JOURNAL OF BACTERIOLOGY, Feb. 1987, p. 785–789
0021-9193/87/020785-05$02.00/0

Vol. 169, No. 2

Loss of Aldehyde Dehydrogenase in an *Escherichia coli* Mutant Selected for Growth on the Rare Sugar L-Galactose

YAN ZHU AND E. C. C. LIN*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Received 11 September 1986/Accepted 4 November 1986

***Escherichia coli* K-12 converts L-fucose to dihydroxyacetone phosphate (C-1 to C-3) and L-lactaldehyde (C-4 to C-6) by a pathway specified by the *fuc* regulon. Aerobically, L-lactaldehyde serves as a carbon and energy source by the action of an aldehyde dehydrogenase of broad specificity; the product, L-lactate, is then converted to pyruvate. Anaerobically, L-lactaldehyde serves as an electron acceptor to regenerate NAD from NADH by the action of an oxidoreductase; the reduced product, L-1,2-propanediol, is excreted. A strain selected for growth on L-galactose (a structural analog of L-fucose) acquired a broadened inducer specificity because of an altered *fucR* gene encoding the activator protein for the *fuc* regulon (Y. Zhu and E. C. C. Lin, J. Mol. Evol. 23:259–266, 1986). In this study, a second mutation that abolished aldehyde dehydrogenase activity was discovered. The L-fucose pathway converts L-galactose to dihydroxyacetone phosphate and L-glyceraldehyde. Aldehyde dehydrogenase then converts L-glyceraldehyde to L-glycerate, which is toxic. Loss of the dehydrogenase averts the toxicity during growth on L-galactose, but reduces by one-half the aerobic growth yield on L-fucose. When mutant cells induced in the L-fucose system were incubated with radioactive L-fucose, accumulation of radioactivity occurred if the substrate was labeled at C-1 but not if it was labeled C-6. Complete aerobic utilization of carbons 4 through 6 of L-fucose depends not only on an adequate activity of aldehyde dehydrogenase to trap L-lactaldehyde as its anionic acid but also on the lack of L-1,2-propanediol oxidoreductase activity, which converts L-lactaldehyde to a readily excreted alcohol.**

Escherichia coli K-12 grows on L-fucose as the sole carbon and energy source via an inducible pathway mediated by the sequential action of L-fucose permease (12), L-fucose isomerase (10), L-fuculose kinase (14), and L-fuculose 1-phosphate aldolase (9). The aldolase cleaves the six-carbon substrate into dihydroxyacetone phosphate and L-lactaldehyde. Aerobically, L-lactaldehyde is oxidized by an NAD-linked dehydrogenase to L-lactate, which is converted to pyruvate by a dehydrogenase of the flavoprotein class. Anaerobically, L-lactaldehyde is reduced by an NADH-linked oxidoreductase to L-1,2-propanediol, which is then excreted (7, 22, 23). The sacrifice of one-half of the carbon skeleton of L-fucose permits more dihydroxyacetone phosphate to be used as a carbon source (Fig. 1). The structural genes *fucP* (encoding the permease), *fucI* (encoding the isomerase), *fucK* (encoding the kinase), *fucA* (encoding the aldolase), and *fucO* (encoding L-1,2-propanediol oxidoreductase) are organized into three operons clustered at min 60.2 (3, 5, 6, 11–13). They are under the control of the *fucR* product, a positive regulator (21; Y. Zhu and Y.-M. Chen, unpublished data) which interacts with L-fuculose 1-phosphate as the effector (J. M. Bartkus and R. P. Mortlock, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, K238, p. 216). The gene *ald*, encoding aldehyde dehydrogenase, is not linked to the *fuc* gene cluster (Y.-M. Chen and Y. Zhu, unpublished data).

L-Galactose and D-arabinose, structural analogs of L-fucose, cannot be utilized by wild-type *E. coli* K-12. Mutants capable of growing on D-arabinose as the sole carbon and energy source have been selected. In these strains, the *fuc* regulon is induced in the presence of either L-fucose or D-arabinose (15). A mutant capable of growing on D-arabinose and on L-galactose, strain ECL469, has recently been partially characterized. A mutation in the *fucR* regulator gene altered the inducer specificity in such a way that the *fuc* regulon became triply inducible by L-galactose, D-arabinose, or L-fucose. However, it was noticed that growth of the mutant on L-fucose agar gave colonies of subnormal size. Moreover, the introduction of $fucR^+$ on a multicopy plasmid diminished growth on L-galactose and D-arabinose without improving the growth yield on L-fucose. This result would suggest that a mutation other than that in *fucR* was responsible for the growth defect on L-fucose (27). The nature of that mutation is the subject of the present study.

MATERIALS AND METHODS

Chemicals. Crystalline L-glycerate was a generous gift of L. N. Ornston. L-Lactaldehyde, synthesized by the reaction of ninhydrin with D-threonine (26), was obtained from Y.-M. Chen. L-Galactose, L-glyceraldehyde, L-fucose, and D-arabinose were purchased from Sigma Chemical Co., St. Louis, Mo. L-[6-^{3}H]fucose (specific activity, 27 Ci/mmol) was purchased from ICN Pharmaceuticals, Inc., Irvine, Calif. L-[1-^{14}C]fucose (specific activity, 59.2 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass. Vitamin-free casein acid hydrolysate was from ICN Nutritional Biochemicals, Cleveland, Ohio. Nobel agar was from Difco Laboratories, Detroit, Mich. All other reagents used were commercial products of the highest grade available.

Bacterial strains. The lineages and genotypes of the various strains used are given in Table 1. Strain ECL709, lacking aldehyde dehydrogenase, was identified as a pink (instead of red) colony on MacConkey–L-fucose agar. Strain ECL710, lacking L-fucose isomerase activity, was identified as a pale colony on the same agar. Both mutants were derived from strain ECL708 after mutagenesis with ethyl methanesulfonate.

Growth of cells and preparation of extracts. Liquid cultures were grown at 37°C in a 300-ml flask containing 100 ml of

* Corresponding author.

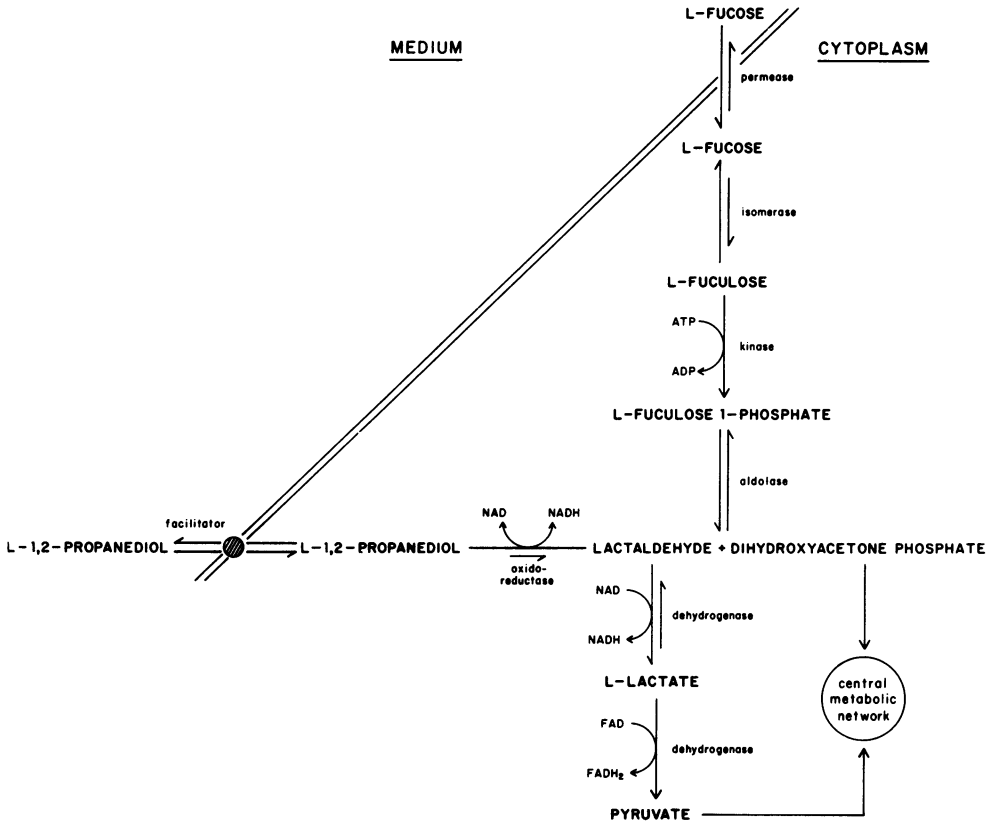

FIG. 1. Pathways for aerobic and anaerobic dissimilation of L-fucose by *E. coli*.

mineral medium (24) with appropriate supplements and agitated on a rotary shaker. Noninducing medium contained 10 mM D-xylose as the carbon and energy source. Inducing media contained 10 mM L-fucose, L-galactose, or D-arabinose as the sole source of carbon and energy. Anaerobic cultures were supplemented additionally with 0.1% casein amino acids and 20 mM pyruvate and grown in 150-ml tightly capped bottles (6).

For the assay of L-fucose uptake, cells in an exponentially growing culture at about 150 Klett units (no. 42 filter) were harvested by centrifugation, washed, and diluted to 100 Klett units with mineral medium. For enzyme assays, cells in an exponentially growing culture at 130 to 190 Klett units were harvested, washed, and suspended in 0.1 M potassium phosphate (pH 7.0). The pellet was weighed and dispersed in 4 volumes of the phosphate buffer. The dispersed cells were disrupted by sonication (6). The cell extract was centrifuged at 100,000 × *g* for 2 h at 4°C, and the supernatant fraction was used.

Substrate uptake and enzyme assays. Uptake and retention of the aldehyde end of L-fucose were determined with the cells suspended in 0.02 mM L-[1-^{14}C]fucose at 32°C by the procedure described for the assay of L-fucose permease activity (4). Retention of the methyl end of L-fucose was assayed by incubation of the cells with L-[6-^{3}H]fucose.

Aldehyde dehydrogenase activity was measured spectrophotometrically (E_{340}) by the rate of NADH formation at 25°C with either 0.05 mM L-lactaldehyde or 0.2 mM L-glyceraldehyde as the substrate (6). Specific enzyme activities are expressed as nanomoles of product formed per minute per milligram of protein. Protein concentration was determined with bovine serum albumin as the standard (18).

Tests for growth and inhibition. Overnight cultures were diluted to contain about 2.5 × 10^4 cells per ml in mineral

TABLE 1. *E. coli* strains

Strain	Source Strain	Relevant genotype[a]	Source or reference
ECL1	K-12	*ald*$^+$ *fucO*$^+$ *fucA*$^+$ *fucP*$^+$ *fucI*$^+$ *fucK*$^+$ *fucR*$^+$	16
ECL40	ECL3	*ald-1 fucO*(Con) *fucA*(Con) *fucP*(Non) *fucI*(Non) *fucK*(Non)	22
ECL469	ECL466	*ald-201 fucO*$^+$ *fucA*$^+$ *fucP*$^+$ *fucI*$^+$ *fucK*$^+$ *fucR**	27
ECL708	ECL459	*ald*$^+$ *fucO*(Con) *fucA*(Con) *fucP*(Con) *fucI*(Con) *fucK*(Con)	4; Y.-M Chen
ECL709	ECL708	*ald-2 fucO*(Con) *fucA*(Con) *fucP*(Con) *fucI*(Con) *fucK*(Con)	This study
ECL710	ECL708	*ald*$^+$ *fucO*(Con) *fucA*(Con) *fucP*(Con) *fucI fucK*(Con)	This study

[a] Genetic symbols: *fucO*, structural gene for L-1,2-propanediol oxidoreductase; *fucA*, structural gene for L-fuculose 1-phosphate aldolase; *fucP*, structural gene for L-fucose permease; *fucI*, structural gene for L-fucose isomerase; *fucK*, structural gene for L-fuculose kinase; *fucR*, structural gene for the activator protein; *fucR**, structural gene for an activator protein with altered effector specificity; *ald*, gene specifying aldehyde dehydrogenase. Con, Constitutivity; Non, noninducibility.

medium, and 10 μl of the diluted culture was spread onto one trisection of the appropriate agar plate. As the carbon source for growth, L-lactaldehyde or L-glyceraldehyde (10 μmol) was added to a sterilized filter disk placed at the center of the agar without other carbon and energy sources. To test for inhibition of growth, L-lactaldehyde or L-glycerate (10 μmol) was added to a disk and placed on agar containing 30 mM acetate. The plates were incubated at 37°C for 3 days. A minimal agar with no added carbon and energy source gave no visible colonies.

RESULTS

Impaired growth ability of the L-galactose-positive mutant on L-fucose. With 10 mM L-fucose as the sole source of carbon and energy in liquid medium, the mutant was normal in its growth rate but gave only one-half of the expected growth yield (Fig. 2). It might also be noted that, per atom of carbon, the mutant gave a higher growth yield on L-galactose and D-arabinose than on L-fucose. After stationary phase was reached, cultures of the mutant grown in L-fucose and L-galactose media declined slightly in density. This decline did not occur with cells grown on D-arabinose. The significance of the drop in culture density is unclear.

Loss of aldehyde dehydrogenase. Full aerobic utilization of L-fucose depends on complete exploitation of both dihydroxyacetone phosphate and L-lactaldehyde. That the mutant suffered no impairment in the metabolism of dihydroxyacetone phosphate was indicated by its normal growth on glycerol, the catabolism of which requires its prior conversion to the same triose phosphate. Further tests showed that the mutant retained the ability to grow on L-lactate but lost the ability to grow on L-lactaldehyde. This finding suggested that the metabolic defect was caused by the loss of aldehyde dehydrogenase activity. Enzymic analysis confirmed and extended this deduction. Whereas extracts of wild-type cells (strain ECL1) contained dehydrogenase activities when assayed with either L-lactaldehyde or L-glyceraldehyde as a substrate, extracts of mutant cells (ECL469) grown on L-fucose, D-arabinose, or L-galactose lacked activity with both of these substrates (Table 2). Moreover, extracts of strain ECL40, isolated as an L-lactaldehyde dehydrogenase-negative mutant (22), also lacked L-glyceraldehyde dehydrogenating activity. The identities of the enzymes active on L-lactaldehyde and on L-glyceraldehyde were further indicated by transduction of the *ald*$^+$ gene from the wild-type strain into the L-galactose-positive mutant ECL469 (selection for tetracycline resistance conferred by a nearby transposon, Tn*10*). Transductants acquiring the enzyme active on L-lactaldehyde also acquired the enzyme active on L-glyceraldehyde (data not shown). The absence of aldehyde dehydrogenase activity in the L-galactose-positive mutant not only explained the impaired growth of the mutant on L-fucose, but also pointed to the basis of selection against cells possessing the enzyme; i.e., during growth on L-galactose, L-glyceraldehyde was probably converted to L-glycerate, which was detrimental to the cell.

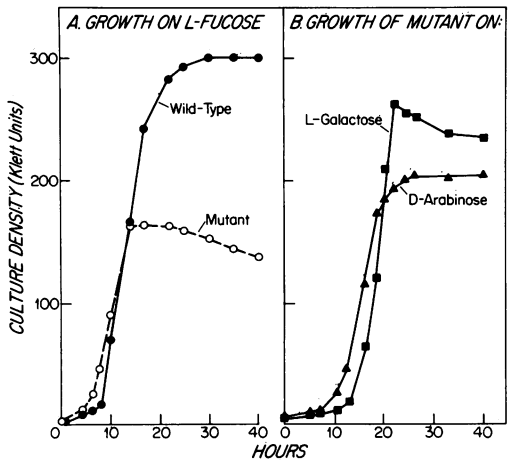

FIG. 2. Growth curves of wild-type strain ECL1 and mutant strain ECL469. (A) Both strains with L-fucose as the sole source of carbon and energy; (B) strain ECL469 with D-arabinose or L-galactose as the sole source of carbon and energy.

TABLE 2. Activity levels of aldehyde dehydrogenase in different strains

Strain	Growth medium[a]	Sp act of aldehyde dehydrogenase assayed with[b]:	
		L-Lactaldehyde	L-Glyceraldehyde
ECL1	D-Xylose	91	52
	L-Fucose	190	200
ECL469	D-Xylose	0	0
	L-Fucose	0	0
	D-Arabinose	0	3
	L-Galactose	0	7
ECL40	D-Xylose	0	0

[a] Cells were grown under aerobic conditions.
[b] Specific activities are expressed in nanomoles per minute per milligram of cells (dry weight) at 25°C.

Toxicity of L-glycerate. To test the hypothesis, growth responses to L-lactaldehyde, L-glyceraldehyde, and L-glycerate were analyzed as a function of the presence or absence of aldehyde dehydrogenase. It was found that L-lactaldehyde served as a sole source of carbon and energy for the wild-type parent (strain ECL1), but not for the L-galactose-positive mutant (ECL469) or for an aldehyde dehydrogenase-negative mutant (ECL40). L-Glyceraldehyde did not support the growth of the wild-type strain and prevented its growth on acetate. The two mutants lacking aldehyde dehydrogenase, however, actually grew on the compound. This finding would suggest that a catabolic pathway existed for L-glyceraldehyde but that its exploitation for growth was prevented by the toxic production of L-glycerate (note the higher growth yield of strain ECL469 on L-galactose than on L-fucose [Fig. 1]). L-Glycerate inhibited the growth of all three test strains on acetate, regardless of whether they possessed aldehyde dehydrogenase activity. Among several carbon and energy sources screened, acetate was found to be the most sensitive test compound, presumably because its poor nutritional value made the cells highly vulnerable. Why L-glycerate should be toxic remains unclear.

Inability of the L-galactose-positive mutant to retain the methyl group of L-fucose. Failure of the L-galactose-positive mutant to utilize the three-carbon fragment derived from the methyl end of the L-fucose molecule was confirmed by testing the radioactivity retained by the cells after incubation with L-fucose labeled at C-1 or C-6. When wild-type cells induced in the *fuc* regulon were incubated with L-fucose labeled at C-6, the amount of radioactivity retained was about 60% of that observed with L-fucose labeled at C-1 (Table 3). (The discrepancy suggests that a metabolite of L-lactaldehyde or a portion of the compound itself was lost to the medium under the experimental condition.) When a

TABLE 3. Accumulation of radioactivity by cells incubated with L-fucose labeled at different positions[a]

Strain	Growth medium	Amt of radioactivity[b] after incubation with:	
		L-[1-^{14}C]fucose	L-[6-^{3}H]fucose
ECL1	D-Xylose	0.2	0
	L-Fucose	64	40
ECL469	D-Xylose	0.2	0.8
	L-Fucose	32	0.6
	D-Arabinose	34	0
	L-Galactose	46	1.4

[a] Cells grown aerobically on various carbon and energy sources were incubated with the labeled substrate for 1 min at 32°C, and the radioactivity retained was determined.

[b] Expressed in nanomoles of L-fucose equivalent retained per milligram of cells (dry weight).

similar comparison was made with the mutant cells preinduced with L-fucose, L-arabinose, or L-galactose, the amount of radioactivity retained after incubation with the compound labeled at C-1 was 30 to 50% lower than that observed with wild-type cells. This suggests that L-fucose permease was not fully inducible because of the *fucR* mutation (see also reference 27). More important, the retention of the C-6 label by the mutant cells was practically nil.

Interference with aerobic assimilation of L-lactaldehyde by L-1,2-propanediol oxidoreductase. The availability of L-fucose labeled at different carbons also permitted an analysis of the influence of L-1,2-propanediol oxidoreductase activity on the retention of carbons 4 through 6 of L-fucose. When strain ECL708, a mutant with a high aerobic constitutive level of activity of this enzyme (4), was incubated under aerobic conditions with L-fucose labeled at C-1 or C-6, retention of the C-6 label was about 30% of that of the C-1 label (Fig. 3A). This percentage is in contrast to the 60% retention of the C-6 label observed with aerobically grown wild-type cells (Table 3), which contained a low activity of L-1,2-propanediol oxidoreductase (12). The presence of an *ald* mutation in strain ECL709 lowered the retention of the C-6 label to 15% (Fig. 3B). A derivative of strain ECL708, strain ECL710 lacking L-fucose isomerase activity and therefore unable to form L-lactaldehyde from L-fucose, was used as a control for this comparison (Fig. 3C). It thus appears that, even under aerobic conditions, the presence of aldehyde dehydrogenase is not by itself sufficient to ensure complete metabolite retention. Diversion of L-lactaldehyde to L-1,2-propanediol, another small uncharged molecule, must be minimized.

This view is also consistent with the results from wild-type cells grown under different physiological conditions. When aerobically grown cells containing a low activity of L-1,2-propanediol oxidoreductase but a high activity of aldehyde dehydrogenase (12) were incubated under aerobic conditions with L-fucose labeled at C-1 or C-6, retention of the C-6 label was about 60% of that of the C-1 label (Fig. 4A). When a parallel set of incubations were carried out with anaerobically grown cells containing a high activity of L-1,2-propanediol oxidoreductase but a low activity of aldehyde dehydrogenase (12), retention of the C-6 label was <1% of that of the C-1 label (Fig. 3B).

DISCUSSION

The *E. coli* enzyme first described as L-lactaldehyde dehydrogenase (22) was subsequently found to play an essential role in the growth of cells on ethylene glycol by catalyzing the oxidation of glycoaldehyde to glycolate (1, 2). Our study showed that this dehydrogenase is also capable of converting L-glyceraldehyde to L-glycerate. Because of its broad substrate specificity and the lack of evidence that its function is limited to the utilization of L-lactaldehyde, the enzyme is more appropriately designated simply as aldehyde dehydrogenase.

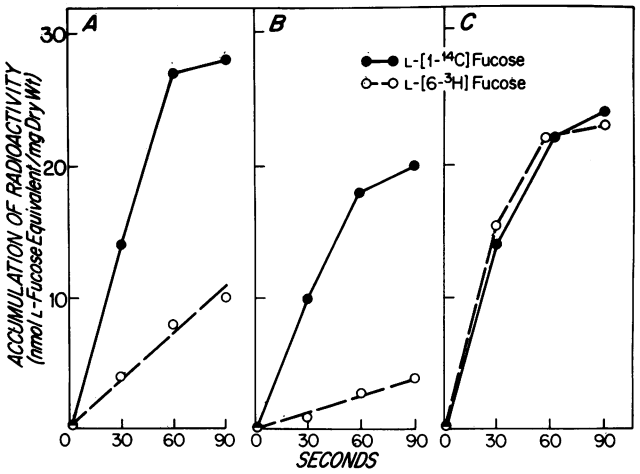

FIG. 3. Effects of metabolic blocks in the L-fucose pathway on the ability of cells to retain the aldehyde or methyl end of L-fucose. Cells grown aerobically in 0.5% casein hydrolysate medium were assayed for retention of radioactivity when incubated with L-fucose labeled at C-1 (●) or C-6 (○) as described in Materials and Methods. (A) Strain ECL708 (elevated aerobic constitutive level of L-1,2-propanediol oxidoreductase activity); (B) strain ECL709 (elevated aerobic constitutive level of L-1,2-propanediol oxidoreductase activity; defective aldehyde dehydrogenase); (C) strain ECL710 (constitutive L-fucose pathway but lacking L-fucose isomerase).

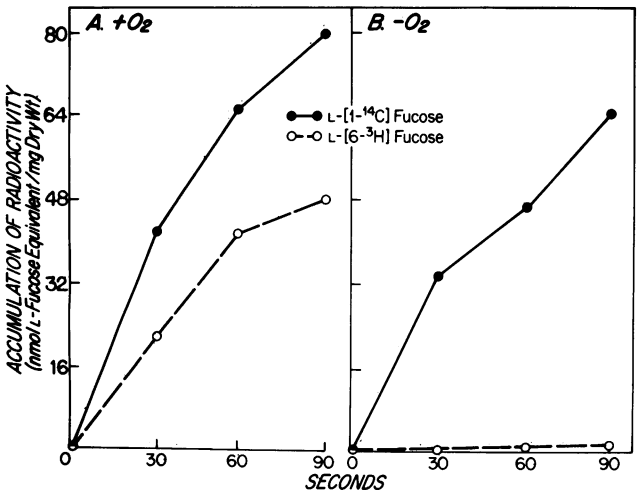

FIG. 4. Effect of respiratory condition of growth on the ability of wild-type strain ECL1 to retain the aldehyde or methyl end of L-fucose. Cells were assayed for retention of radioactivity after incubation with L-fucose labeled at C-1 (●) or C-6 (○) as described in Materials and Methods. (A) Aerobic growth on L-fucose (high aldehyde dehydrogenase specific activity and low L-1,2-propanediol specific activity [12]); (B) anaerobic growth on L-fucose (low aldehyde dehydrogenase specific activity and high L-1,2-propanediol oxidoreductase activity [12]).

The loss of aldehyde dehydrogenase unmasked a pathway for L-glyceraldehyde utilization which enabled strains ECL469 and ECL40 to grow on the compound as the sole source of carbon and energy and enabled strain ECL469 to grow more effectively on L-galactose than on L-fucose. The biochemical steps in the L-glyceraldehyde pathway have yet to be characterized. Phosphorylation to L-glyceraldehyde phosphate, however, can be excluded as a possibility, since L-glyceraldehyde 3-phosphate was shown to be bactericidal (25).

The loss of aldehyde dehydrogenase activity also demonstrated the importance of converting uncharged L-lactaldehyde to anionic L-lactate for effective metabolite retention by the cell (8).

The loss of the enzyme in strain ECL469 provided another example of the acquisition of one function at the expense of another. In previous studies, it was shown that selection for growth on L-1,2-propanediol resulted in the loss of the ability to grow on L-fucose. In that case, the functional loss was a secondary consequence of an alteration of gene expression (17). In the present case, the functional loss was the result of a direct selection against the harmful activity of an enzyme.

The L-galactose-positive mutant of *E. coli* is highly analogous to a mutant of *Klebsiella pneumoniae* (previously classified as *Aerobacter aerogenes*) that acquired the ability to grow on L-mannose, a structural analog of L-rhamnose. It was found that the enzymes encoded by the *rha* genes converted L-mannose to dihydroxyacetone phosphate and L-glyceraldehyde. Moreover, the pathways were inducible by L-mannose. Growth of wild-type cells on the novel carbon source was not possible, because its metabolism gave rise to a toxic compound thought to be either L-glyceraldehyde itself or one of its metabolites (19, 20). In view of what is now known, it seems very likely that the *K. pneumoniae* mutant lost aldehyde dehydrogenase activity.

ACKNOWLEDGMENTS

We thank Y.-M. Chen for helpful discussions.

Studies in this laboratory were supported by grant DMB8314312 from the National Science Foundation and by Public Health Service grant 5-RO1-GM11983 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Boronat, A., E. Caballero, and J. Aguilar.** 1983. Experimental evolution of a metabolic pathway for ethylene glycol utilization by *Escherichia coli*. J. Bacteriol. **153**:134–139.
2. **Caballero, E., L. Baldoma, J. Ros, A. Boronat, and J. Aguilar.** 1983. Identification of lactaldehyde dehydrogenase and glycoaldehyde dehydrogenase as functions of the same protein in *Escherichia coli*. J. Biol. Chem. **258**:7788–7792.
3. **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.
4. **Chen, Y.-M., T. Chakrabarti, and E. C. C. Lin.** 1984. Constitutive activation of L-fucose genes by an unlinked mutation in *Escherichia coli*. J. Bacteriol. **159**:725–729.
5. **Chen, Y.-M., and E. C. C. Lin.** 1984. Post-transcriptional control of L-1,2-propanediol oxidoreductase in the L-fucose pathway of *Escherichia coli* K-12. J. Bacteriol. **157**:341–344.
6. **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* K-12. J. Gen. Microbiol. **129**:3355–3362.
7. **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.
8. **Davis, B. D.** 1958. On the importance of being ionized. Arch. Biochem. Biophys. **87**:497–509.
9. **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.
10. **Green, M., and S. S. Cohen.** 1956. The enzymatic conversion of L-fucose to L-fuculose. J. Biol. Chem. **219**:557–568.
11. **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.
12. **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.
13. **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.
14. **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.
15. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: a new pathway in *Escherichia coli*. J. Bacteriol. **106**:90–96.
16. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30**:535–578.
17. **Lin, E. C. C., and T. T. Wu.** 1984. Functional divergence of the L-fucose system in mutants of *Escherichia coli*, p. 135–164. *In* R. P. Mortlock (ed.), Microorganisms as model systems for studying evolution. Plenum Publishing Corp., New York.
18. **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.
19. **Mayo, J. W., and R. L. Anderson.** 1968. Pathway of L-mannose degradation in *Aerobacter aerogenes*. J. Biol. Chem. **243**:6330–6333.
20. **Mayo, J. W., and R. L. Anderson.** 1969. Basis for the mutational acquisition of the ability of *Aerobacter aerogenes* to grow on L-mannose. J. Bacteriol. **100**:948–955.
21. **Skjold, A. C., and D. H. Ezekiel.** 1982. Regulation of D-arabinose utilization in *Escherichia coli* K-12. J. Bacteriol. **152**:521–523.
22. **Sridhara, S., and T. T. Wu.** 1969. Purification and properties of lactaldehyde dehydrogenase in *Escherichia coli*. J. Biol. Chem. **244**:5233–5238.
23. **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.
24. **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.
25. **Tang, C.-T., R. Engel, and B. E. Tropp.** 1977. L-Glyceraldehyde 3-phosphate, a bactericidal agent. Antimicrob. Agents Chemother. **11**:147–153.
26. **Zagalak, B., P. A. Frey, G. L. Karabatsos, and R. H. Abeles.** 1966. The stereochemistry of the conversion of D and L 1,2-propanediols to propionaldehyde. J. Biol. Chem. **241**:3028–3035.
27. **Zhu, Y., and E. C. C. Lin.** 1986. An evolvant of *Escherichia coli* that employs the L-fucose pathway also for growth on L-galactose and D-arabinose. J. Mol. Evol. **23**:259–266.