JOURNAL OF BACTERIOLOGY, Apr. 1971, p. 90-96

Vol. 106, No. 1
Printed in U.S.A.

Metabolism of D-Arabinose: a New Pathway in *Escherichia coli*[1]

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Received for publication 4 January 1971

Several growth characteristics of *Escherichia coli* K-12 suggest that growth on L-fucose results in the synthesis of all the enzymes necessary for growth on D-arabinose. Conversely, when a mutant of *E. coli* is grown on D-arabinose, all of the enzymes necessary for immediate growth on L-fucose are present. Three enzymes of the L-fucose pathway in *E. coli*, L-fucose isomerase, L-fuculokinase, and L-fuculose-1-phosphate aldolase possess activity on D-arabinose, D-ribulose, and D-ribulose-1-phosphate, respectively. The products of the aldolase, with D-ribulose-1-phosphate as substrate, are dihydroxyacetone phosphate and glycolaldehyde. L-Fucose, but not D-arabinose, is capable of inducing these activities in wild-type *E. coli*. In mutants capable of utilizing D-arabinose as sole source of carbon and energy, these activities are induced in the presence of D-arabinose and in the presence of L-fucose. Mutants unable to utilize L-fucose, selected from strains capable of growth on D-arabinose, are found to have lost the ability to grow on D-arabinose. Enzymatic analysis of cell-free extracts, prepared from cultures of these mutants, reveals that a deficiency in any of the L-fucose pathway enzymes results in the loss of ability to utilize D-arabinose. Thus, the pathway of D-arabinose catabolism in *E. coli* K-12 is believed to be: D-arabinose $\rightleftharpoons$ D-ribulose $\rightarrow$ D-ribulose-1-phosphate $\rightleftharpoons$ dihydroxyacetone phosphate plus glycolaldehyde. Evidence is presented which suggests that the glycolaldehyde is further oxidized to glycolate.

Two pathways are known for the degradation of D-arabinose by bacteria. In *Aerobacter aerogenes* (17) D-arabinose is isomerized to D-ribulose, which is then phosphorylated at carbon 5 and epimerized at carbon 3 to produce D-xylulose-5-phosphate. In *Pseudomonas saccharophila* (19) metabolism of D-arabinose results in the formation of pyruvic acid and glycolic acid by the following pathway: D-arabinofuranose $\xrightarrow[-2(H)]{NAD}$ D-arabino-γ-lactone $\xrightarrow{+H_2O}$ D-arabonic acid $\xrightarrow{-H_2O}$ 2-keto-3-deoxy-D-arabonic acid $\xrightarrow[-2(H),]{NAD}$ pyruvic acid plus glycolic acid. In this paper, evidence for a third pathway for the metabolism of D-arabinose in *Escherichia coli* is presented.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. The parent strain of *Escherichia coli* K-12, the D-arabinose-positive mutant, the minimal salts medium, and general conditions of growth were described in the accompanying paper (13).

To obtain growth curves and to determine the influence of inoculum substrate on the type of growth curve produced, overnight cultures were obtained by incubation of a screw-cap test tube (20 by 130 mm) containing 5.0 ml of minimal salts and 0.3% of the particular compound serving as the inoculum substrate. The tubes were aerated by agitation on a rotary shaker (New Brunswick Scientific Co., Inc., New Burnswick, N.J.). These cultures were transferred to 20 ml of the same medium in a 250-ml side-arm flask and allowed to reach early log phase. The exponentially growing cells were harvested by centrifugation, washed once in sterile minimal salts, and suspended in sterile salts to a density of 0.6 mg (dry weight) per ml of cells. The cell suspensions were transferred to 25 ml of the growth medium to be tested, in 250-ml side-arm flasks, to a density of 0.08 to 0.12 mg (dry weight) per ml of cells. These flasks were incubated in the same manner as the test tubes above, and growth was followed by measuring the increase in turbidity on a Klett-Summerson photocolorimeter equipped with a red filter (660 nm; Klett Manufacturing Co., Inc., New York, N.Y.).

To prepare cell-free extracts for assay of enzymatic activities, all cultures were harvested during late log phase. For detection of constitutive enzyme synthesis, cells were grown on minimal salts containing 1.0%

[1] This paper was presented in part at the 70th Annual Meeting of the American Society for Microbiology, Boston, Massachusetts, 26 April-1 May 1970.

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casein hydrolysate. To detect induced enzyme synthesis, salts and casein hydrolysate were supplemented with 0.3% of the inducing substrate being tested.

In experiments designed to detect the accumulation of glycolate in the growth medium, preparation of inocula and growth of cultures were identical to that described above. In these experiments, 1.0-ml samples were removed at various intervals throughout the growth cycle and treated as described below.

Isolation of L-fucose-deficient mutants. All mutants selected for inability to utilize L-fucose for growth were isolated after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1), followed by penicillin enrichment (5) and replica plating. A double penicillin enrichment was used to increase the percentage of specific mutants obtained.

Preparation of cell-free extracts. Cells were collected by centrifugation at 12,000 × *g* for 10 min at 4 C in an RC-2 refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The pellet was washed twice in minimal salts, once with 20% of the original volume and once with 10%. The washed cells were then resuspended in the appropriate buffer solution (*see below*) in a volume equal to 5 to 10% of the original. The resuspended cells were disrupted directly in the cup of a 10-kc magnetostrictive oscillator (Raytheon Co., South Norwalk, Conn.), by exposure to sonic vibration for 1 min per 2 ml of cell suspension at 5 C at maximum obtainable power. Cellular debris was removed by centrifugation for 10 min at 27,000 × *g*. The supernatant fractions, constituting the crude extracts, were collected in tubes and stored in an ice bath.

The following buffer solutions were used in the preparation of cell-free extracts. (i) Pentose isomerase and pentulokinase assays were carried out in 0.02 M phosphate buffer (*p*H 7.5), (ii) aldolase assays were carried out in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 7.5), 0.004 M ethylenediaminetetraacetic acid, and 0.008 M magnesium acetate.

Determinations of enzymatic activities. Isomerase activity was determined from the rate of pentulose, or methylpentulose, formation (2). Kinase activity was determined from the rate of pentulose disappearance (14).

The rate of cleavage of L-fuculose-1-phosphate and D-ribulose-1-phosphate was measured by a modification of the method of Ghalambor and Heath (7). The assay was carried out in two steps: (i) the enzymatic synthesis of pentulose-1-phosphate by the reaction of purified L-fuculokinase (EC 2.7.1.51) from *E. coli* with either D-ribulose or L-fuculose; (ii) the measurement of dihydroxyacetone phosphate (DHAP) formation after addition of "crude extract."

High NADH oxidase activity made it impossible to detect any aldolase activity in cell-free extracts. The reduced nicotinamide adenine dinucleotide (NADH) oxidase activity was separated, therefore, from aldolase activity by elution from diethylaminoethyl (DEAE) cellulose columns with a stepwise gradient of NaCl. The following procedure was employed to standardize the assay as much as possible, for the purpose of comparing different extracts. Cell-free extracts were prepared from 250 ml of an exponentially-growing culture. All cultures were harvested between 0.7 and 0.85 mg (dry weight) per ml of cells. In all cases, 75 mg of protein were applied to a DEAE cellulose column (20 by 60 mm), previously equilibrated with aldolase buffer solution. After collection of the eluate, three 10-ml fractions each of 0.10, 0.15, and 0.20 M NaCl in aldolase buffer solution were collected. For all extracts tested, 85 to 90% of the NADH oxidase activity was eluted in the first three 0.10 M NaCl fractions. Approximately 70% of the aldolase activity detected was eluted with the second 0.15 M NaCl fraction, which was used as the crude extract. For determination of specific activities, the protein concentration of the second 0.15 M fraction was used.

A unit of activity, for all enzyme assays, was defined as that amount of enzyme which catalyzes the conversion of 1 nmole of substrate to product in 1 min. Protein content of crude extracts was measured by the ratio of absorbancies at 280 and 260 nm (24). Specific activity was defined as units per minute per milligram of protein.

Analytical procedures. By heating with concentrated sulfuric acid, lactic acid was quantitatively converted to acetaldehyde, which was determined by measurement of the purple color formed with *p*-hydroxydiphenyl at 560 nm against a reagent blank (3). The orcinol reaction (16) was used for the quantitative determination of pentose. Ketopentose was measured by the cysteine-carbazole reaction (6). Incubation times for the cysteine-carbazole reaction, at 37 C, were 20 min for D-ribulose and 2 hr for L-fuculose.

Glycolate was quantitated by reaction with chromotropic acid (20) at 580 nm. A sample to be assayed was first treated with $ZnSO_4$ and $Ba(OH)_2$ to remove nucleotides and other organic phosphates, which were found to interfere with the assay. A 30-min heat treatment in a boiling-water bath was found necessary to obtain full color development. The 580 nm reading was found to be increased by the presence of aldopentose. This could be corrected by prior determination of pentose concentration and subtracting the absorbancy reading which would be given by that concentration of pentose from the observed reading.

The identity of the glycolate was confirmed by paper chromatography (23). Samples were applied to Whatman no. 1 filter paper which was developed in a solvent system of ethanol-water-ammonium hydroxide (8:1:1). The developed chromatograms were air-dried and sprayed with a 0.04% solution of bromocresol green in 95% ethanol.

Glycolaldehyde was measured colorimetrically after formation of an osazone (21), and also spectrophotometrically by reduction to ethyleneglycol by using alcohol dehydrogenase (EC 1.1.1.1; reference 11).

α-Glycerol phosphate was determined enzymatically by oxidation with α-glycerophosphate dehydrogenase (EC 1.1.1.8; reference 10). Dihydroxyacetone phosphate was measured spectrophotometrically with α-glycerophosphate dehydrogenase and NADH (7). This same procedure was employed for the determination of fructose diphosphate which was first cleaved to DHAP and glyceraldehyde-3-phosphate by the addition of excess rabbit muscle aldolase (EC 4.1.2.13).

Chemicals. The preparation of D-ribulose and L-fuculose was described in the accompanying paper (13). All other chemicals and reagents were obtained from commercial sources.

RESULTS

Previous work has demonstrated a common identity for D-arabinose isomerase and L-fucose isomerase (EC 5.3.1.3) in *E. coli* B (4, 8, 14). Evidence that other enzymes of the L-fucose pathway may be involved in the metabolism of D-arabinose by *E. coli* K-12 was provided by the growth data illustrated in Table 1.

Wild-type *E. coli* strain 1000 grew at a rate of 0.71 generations per hour on L-fucose, with a lag of 210 min, when transferred from a D-glucose inoculum. At least 5 days were required before growth of strain 1000 could be detected on D-arabinose from a D-glucose inoculum. Strain 1102, a mutant of 1000 selected for ability to utilize D-arabinose as a growth substrate, was capable of exponential growth on D-arabinose at a rate of 0.57, with a lag of only 12 hr from a D-glucose inoculum.

Wild-type cells, preinduced on L-fucose, were able to grow at a linear rate when transferred to a D-arabinose medium. The absence of any lag in growth suggested that the presence of one or more enzymes of the L-fucose pathway was all that was necessary for these cells to acquire the ability to grow on D-arabinose. The growth rate decreased by approximately half at each doubling of cell mass, indicating a dilution of the enzymes and a lack of their induction in the presence of D-arabinose. In contrast to wild-type cells, growth of strain 1102 on D-arabinose, after preinduction by L-fucose, was exponential and did not decrease in rate until the stationary phase was approached. This pattern suggested that the necessary enzymes were being continuously synthesized by the mutant in the presence of D-arabinose. Further evidence that growth of the mutant strain on D-arabinose involved synthesis of the enzymes of the L-fucose pathway was provided by the lag-free growth of this mutant on L-fucose after growth on D-arabinose.

Enzyme activities in cell-free extracts. The pathway for the metabolism of L-fucose by *E. coli* has been well documented. Green and Cohen (8) demonstrated the conversion of L-fucose to L-fuculose by an isomerase. Heath and Ghalambor (9) purified and characterized a kinase which phosphorylates L-fuculose at the 1-carbon position. An aldolase which cleaves L-fuculose-1-phosphate between carbons 3 and 4, producing dihydroxyacetone phosphate and L-lactaldehyde, was partially purified by Ghalambor and Heath (7). These authors provided evidence that L-fucose isomerase, L-fuculokinase, and L-fuculose-1-phosphate aldolase (EC 4.1.2.17) possess enzymatic activity on D-arabinose, D-ribulose, and D-ribulose-1-phosphate, respectively.

The data presented in Table 2 provide evidence that the mutation(s) leading to growth of *E. coli* on D-arabinose permits the induction of at least three enzymes of the L-fucose pathway in the presence of D-arabinose. Cell-free extracts of wild-type *E. coli*, prepared from cells grown in the presence of L-fucose, possess isomerase activity on L-fucose and D-arabinose, kinase activity on L-fuculose and D-ribulose, and aldolase activity on L-fuculose-1-phosphate and D-ribulose-1-phosphate. Extracts prepared from wild-type cells, grown either in the presence of D-arabinose, or on casein hydrolysate alone, exhibit none of the above activities. Cell-free extracts prepared from strain 1102, grown in the presence of either L-fucose or D-arabinose, possess all of the above activities. Evidence that the mutation to growth on D-arabinose does not result in the constitutive synthesis of any of these enzymes is provided by their absence in crude extracts prepared from strain 1102 grown on casein hydrolysate alone.

The ranges of activities presented in Table 2 represent the highest and lowest values obtained. As reported in the accompanying paper (13), the ratio of L-fuculokinase activity on L-fuculose and D-ribulose is 2:1. However, because of the presence of interfering enzymes, particularly adenosine triphosphatase activity, these ratios were not obtained when kinase assays were performed with crude extracts. Aldolase activities were deter-

TABLE 1. *Relationships between growth of Escherichia coli K-12 on L-fucose and on D-arabinose*

Strain	Inoculum medium[a]	Growth medium[a]	Lag phase	Growth rate[b]
1000	D-Glucose	L-Fucose	210 min	0.71
	D-Glucose	D-Arabinose	5 days	0.38, 0.50
	L-Fucose	D-Arabinose	0	0.36, 0.16, 0.063
1102	D-Glucose	D-Arabinose	12 hr	0.57
	L-Fucose	D-Arabinose	0	0.56
	D-Arabinose	L-Fucose	0	0.80

[a] All cells were removed from inoculum media during exponential growth, washed with sterile minimal salts, and then transferred to growth medium.

[b] Growth rate was defined as number of doublings of cell mass per hour.

TABLE 2. *Isomerase-, kinase-, and aldolase-specific activities in cell-free extracts of Escherichia coli K-12*[a]

Activity measured	Strain	Enzyme substrate	Carbohydrate inducer		
			L-Fucose	D-Arabinose	None
Isomerase	1000	L-Fucose	62–249	3–7	1–7
		D-Arabinose	30–87	1–2	1–2
	1102	L-Fucose	110–214	165–449	1–8
		D-Arabinose	40–72	74–177	1–2
Kinase	1000	L-Fuculose	40–45	4	1–3
		D-Ribulose	60–80	4	4–7
	1102	L-Fuculose	25–31	28–30	4
		D-Ribulose	22–29	20–29	4
Aldolase	1000	L-Fuculose-1-P	222	6	6
		D-Ribulose-1-P	51	1	1
	1102	L-Fuculose-1-P	206	340	15
		D-Ribulose-1-P	37	59	1

[a] All specific activities are expressed as nanomoles per minute per milligram of protein.

mined by assaying for dihydroxyacetone phosphate production, since this product is common to the cleavage of both L-fuculose-1-phosphate and D-ribulose-1-phosphate.

Further degradation of D-ribulose-1-phosphate. The products of L-fuculose-1-phosphate aldolase action on D-ribulose-1-phosphate should be dihydroxyacetone phosphate and glycolaldehyde. The ability to demonstrate aldolase activity with D-ribulose-1-phosphate by assaying for the production of either dihydroxyacetone phosphate or glycolaldehyde was established in the accompanying paper (13). The route for further metabolism of triose phosphate is well known. Glycolaldehyde might be further oxidized to glycolate, perhaps by a nonspecific aldehyde dehydrogenase constitutively synthesized by *E. coli* K-12 (22). The first reaction in the metabolism of glycolate is oxidation to glyoxylate by glycolate oxidase (12).

Assuming that under strictly anaerobic conditions glycolate should not be further metabolized, the following experiment was performed. Exponentially growing cells of strain 1102 in 250 ml of minimal salts, containing either 0.3% D-arabinose or 0.3% L-fucose, were used for the preparation of cell-free extracts in Tris buffer (*p*H 7.8). In both cases, 5 ml of extract was added to a Warburg flask containing, in a volume of 5 ml, 200 μmoles of $MgCl_2$, 100 μmoles of NaF, 50 μmoles of $MnCl_2$, 1 μmole of nicotinamide adenine dinucleotide (NAD), 0.01 ml of lactic acid dehydrogenase (Sigma Chemical Co.), 100 μmoles of D-arabinose, and 300 μmoles of adenosine triphosphate (ATP). The flasks were incubated under anaerobic conditions, in a nitrogen atmosphere, at 37 C for 2 hr. The reaction mixtures were deproteinized with perchloric acid and assayed for fermentation products.

The results of this experiment can be seen in Table 3. In both cases glycolate and glycolaldehyde were produced. In the case of the D-arabinose-grown cells these two-carbon units accounted for 36% of the total carbon utilized; in the L-fucose-grown extract, 33.3% of the carbon

TABLE 3. *Products of D-arabinose degradation by cell-free extracts of Escherichia coli strain 1102 under anaerobic conditions*

Compound	D-Arabinose-grown cells		L-Fucose-grown cells	
	Micromoles	Per cent C recovery	Micromoles	Per cent C recovery
Used				
D-Arabinose	44.7		74.4	
Produced				
D-Ribulose	3.9	8.7	0.6	0.8
Glycolate	15.3	13.7	35.0	18.8
Glycoladehyde	24.9	22.3	25.6	14.5
Lactic acid	2.5	3.5	7.8	4.2
α-Glycerol-P	20.8	27.9	39.5	31.8
Dihydroxyacetone-P	2.7	3.7	6.3	5.1
Fructose-1,6-di-P	1.6	4.3	6.2	10.1
Total carbon recovery		84.1		85.3

utilized could be accounted for. The maximum theoretical recovery, if these two-carbon units were produced as a result of the aldolase action, would be 40%. It was evident from this experiment that growth of *E. coli* on either L-fucose or D-arabinose leads to the synthesis of enzymes resulting in similar metabolism of D-arabinose, and most likely by the same pathway in both cases.

Production of glycolate during growth on D-arabinose. Glycolate was produced by *E. coli* during growth on D-arabinose, as shown by the following experiment. A 1.0-ml sample of growth medium was removed from a culture of *E. coli* strain 1102 growing on D-arabinose at various intervals during the growth cycle. After removal of cells by centrifugation, the supernatant fluid was assayed for the presence of D-arabinose, glycolaldehyde, and glycolate. Typical results are shown in Fig. 1. Although glycolaldehyde could not be detected in the medium, glycolate did accumulate. The concentration of glycolate was found to increase until the supply of D-arabinose was exhausted. Growth continued beyond the point at which D-arabinose could no longer be detected in the medium, and ceased only when the glycolate had also disappeared.

Strain 1102, whether previously grown on L-fucose or on D-glucose, produced glycolate when transferred to a D-arabinose growth medium. The parent strain, 1000, accumulated glycolate in a D-arabinose growth medium if cells had been previously grown on L-fucose. Since strain 1000 could not grow on D-arabinose following growth on D-glucose, this strain could not be compared to 1102 under these conditions. Evidence that glycolate accumulation is specific for growth on D-arabinose was provided by the inability to detect glycolate in the medium of either strain 1000 or 1102 during growth on L-arabinose via the nonoxidative pentose monophosphate pathway. These results are summarized in Table 4.

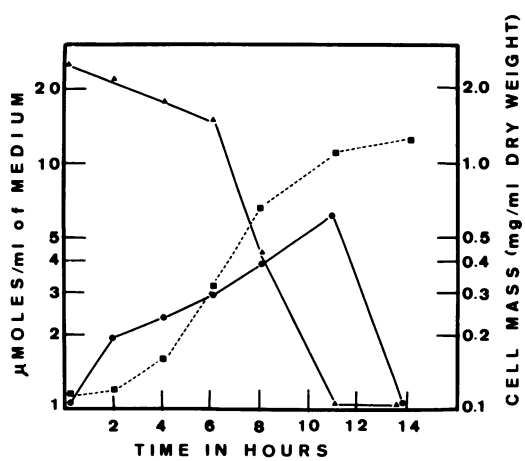

FIG. 1. *Correlation of D-arabinose disappearance with glycolate accumulation in the medium of Escherichia coli strain 1102 growing on D-arabinose. Symbols: ●, micromoles of glycolate per milliliter of medium; ▲, micromoles of D-arabinose per milliliter of medium; ■, cell mass, milligrams (dry weight) per milliliter.*

Characterization of L-fucose-deficient mutants. Several mutants were selected for inability to grow on L-fucose from strains originally capable of growth on both L-fucose and D-arabinose. Every isolate which lost the ability to grow on L-fucose also lost the ability to grow on D-arabinose (Table 5). Strain 1401 was derived from strain 1102. Strains 1404, 1405, and 1407 were derived from strain 1602, an L-ribulokinase-deficient derivative of strain 1102.

Strain 1401 lost all three enzymes of the L-fucose pathway: isomerase, kinase, and aldolase. Three other separate isolates were found to have the same characteristics. Three possibilities were suggested by these mutants. (i) Permease activity was lost, thus resulting in the inability of inducer to enter the cell; (ii) a mutation in any one of the three structural genes resulted in an extremely polar effect; or (iii) the mutation occurred in the isomerase structural gene, the activity of which was required for induction of an entire regulon (15) composed of all three structural genes.

Mutant 1404, which retained both isomerase and kinase activities but lost aldolase activity, and mutant 1407, which retained both isomerase and aldolase activities, but had markedly reduced kinase activity, provided further evidence that the kinase and aldolase of the L-fucose pathway are required for growth of *E. coli* on D-arabinose.

Nine other mutants selected for inability to

TABLE 4. *Production of glycolate during growth of Escherichia coli K-12 on D-arabinose*

Strain	Inoculum medium[a]	Growth medium[a]	Glycolate (μmoles)[b]
1000	L-Fucose	D-Arabinose	2.5
	L-Fucose	L-Arabinose	0.0
	D-Glucose	L-Arabinose	0.0
1102	L-Fucose	D-Arabinose	6.2
	L-Fucose	L-Arabinose	0.0
	D-Glucose	D-Arabinose	4.0
	D-Glucose	L-Arabinose	0.0

[a] All cells were removed from inoculum media during exponential growth, washed with sterile minimal salts, and then transferred to growth medium.

[b] Concentrations of glycolate are given in micromoles per milliliter of medium. Numbers refer to maximum amount of glycolate detected during the growth cycle.

TABLE 5. *Characterization of L-fucose-negative mutants of Escherichia coli K-12*

Strain	Isomerase activity[a]		Kinase activity[a]		Aldolase activity[a]		Growth[b]	
	L-Fucose	D-Arabinose	L-Fuculose	D-Ribulose	L-Fuculose-1-P	D-Ribulose-1-P	L-Fucose	D-Arabinose
1102	252	110	30	22	206	37	+	+
1602	380	180	30	36	80	14	+	+
1401	0	0	0	0	4	0	−	−
1404	840	260	39	31	0	0	−	−
1405	750	150	33	24	20	0	−	−
1407	250	110	7	4	50	10	−	−

[a] All activities were obtained from cell-free extracts prepared from cultures grown on 1.0% casein hydrolysate in the presence of 0.3% D-arabinose. All activities are in nanomoles per minute per milligram of protein.

[b] Growth was recorded as negative if there was no increase in turbidity within a period of 48 hours.

grow on L-fucose, but not subjected to enzymatic analysis, also lost the ability to grow on D-arabinose.

DISCUSSION

The data presented in this paper indicate that D-arabinose is metabolized by *E. coli* K-12 by the action of at least three enzymes common to the metabolism of L-fucose by this organism, isomerase, kinase, and aldolase. The products of the aldolase formed during growth on D-arabinose, dihydroxyacetone phosphate and glycolaldehyde, are most likely further degraded by well-known pathways. The dihydroxyacetone phosphate can be further metabolized via enzymes of the glycolytic pathway. Although no enzyme responsible for the oxidation of glycolaldehyde to glycolate was demonstrated in *E. coli*, that such an oxidation does actually occur is suggested by two types of results. (1) Glycolate is a major product of the anaerobic degradation of D-arabinose by cell-free extracts of *E. coli*; (ii) glycolate accumulates in the medium of a culture of *E. coli* growing on D-arabinose. This accumulation of glycolate is probably due to a delay in the induction of enzymes necessary for its further metabolism, since it disappears from the medium during later stages of the growth cycle. An aldehyde dehydrogenase capable of acting on L-lactaldehyde, constitutively synthesized by *E. coli* (22), might be an excellent candidate for oxidation of glycolaldehyde to glycolate. Pathways for the further metabolism of glycolate were recently summarized (18).

At least two explanations can be proposed for the ability to detect glycolate, but not glycolaldehyde, in the medium during growth on D-arabinose. Glycolaldehyde may be rapidly oxidized to glycolate, and thus never be detected, or if not immediately oxidized, it may be retained within the cell, rather than excreted into the medium.

Mutants of *E. coli*, capable of growth on D-arabinose, are routinely obtained by inoculating 5 ml of a minimal medium supplemented with 0.3% D-arabinose with 10^7 to 10^8 cells. Such cultures reproducibly provide these mutants within 5 to 7 days when incubated aerobically at 37 C. These results suggest that the frequency of mutation to growth on D-arabinose is probably high, and that such reproducibility could be accounted for by the necessity for only a single mutational event.

The mutation which permits growth of *E. coli* on D-arabinose leads to the synthesis of at least three enzymes of the L-fucose pathway in the presence of D-arabinose. One type of mutation which might permit this to occur would be a mutation in a single regulatory gene which controlled the synthesis of all three enzymes, and resulted in recognition of a new substrate as the inducer. In this case, the regulatory gene would retain its affinity for the normal inducer, L-fucose, or an intermediate in its degradation. Since all three of the L-fucose pathway enzymes possess activity on D-arabinose or its relevant intermediate in wild-type *E. coli*, it is not necessary to invoke altered substrate specificities for metabolism of D-arabinose.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-06848 from the National Institute of Allergy and Infectious Diseases. D. J. L. was supported by Public Health Service predoctoral fellowship 5 F01 GM-36, 120-03 from the National Institute of General Medical Sciences.

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