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NAD-Linked Aldehyde Dehydrogenase for Aerobic Utilization of L-Fucose and L-Rhamnose by *Escherichia coli*

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Mutant analysis revealed that complete utilization of L-fucose and L-rhamnose by *Escherichia coli* requires the activity of a common NAD-linked aldehyde dehydrogenase which converts L-lactaldehyde to L-lactate. Mutations affecting this activity mapped to the *ald* locus at min 31, well apart from the *fuc* genes (min 60) encoding the trunk pathway for L-fucose dissimilation (as well as L-1,2-propanediol oxidoreductase) and the *rha* genes (min 88) encoding the trunk pathway for L-rhamnose dissimilation. Mutants that grow on L-1,2-propanediol as a carbon and energy source also depend on the *ald* gene product for the conversion of L-lactaldehyde to L-lactate.

Growth of *Escherichia coli* on three compounds as the sole sources of carbon and energy is known to involve the conversion of L-lactaldehyde to L-lactate. Two of these compounds, L-fucose and L-rhamnose, are utilized by existing physiological pathways. The third compound, L-1,2-propanediol, is utilized by mutants selected for this special ability (for reviews, see references 24 and 25).

Fucose and rhamnose are metabolized through parallel trunk pathways, each of which is sequentially mediated by a permease, an isomerase, a kinase, and an aldolase (Fig. 1). The two sugars differ in stereoconfiguration at carbons 2 and 4, but structural differences between the corresponding intermediates disappear with cleavage of the phosphorylated ketose, yielding in both cases dihydroxyacetone phosphate and lactaldehyde. Aerobically, lactaldehyde is converted by an NAD-linked reaction to lactate, which is oxidized to pyruvate for further use (15, 33). Anaerobically, lactaldehyde is reduced by an NADH-linked reaction to propanediol, which is excreted into the medium (15). The sacrifice of the aldehyde as a hydrogen sink increases the portion of dihydroxyacetone phosphate that can be utilized as a carbon and energy source.

The catalytic proteins of each trunk pathway and its activator protein are encoded by a single gene cluster: the *fuc* locus at min 60.2 (1, 10, 17, 31, 32) and the *rha* locus at min 87.7 (1, 17, 29). The structural genes of the *fuc* system appear to be organized as a regulon comprising at least three operons: *fucO*, encoding propanediol oxidoreductase; *fucA*, encoding L-fuculose 1-phosphate aldolase; and *fucPIK*, encoding L-fucose permease, L-fucose isomerase, and L-fuculose kinase (10, 18–20). Whereas the *fuc* system responds to fuculose 1-phosphate as the effector (3), the *rha* system responds to rhamnose as the effector (29).

The reduction of lactaldehyde to propanediol during anaerobic growth on fucose or rhamnose is catalyzed by the same oxidoreductase. This enzyme is encoded by *fucO*, a member of the *fuc* gene cluster (6, 7, 12, 14, 15). Anaerobic growth on rhamnose cross induces *fucO* together with the other genes of the *fuc* system either because lactaldehyde is an alternative effector or because it is converted to fuculose 1-phosphate by the reversible reaction catalyzed by its aldolase (Y.-M. Chen, J. F. Tobin, Y. Zhu, R. F. Schleif, and E. C. C. Lin, submitted for publication; Y. Zhu, and E. C. C. Lin, submitted for publication). Thus, anaerobic growth on rhamnose depends on an enzyme of the *fuc* system.

Propanediol, the fermentation product, cannot serve aerobically as a sole carbon and energy source by being reconverted to lactaldehyde. First, *fucO* is not induced. Second, even when the gene is expressed (13, 14), the protein synthesized aerobically is enzymatically inactive (7). By serial selection for aerobic growth on propanediol, mutants such as strain ECL3 that constitutively synthesize a catalytically active oxidoreductase [*fucO*(Con)] were isolated (34). As a mechanistic consequence of one of the mutations, L-fucose permease, L-fucose isomerase, and L-fuculose kinase became noninducible [*fucPIK*(Non)]. Propanediol-positive mutants thus became fucose negative. The mutations giving this complex phenotype map at the *fuc* locus (15, 19, 20).

The objective of this study was to determine whether a common enzyme is responsible for the dehydrogenation of lactaldehyde to lactate in aerobic dissimilation of fucose, rhamnose, and propanediol by wild-type and mutant strains and to determine the map position of the gene(s) involved.

MATERIALS AND METHODS

Chemicals. L-Lactaldehyde was prepared by the reaction of ninhydrin with D-threonine (38). L-Fucose, L-rhamnose, D-xylose, DL-1,2-propanediol, succinate semialdehyde, and cyclic 3′,5′-AMP were purchased from Sigma Chemical Co., St. Louis, Mo. Vitamin-free casein acid hydrolysate (CAA) was from ICN Nutritional Biochemicals, Cleveland, Ohio. All other chemicals used were commercial products of reagent grade.

Bacteria and bacteriophages. The genotypes and sources of *E. coli* K-12 strains used are given in Table 1. Strain ECL708, with all the *fuc* genes constitutively expressed, was derived by P1 *vir* transduction (28) of the *fuc* locus from strain ECL459 to strain ECL116. The transductants were selected for the ability to grow on propanediol (19, 20) and tested for the constitutive synthesis of L-fucose permease, L-fucose isomerase, L-fuculose kinase, L-fuculose 1-phosphate aldolase, and propanediol oxidoreductase by assays previously described (11).

To isolate mutants with a Tn*10* placed near *ald*$^+$, a pool of Tn*10* insertions at random chromosomal positions was first prepared by transposition of the Tn*10* from lambda NK55 to

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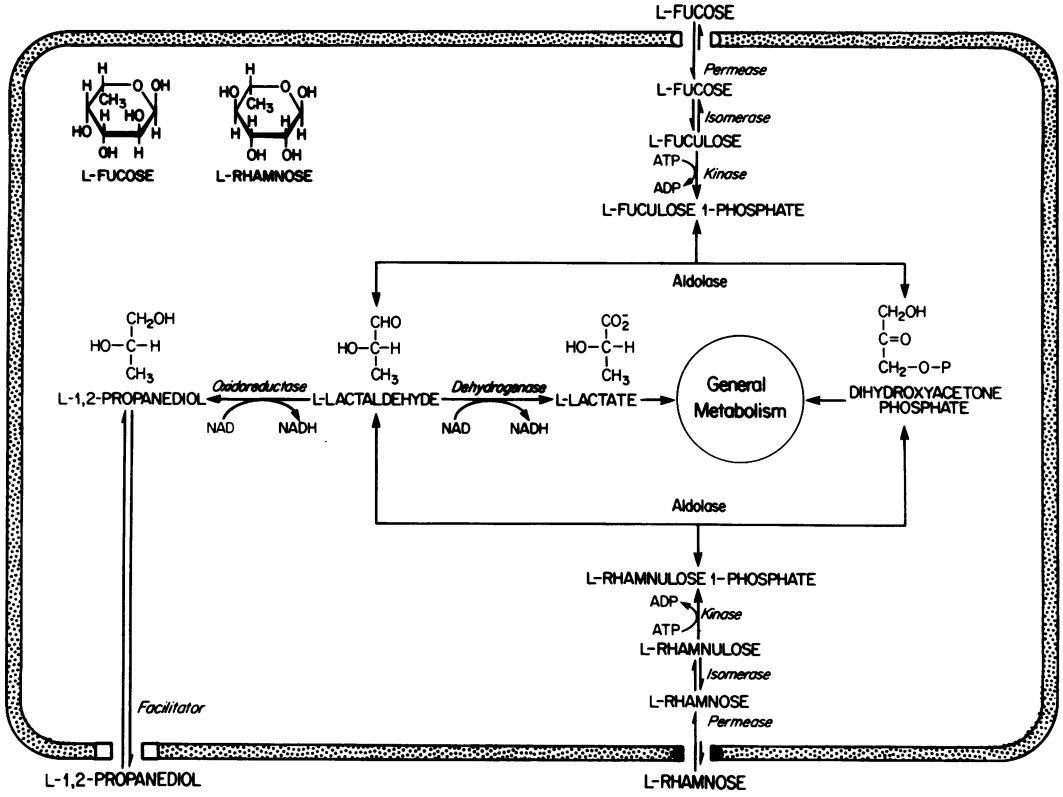

FIG. 1. Pathways of L-fucose and L-rhamnose dissimilation by *E. coli*.

the genome of a host population (4). The tetracycline-resistant (Tc^r) colonies were pooled, and a P1 lysate was used to transduce strain ECL40 [*ald-1 fucO*(Con)], which was unable to grow on propanediol because of the *ald* mutation. Transductants (*ald*$^+$ Tn*10*) were selected on propanediol as the sole carbon and energy source in the presence of tetracycline and purified on the same agar. The Tn*10* in the transductants was transduced into strain ECL40 by selection for Tc^r to determine the linkage of the transposon to *ald*$^+$. Strains ECL471 (*ald-1 zbd-1*::Tn*10*) and strain ECL486 (*ald*$^+$ *zbd-1*::Tn*10*) were derived by this procedure.

Strain ECL494 was constructed by transducing the *ald*(Con) from strain ECL430 into strain ECL471, selecting for growth on propanediol, and scoring for loss of Tn*10*. Strain ECL495 was constructed by transducing *ald*$^+$ of strain ECL3 into strain ECL471, selecting for growth on propanediol, and scoring for loss of Tn*10*.

Growth conditions and preparation of cell extracts. To test for growth, about 500 cells were spread uniformly on a minimal agar containing the appropriate carbon and energy source and incubated for 2 days at 37°C. When succinate semialdehyde was tested as the carbon and energy source, a sterile filter disk impregnated with 10 μmol of the compound was placed on the center of a mineral agar plate.

Strain ECL289 (*argA*::Tn*10 eno*) was grown on arginine-supplemented (20 μg/ml) mineral medium containing glycerol and succinate as combined carbon and energy sources (21, 22).

For enzyme assays, the cells were grown in 100 ml of mineral medium (35) with appropriate supplements. The culture was aerated at 37°C in a 500-ml flask agitated on a rotary shaker. Unless otherwise specified, fucose, rhamnose, xylose, glycerol, glucose, and succinate were added at 0.2%, and CAA was added at 0.5%. To determine the induced activity of succinate semialdehyde dehydrogenase, cells were grown for 3 h in a medium containing 15 mM succinate and 0.01% CAA with or without 3 mM succinate semialdehyde (16). Cells harvested from an exponentially grown culture (100 to 150 Klett units; no. 42 filter) were centrifuged and washed once with 0.1 M potassium phosphate (pH 7.0). The final pellet was weighed and dispersed in 4 volumes of the same buffer, and the cells were disrupted (1 min/ml of suspension) in a tube by a model 60-W ultrasonic disintegrater (Measuring and Scientific Equipment, Ltd., London, England) while being chilled in a −10°C bath. The extract was centrifuged at 100,000 × *g* for 2 h at 4°C, and the supernatant fraction was used for enzyme assays.

Enzyme assays. Aldehyde dehydrogenase activity was measured by the rate of increase in A_{340} in a solution of 0.05 mM L-lactaldehyde, 0.66 mM NAD, 1 mM glutathione, and 20 mM sodium glycine (pH 10.5) in a final volume of 1 ml. Glycolaldehyde was tested as the substrate at 1 mM. Succinate semialdehyde dehydrogenase activity was measured by the rate of increase in A_{340} in a solution of 0.05 mM succinate semialdehyde, 0.66 mM NAD, and 0.1 M potassium phosphate at pH 8.0 (30). Protein concentrations were determined with bovine serum albumin as standard (27). Enzyme assays were carried out at 25°C, and the unit of specific activity was expressed in nanomoles of product formed per minute per milligram of protein.

RESULTS

Aldehyde dehydrogenase and growth on fucose or rhamnose. Strain ECL3 [*ald*$^+$ *fucO*(Con) *fucPIK*(Non)], the mutant that acquired the ability to grow on propanediol but

TABLE 1. *E. coli* K-12 strains used

Strain	Derived from:	Genotype[a]	Source or reference
BW6160	Broda 8	Hfr Broda 8 *zdh-57*::Tn*10* λ^r *metB1 relA1 spoT1*	B. Bachmann (36)
BW7620	KL99	Hfr KL99 *zed-977*::Tn*10 lac-42 relA1 spoT1 thi-1*	B. Bachmann (36)
KL706		F'133 *argG6 gal-6 hisG1 layY1 leuB6 malA1 metB1 mtl-2 recA1 rpsL104 supE44 fhuA2 tsx-1 xyl-7*	B. Bachmann
KL731		F'116 *argG6 gal-6 hisG1 lacY1 leuB6 malA1 metB1 mtl-2 recA1 rpsL104 supE44 thyA23 fhuA2 tsx-1 xyl-7*	B. Bachmann
N3041		*eda-51*::Tn*10* IN(*rnnD-rnnE*)*1*	B. Bachmann
NK6702		*man-6*::Tn*10* IN(*rnnD-rnnE*)*1*	B. Bachmann
PLK1253		F$^-$ *zdd230*::Tn*9zde-234*::Tn*10 arg-59 deo-70 his-85 ilv-632 pro-48 thyA714 trpA9605 trpR55 tsx-84* rac$^-$	B. Bachmann (5)
PLK1269		F$^-$ *zdc-235*::Tn*10 zdd-230*::Tn*9 arg-59 deo-70 his-85 ilv-632 pro-48 thyA714 trpA9605 trpR55 tsx-84* rac$^-$	B. Bachmann (5)
PLK1165		F$^-$ *trg-2*::Tn*10 arg deoB* or *deoC his-29 ilv pro thyA714 trpA9605 trpR55 tsx* rac$^-$	T. Griffin (5)
ECL1		HfrC *phoA8 relA1 fhuA22* T2^r (λ)	23
ECL3	ECL1	HfrC *fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	34
ECL40	ECL3	HfrC *ald-1 fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	34
ECL116	K-12	F$^-$ *endA hsdR* Δ(*argF-lac*)*U169 thi*	2, 13
ECL289	ECL248	HfrC *argA*::Tn*10 eno phoA8 relA1 fhuA22* T2^r (λ)	10
ECL339	ECL116	F$^-$ Δ(*rha-pfkA*)*15 zig*::Tn*10 endA hsdR* Δ *lacU169 thi*	13
ECL430	ECL3	HfrC *ald*(Con) *fucO*(Con) *fucA*(Non) fucPIK(Non) *phoA8 relA1 fhuA22* T2^r (λ)	18
ECL459	ECL421	HfrC *fucO*(Con) *fucA*(Con) *fucPIK*(Con) *phoA8 relA1 fhuA22* T2^r (λ)	11
ECL471	ECL40	HfrC *ald-1 zdb-1*::Tn*10 fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	This study
ECL486	ECL40	HfrC *zdb-1*::Tn*10 fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	This study
ECL492	ECL40	HfrC *trg-2*::Tn*10 fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	This study
ECL493	ECL40	HfrC *ald*-1 *trg*-2::Tn*10 fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	This study
ECL494	ECL471	HfrC *ald*(Con) *fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	This study
ECL495	ECL471	HfrC *fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 fhuA22* T2^r (λ)	This study
ECL708	ECL116	F$^-$ *fucO*(Con) *fucA*(Con) *fucPIK*(Con) *endA hsdR* Δ *lacU169 thi*	This study
ECL709	ECL708	F$^-$ *ald-2 fucO*(Con) *fucA*(Con) *fucPIK*(Con) *endA hsdR* Δ *lacU169 thi*	39
ECL719	ECL709	F$^-$ *ald-2 fucO*(Con) *fucA*(Con) *fucPIK*(Con) *rpsL endA hsdR* Δ *lacU169 thi*	This study

[a] Symbols: *ald*, gene for aldehyde dehydrogenase; *fucO*, gene for L-1,2-propanediol oxidoreductase; *fucA*, gene for L-fuculose 1-phosphate aldolase; *fucPIK*, operon encoding L-fucose permease, L-fucose isomerase, and L-fuculose kinase; Con, constitutivity; and Non, noninducibility.

lost the ability to grow on fucose, remained normal in its growth on rhamnose agar when compared with the parental wild-type strain ECL1 (the average diameter of colonies after 2 days of incubation at 37°C was 1.5 mm). Strain ECL40 [*ald-1*, *fucO*(Con) *fucPIK*(Non)], isolated from strain ECL3 as a mutant that lost the ability to grow on propanediol because lactaldehyde dehydrogenase activity was missing (33), grew subnormally on rhamnose (the average diameter of colonies was 0.8 mm). Strains ECL3 and ECL40, however, both grew normally on glucose.

Strain ECL708 [*ald*$^+$ *fucO*(Con)], a propanediol-positive mutant which expressed all the genes in the *fuc* cluster constitutively (11), grew normally on fucose and on rhamnose. Strain ECL709 [*ald-2 fucO*(Con)], isolated from strain ECL708 as a mutant impaired in its growth on fucose and found to be missing lactaldehyde dehydrogenase activity (39), also grew subnormally on rhamnose (the average diameter of colonies was 0.8 mm). Growth on glucose was normal for both strains ECL708 and ECL709. (The subnormal growth on fucose or rhamnose proved to be a useful trait for screening *ald* transductants.) The phenotypic changes of strains ECL40 and ECL709 suggested that the same enzyme was responsible for converting lactaldehyde to lactate during growth on fucose, rhamnose, or propanediol.

Aldehyde dehydrogenase activities in wild-type and mutant strains grown on fucose and rhamnose. The wild-type strain ECL1, when grown aerobically on fucose or rhamnose, showed specific activities of aldehyde dehydrogenase slightly higher (1.4- to 2-fold) than when grown on xylose or CAA (Table 2). Similar results were obtained with strain ECL708 (*ald*$^+$). Strain ECL3 (*ald*$^+$), which did not grow on fucose, gave a higher aldehyde dehydrogenase activity, when grown on rhamnose than when grown on xylose and CAA. Strain ECL40 (*ald*-1), which likewise could not grow on fucose, gave no detectable aldehyde dehydrogenase activity when grown on rhamnose, xylose, or CAA. No activity was found in strain ECL709 (*ald*-2) when it was grown on any of the four carbon and energy sources. The enzymatic data therefore support the conclusion that the same enzyme was responsible for converting lactaldehyde to lactate during growth on fucose, rhamnose, or propanediol. Moreover, the basal aldehyde dehydrogenase activity in wild-type cells and the increment in the activity in cells grown on fucose and rhamnose are attributable to the same enzyme.

Since aldehyde dehydrogenase was reported to be responsible also for the conversion of glycolaldehyde to glycolate in mutants capable of growing on ethylene glycol (8, 9), extracts of strains ECL1, ECL3, ECL40, and ECL709 grown on CAA were also assayed with glycolaldehyde as substrate. As expected, a high basal activity was found in strains ECL1 and ECL3, but the activity was missing in strains ECL40 and ECL709 (data not shown).

Catabolite repression by glucose. It was reported that the

TABLE 2. Aldehyde dehydrogenase activities in wild-type and mutant strains grown on different carbon and energy sources

Strain	Relevant genotype[a]	Sp act of aldehyde dehydrogenase of cells grown on:			
		Fucose	Rhamnose	Xylose	CAA
ECL1	*ald*$^+$	200	140	100	110
ECL3	*ald*$^+$ *fucO*(Con) *fucPIK*(Non)	NG[b]	150	100	110
ECL40	*ald-1 fucO*(Con) *fucPIK*(Non)	NG	0	0	0
ECL708	*ald*$^+$ *fucO*(Con)	130	110	80	100
ECL709	*ald-2 fucO*(Con)	0	0	0	0

[a] See Table 1, footnote *a*.
[b] NG, No growth.

aldehyde dehydrogenase activity level in wild-type cells grown on glucose as the sole carbon and energy course was sixfold lower than that in cells grown on CAA (19). To test whether or not the low enzyme level in glucose-grown cells reflected catabolite repression, the specific activity of aldehyde dehydrogenase was analyzed in cells of strain ECL1 grown under three conditions: on fucose alone, on fucose plus glucose, or on fucose plus glucose in the presence of cyclic AMP (2 mM). The presence of glucose in the medium reduced the activity level more than 10-fold, but less than 3-fold repression occurred in the presence of cyclic AMP.

***ald* gene not linked to *fuc* or *rha*.** Since *fucO*, encoding the sole enzyme responsible for the reduction of lactaldehyde to propanediol, is linked to the rest of the *fuc* genes, it seemed possible that the *ald* gene specifying aldehyde dehydrogenase is linked to either the *fuc* or the *rha* gene cluster.

To find out whether *ald* is linked to *fuc*, we used strains ECL40 [*ald-1* fucO(Con)] and ECL709 [*ald-2 fucO*(Con)] as transduction donors and strain ECL289 (*ald*$^+$), with the markers *eno* (min 59.6) and *argA* (min 60.5) flanking a *fuc*$^+$ (min 60.2), as the recipient. Transductants were selected for *eno*$^+$ and *arg*$^+$ (growth on glucose without arginine supplementation) and scored for the ability to grow on propanediol. If the *ald-1* or *ald-2* mutations were within the segment of the genome selected, the transductants should have been propanediol negative despite the inheritance of *fucO*(Con). In each of the two crosses, 100 of 100 of the transductants scored were propanediol positive. Thus, incorporation of the segment encompassing *eno* and *argA* did not displace the *ald*$^+$ gene in the recipient.

To find out whether the *ald* gene is at the *rha* locus, we transduced the *rha*$^+$ region from a wild-type strain into strains ECL40 (*ald-1*) and ECL709 (*ald-1*) in two steps. First, a deletion of *rha-pfka*, 80% linked to a Tn*10* insertion, was transduced from strain ECL339 into strains ECL40 and ECL709 by selecting for Tc^r and scoring for loss of ability to grow on rhamnose. Second, a Tc^r and Rha$^-$ transductant of ECL40 and a similar transductant of ECL709 were treated with a P1 lysate of ECL1 (*rha*$^+$). The transductants were selected for Rha$^+$. As expected, about 80% of the Rha$^+$ transductants became Tc^s. When 5 Tc^r Rha$^+$ and 10 Tc^s Rha$^+$ transductants from each of the two crosses were compared with the wild-type strain on rhamnose agar, all of the transductant colonies were subnormal in size, like strains ECL40 and ECL709. When two Tc^s Rha$^+$ transductants from each cross were assayed for aldehyde dehydrogenase activity, none was found. Thus, the inheritance of the *rha*$^+$ locus from the wild-type strain was not associated with the replacement of the *ald-1* and *ald-2* by *ald*$^+$.

The conclusions based on the transduction experiments were confirmed by the observation that F'116 covering the *fuc*$^+$ region (from strain KL731) and F'133 covering the *rha*$^+$ region (from strain KL706) did not complement the *ald-1* or *ald-2* mutations.

Mapping of *ald*. Preliminary timed mating experiments (26) with several different Hfr strains (36) suggested that *ald* was located in the region of min 31 to 42. A more precise location was identified by measuring transductional frequencies between the gene and genes known to be in that area. No cotransduction was observed with markers at min 43 (*zed-977*::Tn*10* of strain BW7620), min 41 (*eda-51*::Tn*10* of strain N3041), min 37 (*zdh-57*::Tn*10* of strain BW6160), min 36 (*man-6*::Tn*9* of strain NK6702), and min 34.2 (*zde-234*::Tn*10* of strain PLK1253). Cotransduction with increasing frequency (Table 3) was observed with markers occupying positions min 34 to 31, i.e., at min 33.3 (*zdd-230*::Tn*9* of strain PLK1253), 1%; at min 32.3 (*zdc-235*::Tn*10* of strain PLK1269), 7 to 8%; at min 31.1 (*trg-2*::Tn*10* of strains PLK1165, ECL492, and ECL493), about 88%. According to the formula devised by Wu (37), *ald* should be about 1.18 min from *zdc-235*::Tn*10* and 0.08 min from *trg*. Since a slight linkage was also shown between *ald* and *zdd-230*::Tn*9* at min 33.3, it is more likely that *ald* is located between *trg* and *zdc-235*::Tn*10* at about min 31.2 (Fig. 2).

When strain ECL486 (*zdb-1*::Tn*10*) was used as a transduction donor and strains ECL40 (*ald-1*) and ECL709 (*ald-2*) were used as recipients, selection for Tn*10* gave about 60%

TABLE 3. Phage P1-mediated transduction of the aldehyde dehydrogenase gene (*ald*)[a]

Donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency (%)
PLK1253 (*zdd-230*::Tn*9*)	ECL719 [*ald-2 fucO*(Con)]	Tn*9*	*ald*$^+$	2/200 (1)
PLK1269 (*zdc-235*::Tn*10*)	ECL719 [*ald-2 fucO*(Con)]	Tn*10*	*ald*$^+$	19/280 (7)
PLK1269 (*zdc-235*::Tn*10*)	ECL40 [*ald-1 fucO*(Con)]	Tn*10*	*ald*$^+$	14/180 (8)
PLK1165 (*trg-2*::Tn*10*)	ECL719 [*ald-2 fucO*(Con)]	Tn*10*	*ald*$^+$	10/11 (91)
PLK1165 (*trg-2*::Tn*10*)	ECL40 [*ald-1 fucO*(Con)]	Tn*10*	*ald*$^+$	28/33 (85)
ECL492 (*trg-2*::Tn*10*)	ECL719 [*ald-2 fucO*(Con)]	Tn*10*	*ald*$^+$	211/240 (88)
ECL493 (*trg-2*::Tn*10 ald-1*)	ECL3 [*ald*$^+$ *fucO*(Con)]	Tn*10*	*ald-1*	45/60 (75)

[a] See Table 1, footnote *a*.

FIG. 2. Genetic map of *E. coli* showing the sites of relevant genes and transposon insertions in the region of *ald*. Minutes on the map are shown in parenthesis.

cotransduction of *ald*$^+$. Thus, the two mutant loci are probably allelic. Seven other independent *ald* mutations induced by ethyl methanesulfonate in strain ECL708 (detected by the pale color of the colonies on MacConkey-propanediol agar) also gave about 60% cotransduction with *zdb-1*::Tn*10*. The same frequency was found with the *ald-201* mutation that conferred resistance to L-glyceraldehyde during growth of a mutant on L-galactose (39).

***ald* gene distinct from *sad* gene.** The *sad* gene, encoding succinate semialdehyde dehydrogenase, is located at min 34.1 (30). Because of the proximity of this gene to the *ald* gene and the fact that both genes encode NAD-dependent aldehyde dehydrogenases, it was prudent to verify the existence of two distinct systems. For this purpose, we examined extracts of strains ECL1 (*ald*$^+$), ECL40 (*ald-1*), and ECL709 (*ald-2*) grown in the presence or absence of succinate semialdehyde for succinate semialdehyde and lactaldehyde dehydrogenating activities. Whereas aldehyde dehydrogenase activity was found only in strain ECL1 (about 130 U when grown in the presence or absence of succinate semialdehyde), succinate semialdehyde dehydrogenase activity was induced in all three strains (from about 15 to about 220 U). In addition, it was found that strains ECL40 and ECL709 grew on succinate semialdehyde despite a deficiency of the *ald* gene product.

The *ald* and *sad* genes are also distinguishable by transduction mapping. The *sad* locus was shown to be 87 and 30% cotransducible with *zde-234*::Tn*10* and *zdd-230*::Tn*9*, respectively (30). In this study, we showed that *ald* was not cotransducible with *zde-234*::Tn*10* and was <1% cotransducible with *zdd-230*::Tn*9*. Moreover, whereas *sad* was less than 2.5% cotransducible with *trg* (30), *ald* was 88% cotransducible with the same marker (Table 3).

Analysis of *ald*(Con) mutation in strain ECL430. Strain ECL430 was isolated from strain ECL3 by repeated selection for increased propanediol scavenging power. In strain ECL430, aldehyde dehydrogenase was produced at an elevated constitutive level. It therefore appears that this mutation conferred an advantage to strain ECL40 by increasing its ability to trap lactaldehyde as lactate (18). The mutant allele responsible for the elevated production of aldehyde dehydrogenase is designated *ald*(Con). In a transduction experiment, it was shown that *ald*(Con) was closely linked to *ald-1*. The ability to grow on propanediol, dependent on *fucO*(Con) and *ald*$^+$ or *ald*(Con), was transduced from strains ECL3 and ECL430 to strain ECL471 [*fucO*(Con) *ald-1 zdb-1*::Tn*10*], in which the *ald-1* mutation was linked to a Tn*10*. The same cotransduction frequency of Tn*10* was found in both crosses. From each cross two Tc^s and two Tc^r transductants were assayed for aldehyde dehydrogenase activity. With strain ECL3 as donor, the transductants all showed wild-type aldehyde dehydrogenase activity (strain ECL495 [Table 4] is an example). With strain ECL430 as donor, the transductants all showed an elevated constitutive level of the enzyme (strain ECL494 [Table 4] is a representative).

TABLE 4. Aldehyde dehydrogenase activities in the *ald*(Con) mutant and its transductants[a]

Strain	Genotype	Phenotype	Sp act of aldehyde dehydrogenase
ECL1	*ald*$^+$ *fuc*$^+$	Ald$^+$ Fuc$^+$ Prd$^-$	110
ECL3	*ald*$^+$ *fucO*(Con) *fucPIK*(Non)	Ald$^+$ Fuc$^-$ Prd$^+$	110
ECL430	*ald*(Con) *fucO*(Con) *fucPIK*(Non)	Ald(Con) Fuc$^-$ Prd$^+$	300
ECL471	*ald-1 fucO*(Con) *fucPIK*(Non)	Ald$^-$ Fuc$^-$ Prd$^-$	0
ECL494	*ald*(Con) *fucO*(Con) *fucPIK*(Non)	Ald(Con) Fuc$^-$ Prd$^+$	370
ECL495	*ald*$^+$ *fucO*(Con) *fucPIK*(Non)	Ald$^+$ Fuc$^-$ Prd$^+$	80

[a] Cells were grown in CAA medium. See Table 1, footnote *a*.

DISCUSSION

It was previously shown that aldehyde dehydrogenase activity was present at a basal level of about 100 activity units (lactaldehyde as substrate) in cells grown aerobically on CAA. Growth on fucose increased the level less than threefold, and growth on glucose reduced the activity more than sixfold. Anaerobic growth on CAA plus pyruvate reduced the activity to a nondetectable level (19). In this study, we showed that catabolite repression was at least in part responsible for the glucose effect. The apparent high basal levels of the enzyme (in cells grown on CAA or xylose) and its repressibility by glucose would suggest that the enzyme has catabolic functions in addition to that of acting on lactaldehyde. The enzyme is likely to be internally induced by more than one effector under a number of growth conditions.

Although the enzyme was first discovered as an L-lactaldehyde dehydrogenase, it was already recognized at the time that it possessed a broad substrate specificity (33). This broad specificity was also made manifest in vivo. A mutant selected from strain ECL3 for its ability to grow on ethylene glycol was found to use aldehyde dehydrogenase to convert glycolaldehyde to glycolate (8, 9). During the study of a mutant that acquired the ability to grow on the rare sugar L-galactose, it was discovered that the dehydrogenase also catalyzed the conversion of L-glyceraldehyde to L-glycerate, which is toxic to the cell (39).

In view of the regulatory patterns of the enzyme synthesis and the wide separation of *ald* from *fuc* and *rha*, the enzyme is more appropriately referred to simply as aldehyde dehydrogenase. Although direct evidence is yet to be obtained, the location at min 31 of all 10 independent *ald* tested strongly suggests that the structural gene is there. It is not known whether the *ald*(Con) mutation lies in the promoter region of the *ald* structural gene or in a closely linked regulator gene. In the meantime, because *ald* is in a region of the *E. coli* chromosome that contains very few known genes (1), it might serve as a useful marker.

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