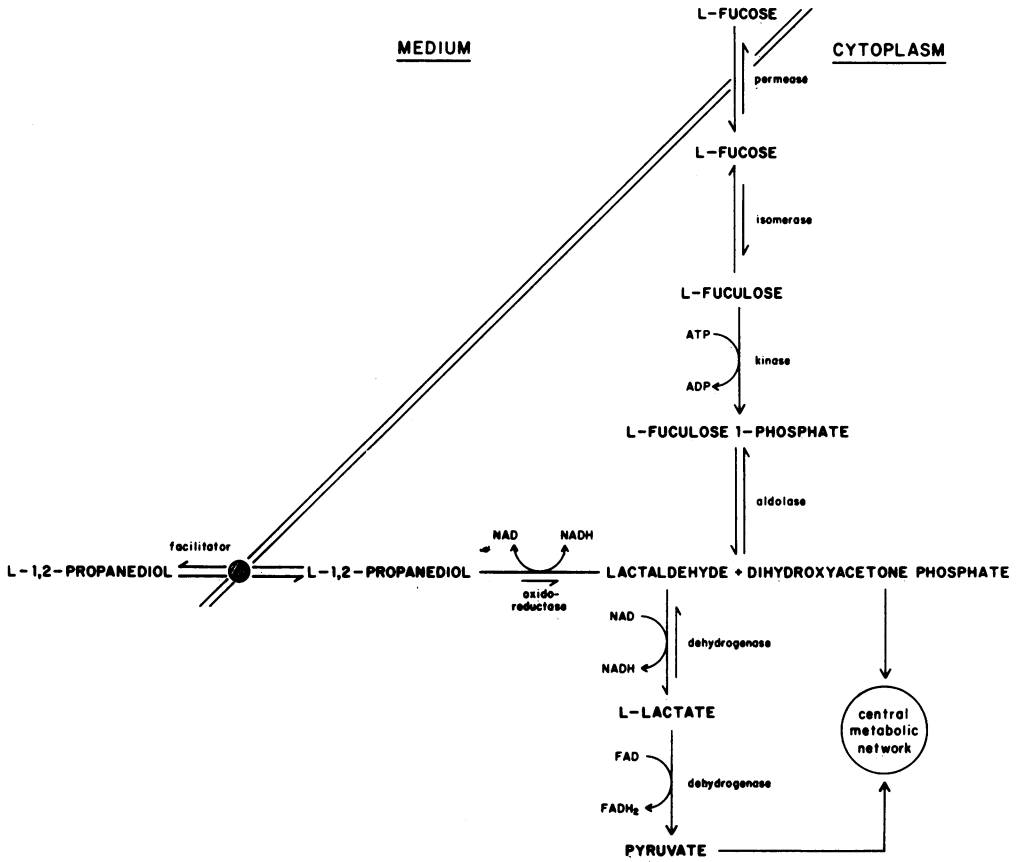

FIG. 1. Pathways for aerobic and anaerobic dissimilation of L-fucose by *E. coli*. The permease, the isomerase, the kinase, and the aldolase are inducible by L-fucose both aerobically and anaerobically. L-1,2-Propanediol oxidoreductase is fully induced anaerobically, whereas L-lactaldehyde dehydrogenase is fully induced aerobically.

carried out at 37°C in a 2-liter flask agitated on a rotary platform, and the cells were harvested by centrifugation, were washed with 50 mM potassium phosphate (pH 7.0), recentrifuged, and resuspended in 20 ml of the same buffer containing 2 mM glutathione. The dispersed cells were divided into portions and disrupted (for 1 min/ml of suspension) in a model 60W ultrasonic disintegrator (MSE) while being chilled in a −10°C bath. The treated preparations were pooled and centrifuged at 5,000 × *g* for 30 min at 4°C. The clear extract was recovered and chilled in ice. Protamine sulfate was added dropwise with mixing to give a final concentration of 1.5 mg/ml, and the precipitate was removed by centrifugation. The recovered supernatant fraction was kept at 0°C, while ammonium sulfate was gradually added to 45% saturation. After neutralization with dilute KOH, the mixture was equilibrated for 30 min by magnetic stirring. The precipitate was removed by centrifugation, and the supernatant fraction was brought to 60% ammonium sulfate saturation. The precipitate was collected, dissolved in 1.5 ml of the glutathione-phosphate buffer, and loaded onto an AcA 44 Ultrogel (LKB Instruments, Inc., France) column (2 by 56 cm; void volume 95 ml) previously washed with water and equilibrated with the glutathionine-phosphate buffer. Elution was carried out with the same buffer at a rate of 0.5 ml/min, and the eluent was collected in 2.5-ml fractions. Fractions 91 through 100 were pooled and concentrated to 7.5 ml by pressurized filtration. A total of 8.8×10^5 enzyme units (nanomoles per minute) was recovered in 55 mg of protein. No L-fuculose aldolase activity was detectable in a sample containing 250 units of the purified L-fucose isomerase, and polyacrylamide gel electrophoresis under nondenaturing conditions (22) revealed a single band.

Bacteria and phage. Sources and relevant properties of the various *Escherichia coli* K-12 strains used in the study are summarized in Table 1. Transduction with phage P1 *vir* was according to Luria et al. (16). Strain ECL289 with the *eno* and *argA*::Tn*10* markers was grown on agar containing glycerol (2 mg/ml) and succinate (2 mg/ml) as combined carbon and energy sources (11, 12) and supplemented with arginine (20 μg/ml). Double *eno*$^+$ and *arg*$^+$ transductants of strain ECL289 with various donors were selected on glucose (2 mg/ml) minimal agar (*eno* mutants cannot grow on glucose because of the enolase defect). The ability of transductants to utilize L-1,2-propanediol was scored on minimal agar (0.4% DL-1,2-propanediol; only the L-isomer is utilizable [20]), and their ability to utilize L-fucose was scored on MacConkey agar with L-fucose (1%).

Growth of cells and preparation of extracts. Liquid cultures were grown aerobically at 37°C in a 500-ml flask containing 100 ml of mineral medium (21) with appropriate supplements and agitated on a rotary shaker. Inducing medium contained 0.2% L-fucose and 0.5% CAA, and noninducing medium contained 0.5% CAA alone. For assay of L-fucose permease, cells were harvested in the exponential phase of growth by centrifugation, washed with mineral medium, and suspended at ca. 10^8 cells per ml of mineral medium containing chloramphenicol (100 μg/ml). For enzyme assays, cells were harvested from exponentially growing cultures at 100 to 150 Klett

TABLE 1. Genealogy of *E. coli* K-12 strains

Strain	Derived from strain	Selection or isolation	Genotype or phenotype[a]	Source (reference)
ECL1	*E. coli* K-12	Deletion of *phoA*	HfrC *phoA8 relA1 tonA22* $T2^r$ (λ)	(14)
ECL3	ECL1	Growth on propanediol (EMS^b-induced and spontaneous mutations)	$FucO^c$ $FucA^c$ $FucP^n$ $FucI^n$ $FucK^n$	(19)
ECL56	ECL3	Fucose-positive revertant (spontaneous)	$FucO^c$ $FucA^c$ $FucP^c$ $FucI^c$ $FucK^c$	(8)
ECL289	ECL248	Transduction of *argA*::Tn*10*	HfrC *eno argA*::Tn*10 relA1 tonA22* $T2^r$ (λ)	(1)
ECL421	ECL1	Growth on propanediol (spontaneous mutations, two steps)	$FucO^c$ $FucA^c$ $FucP^n$ $FucI^n$ $FucK^n$	(9)
ECL452	ECL289	Transduction of arg^+ eno^+ with ECL3 as donor	$FucO^c$ $FucA^c$ $FucP^n$ $FucI^n$ $FucK^n$	This study
ECL453	ECL289	Transduction of arg^+ eno^+ with ECL56 as donor	$FucO^c$ $FucA^c$ $FucP^n$ $FucI^n$ $FucK^n$	This study
ECL459	ECL421	Fucose-positive revertant (spontaneous)	$FucO^c$ $FucA^c$ $FucP^c$ $FucI^c$ $FucK^c$	This study
ECL463	ECL289	Transduction of arg^+ eno^+ with ECL421 as donor	$FucO^c$ $FucA^c$ $FucP^n$ $FucI^n$ $FucK^n$	This study
ECL464	ECL289	Transduction of arg^+ eno^+ with ECL459 as donor	$FucO^c$ $FucA^c$ $FucP^c$ $FucI^c$ $FucK^c$	This study

[a] FucO, Oxidoreductase; FucA, aldolase; FucP, permease; FucI, isomerase; FucK, kinase. Superscript c, constitutivity; superscript n, noninducibility.
[b] EMS, Ethyl methanesulfonate.

units (no. 42 filter) and washed once with 0.1 M potassium phosphate (pH 7.0). The pellet was weighed and dispersed in 4 volumes of 2.5 mM glutathionine and 0.1 M potassium phosphate (pH 7.0). The dispersed cells were disrupted by sonication as already described. The treated preparation was centrifuged at 100,000 × *g* for 2 h at 4°C, and the supernatant fraction was used.

Transport and enzyme assays. L-Fucose permease activity was determined at 37°C from the rate of L-[6-^{3}H]fucose uptake by whole cells. The assay was started by mixing 0.1 ml of cell suspension with 0.1 ml of mineral medium containing L-[6-^{3}H]fucose (0.5 μCi, 4 nmol). A sample of 0.05 ml was withdrawn at zero time and another one after 1 min of incubation. The sample was delivered onto a membrane filter (0.45-μm pore diameter) previously wetted with mineral medium, and the filter was immediately washed twice with 5 ml of mineral medium. After drying, the radioactivity on the filter was determined by scintillation counting. Cellular uptake of the labeled substrate was linear for 1.5 min.

L-Fucose isomerase activity was determined at 25°C from the initial rate of L-fuculose formation by the cysteine carbozole method (23). L-Fuculose kinase activity was determined at 30°C from the rate of L-[6-^{3}H]fuculose 1-phosphate formation in the following manner. A mixture (80 μl) containing labeled L-fucose (0.25 μCi, 1 μmol), purified L-fuculose isomerase (240 nmol/min), $MgCl_2$ (0.5 μmol), and Tris-hydrochloride (pH 7.8) (5 μmol) was preincubated for 15 min to allow equilibration of the isomerization reaction. To initiate the reaction, ATP (1 μmol) and cell extract were added to give a final volume of 100 μl. After 15 min, 50 μl of the assay mixture was withdrawn and spotted on a Whatman DE81 filter paper disk (2.5-cm diameter) which was then dropped into 80% ethanol, washed with water, dried, and determined for its radioactivity by scintillation counting. One sample withdrawn from a separate reaction mixture at zero time served as the blank. Under the conditions used, the rate of L-fuculose 1-phosphate formation remained linear up to 20 min, and the activity was proportional to the concentration of cellular protein. The assay of L-fuculose 1-phosphate aldolase activity was dependent on the formation of dihydroxyacetone phosphate at 25°C (5). L-1,2-Propanediol oxidoreductase activity was measured by the rate of L-lactaldehyde-dependent oxidation of NADH at 25°C (2).

L-Fucose permease activity is expressed as nanomoles of substrate uptake per minute per milligram (dry weight) of cells. Enzyme activities are expressed as nanomoles of product formed per minute per milligram of protein. Protein concentration was determined by the method of Lowry et al. (15).

RESULTS

Transductional analysis. Previous studies showed that all known mutations affecting L-fucose dissimilation are closely linked (for references see reference 1). In the series of transductions to be described, strain ECL289, bearing the fuc^+ locus (minute 60.2 [4]) flanked by the markers *eno* (minute 59.6) and *argA* (minute 60.5), was used as the recipient. Selection for eno^+ and $argA^+$ with the various donor strains gave the results presented in Table 2. With strain ECL3, the L-fucose$^-$ and L-1,2-propanediol$^+$ mutant,

TABLE 2. Transductional analysis selecting for eno^+ and arg^+ with strain ECL289 as the recipient

Donor	No. of transductants analyzed	Unselected traits[a]				% Cotransduction of growth phenotype
		Fuc^+ Prd^-	Fuc^+ Prd^-	Fuc^- Prd^+	Fuc^- Prd^-	
ECL3	100	0	4	96	0	96
ECL56	94	0	0	94	0	0
ECL421	100	1	1	98	0	98
ECL459	100	99	0	0	1	99

[a] Fuc, Ability to grow on L-fucose; Prd, ability to grow on L-1,2-propanediol.

96% of the transductants inherited the donor growth pattern. Unexpectedly, with strain ECL56, the L-fucose$^+$ revertant of strain ECL3, none of the transductants inherited the donor growth pattern; instead, all inherited the pattern of strain ECL3. Strain ECL421, an independent L-fucose$^-$ and L-1,2-propanediol$^+$ mutant, gave the same transduction pattern (98% inheritance of the donor growth phenotype) as strain ECL3. However, with ECL459, the L-fucose$^+$ revertant of strain ECL421, 99% of the transductants did inherit the donor growth pattern and not the pattern of strain ECL421.

Thus, the mutation in strain ECL56 that restored the growth ability on L-fucose is outside of the *eno-argA* region. In contradistinction, the mutation in strain ECL459 that restored the growth ability on L-fucose is within the region. The minority phenotypes of the transductants given in Table 2 probably arose from quadruple crossovers.

Enzymic profiles. L-Fucose permease, L-fucose isomerase, L-fuculose kinase, L-fuculose 1-phosphate aldolase, and L-1,2-propanediol oxidoreductase activities were assayed for the wild-type strain, the L-1,2-propanediol$^+$ and L-fucose$^-$ mutants, their L-fucose$^+$ revertants, and transductants of strain ECL289 inheriting the *eno-argA* region from the mutants and revertants, all grown aerobically. The data presented in Table 3 show that strain ECL3 and strain ECL452, the transductant receiving the *eno-argA* region from ECL3, exhibited essentially the same enzyme profile: the permease, the isomerase, and the kinase were noninducible, whereas the aldolase and the oxidoreductase were constitutive. The same holds for strain ECL421 and strain ECL463, the transductant receiving the *eno-argA* region from strain ECL421.

In both strains ECL56 (the L-fucose$^+$ revertant of strain ECL3) and ECL459 (the L-fucose$^+$ revertant of strain ECL421), all the tested enzyme activities were expressed constitutively, although the activity levels of the permease, the isomerase, and the kinase were lower than those seen in the wild-type strain ECL1 induced with L-fucose. Strain ECL453, the transductant that received the *eno-argA* region from strain ECL56, however, differed strikingly from strain ECL464, the transductant that received the corresponding region from strain ECL459. Transductant ECL453 was nonducible for the permease, the isomerase, and the kinase, whereas transductant ECL464 showed constitutive expression of these gene products. The enzymic patterns of the various strains studied, therefore, are consistent with their growth phenotypes and support the conclusion that the L-fucose$^+$ suppressor mutations in strains ECL56 and ECL459 occurred in different genes.

DISCUSSION

A pivotal event in the progressive evolution of the L-1,2-propanediol-utilizing capacity is the constitutive expression of *fucO* encoding the oxidoreductase and *fucA* encoding the aldolase. This change is associated with the noninducibility of *fucP* encoding the permease, *fucI* encoding the isomerase, and *fucK* encoding the kinase (9). In view of the positive control of the *fuc* system by an activator protein, the product of the closely linked *fucR* gene (13, 17, 18), the following working hypothesis might be proposed to account for the various phenotypes so far observed.

Under continued selective pressure for increased oxidoreductase activity during aerobic growth, a mutant with an altered *fucR* protein that allows constitutive expression of *fucO* inevitably emerges. The nature of the altered activator is such that the protein now permits the constitutive expression of the *fucA* gene as well but no longer permits the expression of *fucP*, *fucI*, and *fucK*, even in the presence of the inducer. The last three genes, however, can be activated by at least two different suppressor mechanisms.

In strain ECL56, a mutated unlinked gene provides a substitute for the altered *fucR* protein, thus allowing constitutive expression of the *fucP*, *fucI*, and *fucK* genes. Other models can also be proposed. For example, the regulator protein encoded by the external suppressor gene might interact with the altered *fucR* protein to give an activator protein complex. Although the position of this unlinked gene has not yet been mapped, it is known not to be a member of

TABLE 3. Specific activities of L-fucose pathway enzymes in mutants, revertants, and transductants grown aerobically

Strain[a]	Inducer in growth medium[b]	Sp act[c]				
		L-Fucose permease	L-Fucose isomerase	L-Fuculose kinase	L-Fuculose 1-phosphate aldolase	L-1,2-Propanediol oxidoreductase
ECL1 (wild type)	+	12	900	23	380	150
ECL3 (mutant of ECL1)	−	0.2	10	0.1	380	1,200
	+	0.3	11	0.08	430	1,300
ECL452 (transductant of ECL3)	−	0.2	15	0.02	400	1,400
	+	0.3	30	0.05	420	1,200
ECL56 (revertant of ECL3)	−	5.6	450	6.5	270	1,000
	+	6.3	420	8.8	290	1,100
ECL453 (transductant of ECL56)	−	0.2	20	0.01	480	1,600
	+	0.2	30	0.05	460	1,200
ECL421 (mutant of ECL1)	−	0.2	20	0.02	500	4,400
	+	0.3	35	0.1	480	4,000
ECL463 (transductant of ECL421)	−	0.2	15	0.05	440	4,200
	+	0.2	20	0.1	470	4,000
ECL459 (revertant of ECL421)	−	5.5	450	3.0	470	4,100
	+	5.0	430	4.0	460	4,000
ECL464 (transductant of ECL459)	−	5.6	520	4.0	420	4,300
	+	5.4	480	3.7	430	4,100

[a] Mutants ECL3 and ECL421 were selected for growth on L-1,2-propanediol; their respective revertants, strains ECL56 and ECL459, were selected for recovery of the ability to grow on L-fucose; transductants were selected for *eno*$^+$ and *arg*$^+$ with strain ECL289 as the recipient.

[b] CAA served as the carbon and energy source in the noninducing medium. The inducing medium additionally contained L-fucose.

[c] Specific enzyme activities are expressed as nanomoles of products formed per minute per milligram of protein, and specific permease activity is expressed as nanomoles of substrate uptake per minute per milligram (dry weight) of cells.

the L-rhamnose system, since deletion of the *rha* locus did not prevent strain ECL56 from growing on L-fucose (unpublished data). In strain ECL421, the altered *fucR* gene might have mutated further, resulting in constitutive expression of all the *fuc* structural genes. Alternatively, if the *fucP*, *fucI*, and *fucK* genes belong to the same operon, a mutation in the promoter region occurred, resulting in constitutive transcription. Conclusive interpretations of the nature of the mutations in strains ECL3, ECL56, ECL421, and ECL459 await understanding of the organization of the entire L-fucose system in greater detail. Analysis of cloned fragments of the *fuc* region is in progress.

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LITERATURE CITED

1. **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.
2. **Chen, Y.-M., E. C. C. Lin, J. Ros, and J. Aguilar.** 1983. Use of operon fusions to examine the regulation of the L-1,2-propanediol oxidoreductase gene of the fucose system in *Escherichia coli* K12. J. Gen. Microbiol. **129**:3355–3362.
3. **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.
4. **Falkow, S., H. Schneider, L. S. Baron, and S. B. Formal.** 1963. Virulence of *Escherichia-Shigella* genetic hybrids for the guinea pig. J. Bacteriol. **86**:1251–1258.
5. **Ghalambor, M. A., and E. C. Heath.** 1962. The metabolism of L-fucose. II. The enzymatic cleavage of L-fuculose-1-phosphate. J. Biol. Chem. **237**:2427–2433.
6. **Green, M., and S. S. Cohen.** 1956. The enzymatic conversion of L-fucose to L-fuculose. J. Biol. Chem. **219**:557–568.
7. **Hacking, A. J., J. Aguilar, and E. C. C. Lin.** 1978. Evolution of propanediol utilization in *Escherichia coli*: mutant with improved substrate-scavenging power. J. Bacteriol. **136**:522–530.
8. **Hacking, A. J., and E. C. C. Lin.** 1976. Disruption of the fucose pathway as a consequence of genetic adaptation to propanediol as a carbon source in *Escherichia coli*. J. Bacteriol. **126**:1166–1172.
9. **Hacking, A. J., and E. C. C. Lin.** 1977. Regulatory changes in the fucose system associated with the evolution of a catabolic pathway for propanediol in *Escherichia coli*. J. Bacteriol. **130**:832–838.
10. **Heath, E. C., and M. A. Ghalambor.** 1962. The metabolism of L-fucose. I. The purification and properties of L-fuculose kinase. J. Biol. Chem. **237**:2423–2426.
11. **Hillman, J. D., and D. G. Fraenkel.** 1975. Glyceraldehyde 3-phosphate dehydrogenase mutants of *Escherichia coli*. J. Bacteriol. **122**:1175–1179.
12. **Irana, M., and P. K. Maitra.** 1974. Isolation and characterization of *Escherichia coli* mutants defective in enzymes in glycolysis. Biochem. Biophys. Res. Commun. **56**:127–133.
13. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: a new pathway in *Escherichia coli*. J. Bacteriol. **106**:90–96.
14. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30**:535–578.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. G. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Luria, S. E., J. N. Adams, and R. C. Ting.** 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348–390.
17. **St. Martin, E. J., and R. P. Mortlock.** 1976. Natural and altered induction of the L-fucose catabolic enzymes in *Klebsiella aerogenes*. J. Bacteriol. **127**:91–97.
18. **Skjold, A. C., and D. H. Ezekiel.** 1982. Regulation of D-arabinose utilization in *Escherichia coli* K-12. J. Bacteriol. **152**:521–523.
19. **Sridhara, S., and T. T. Wu.** 1969. Purification and properties of lactaldehyde dehydrogenase in *Escherichia coli*. J. Biol. Chem. **244**:5233–5238.
20. **Sridhara, S., T. T. Wu, T. M. Chused, and E. C. C. Lin.** 1969. Ferrous-activated nicotinamide adenine dinucleotide-linked dehydrogenase from a mutant of *Escherichia coli* capable of growth on 1,2-propanediol. J. Bacteriol. **98**:87–95.
21. **Tanaka, S., S. A. Lerner, and E. C. C. Lin.** 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferase by a nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. **93**:642–648.
22. **Tang, C.-T., F. E. Ruch, Jr., and E. C. C. Lin.** 1979. Purification and properties of a nicotinamide adenine dinucleotide-linked dehydrogenase that serves an *Escherichia coli* mutant for glycerol catabolism. J. Bacteriol. **140**:182–187.
23. **Zagalak, B., P. A. Frey, G. L. Karabatsos, and R. M. Abeles.** 1966. The stereochemistry of the conversion of D and L-1,2-propanediols to propionaldehyde. J. Biol. Chem. **241**:3028–3035.