

Growth on D-Arabitol of a Mutant Strain of *Escherichia coli* K12 Using a Novel Dehydrogenase and Enzymes Related to L-1,2-Propanediol and D-Xylose Metabolism

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SUMMARY

Escherichia coli K12 cannot grow on D-arabitol, L-arabitol, ribitol or xylitol (Reiner, 1975). Using a mutant of *E. coli* K12 (strain 3; Sridhara *et al.*, 1969) that can grow on L-1,2-propanediol, a second-stage mutant was isolated which can utilize D-arabitol as sole source of carbon and energy for growth. D-Arabitol is probably transported into the bacteria by the same system as that used for the transport of L-1,2-propanediol. The second-stage mutant constitutively synthesizes a new dehydrogenase, which is not present in the parent strain 3. This enzyme, whose native substrate may be D-galactose, apparently dehydrogenates D-arabitol to D-xylulose, and its structural gene is located at 68.5 ± 1 min on the *E. coli* genetic map. D-Xylulose is subsequently catabolized by the enzymes of the D-xylose metabolic pathway.

INTRODUCTION

Since Horowitz' (1945) original proposal of stepwise evolution of biochemical syntheses, the evolution of enzymes for the utilization of novel substrates has been extensively studied in a number of bacteria (Hegeman & Rosenberg, 1970; Clarke, 1974). Several workers have isolated mutant strains of bacteria able to grow on substrates which are not metabolized by the wild-type organisms: examples include mutants of *Klebsiella* (*Aerobacter*) *aerogenes* able to grow on xylitol and L-arabitol (Lerner, Wu & Lin, 1964; Mortlock, Fossitt & Wood, 1965; Wu, Lin & Tanaka, 1968; Rigby, Burleigh & Hartley, 1974; Burleigh, Rigby & Hartley, 1974) and mutants of *Pseudomonas aeruginosa* with an extended range of utilization of amides (Brown, Brown & Clarke, 1969; Clarke, 1974). Clarke (1974) pointed out that *E. coli* has remained pre-eminent in the genetic analysis of the evolution of such enzymes, although recently a genetic system has been developed in *P. aeruginosa* to analyse the amidase mutants (Betz *et al.*, 1974), and another in *K. aerogenes* to study the catabolic pathways of ribitol (Charnetzky & Mortlock, 1974a) and D-arabitol (Charnetzky & Mortlock, 1974b).

Possibly because of its selected environment, *E. coli* grows on a relatively restricted range of substrates. For example, *E. coli* strain K12 cannot grow on any of the four polyhydric pentitols, D-arabitol, L-arabitol, ribitol and xylitol (Reiner, 1975), which are presumably not present in large quantities in the human intestine. As an additional approach to the investigation of biochemical and genetic changes involved in the evolution of new catabolic pathways, we have tried to isolate mutants of *E. coli* K12 able to grow on D-arabitol. Direct attempts were unsuccessful, but by using a mutant selected for growth on L-1,2-propanediol

Table 1. *Strains of Escherichia coli* K12

Strain	Description
1	Also known as E15 (Bachmann, 1972)
3	Selected from strain 1 for growth on L-1,2-propanediol (Sridhara <i>et al.</i> 1969)
152	Derived in Professor E. C. C. Lin's laboratory. It is Lac ⁻ , Thi ⁻ , Arg ⁻ , Met ⁻ , Ilv ⁻ , Mtl ⁻ , Xyl ⁻ , Mal ⁻ , Str ^R , His ⁻ and Gal ⁻
162	Strain 152 with λ_{vir} -resistance marker transduced from strain 911
163	Recombinant of strains 3 and 152 selected for Str ^R and Prd ⁺ . It is also Lac ⁻ , Arg ⁻ , Met ⁻ , Ilv ⁻ , Mtl ⁻ , Xyl ⁺ , His ⁻ and Gal ⁻
911	Selected from strain 3 for growth on D-arabitol
921	Revertant of strain 911 selected for loss of ability to grow on D-arabitol. It has also lost the dehydrogenase activity
951	Revertant of strain 911 selected for loss of ability to grow on D-arabitol. It has also lost the ability to grow on L-1,2-propanediol
952	Revertant of strain 911 selected for loss of ability to grow on D-arabitol. It has also lost the ability to grow on D-xylose

(Sridhara *et al.*, 1969; Sridhara & Wu, 1969; Cocks, Aguilar & Lin, 1974), we have succeeded in deriving a second-stage mutant which can utilize D-arabitol as sole source of carbon and energy for growth.

METHODS

Bacteria. The strains used are listed in Table 1. Strains 152, 162 and 163 are female, and the others are HfrC. Strains 1, 3 and 152 were kindly provided by Professor E. C. C. Lin, Harvard Medical School, Boston, Massachusetts 02115, U.S.A. In preliminary experiments, treatment of strain 3 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Adelberg, Mendel & Chen, 1965) yielded mutants which could grow on D-arabitol. Ten of these mutants were isolated; and had lost their Hfr characteristics and were also λ_{vir} -resistant. The fortuitous connection of λ_{vir} -resistance and the ability to grow on D-arabitol was used to isolate strain 911. Strain 3 was first infected with λ_{vir} -phage at a multiplicity of infection of about 10. The λ_{vir} -resistant mutants were serially diluted and plated on D-arabitol medium as well as on rich medium. Comparison of the plates showed that one in 10^4 of the λ_{vir} -resistant mutants grew on D-arabitol. Many of these mutants were then isolated on D-arabitol medium, and each was tested for Hfr characteristics. Only about 10 % retained the Hfr property and thus could not be contaminants. Among the good Hfr mutants able to grow on D-arabitol, one was designated as strain 911.

Strain 911 was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Adelberg *et al.*, 1965) and cultured in glucose medium. Isolated colonies were patched on glucose and D-arabitol media. Among about 10^4 patches, seven have lost the ability to grow on D-arabitol. Two of these could not grow on DL-1,2-propanediol, three could not grow on D-xylose, but the remaining two could grow on both substrates. They were subsequently tested for the presence of the new dehydrogenase activity; it was absent from the last two mutants. One representative was chosen from each group and they were designated as strains 951, 952 and 921 respectively.

Bacteriophages. We are grateful to Professor J. Beckwith, Harvard Medical School, Boston, Massachusetts 02115, U.S.A., for providing the λ_{vir} -phage; and Dr L. B. Dumas, Northwestern University, Evanston, Illinois 60201, U.S.A., for the P1kc-phage.

Chemicals. DL-1,2-Propanediol was purchased from Fisher Scientific Company, Fairlawn, New Jersey, U.S.A.; D-arabitol from Pfanstiehl Laboratories, Waukegan, Illinois, U.S.A.; γ -D-galactonolactone, D-xylulose, NAD, NADH, NADP and lactic acid dehydro-

genase with pyruvate kinase from Sigma; D-ribulose from P-L Biochemicals, Milwaukee, Wisconsin, U.S.A.; antibiotic medium no. 3, bacto-agar, casein acid hydrolysate, bacto-peptone and yeast extract from Difco; ammonium sulphate (enzyme grade) from Schwarz/Mann, Orangeburg, New Jersey, U.S.A.; D-[1-¹⁴C]arabitol and D-[6-³H]galactose from New England Nuclear Corp., Boston, Massachusetts, U.S.A.; and D-[1-¹⁴C]galactose and [2-¹⁴C]glycerol from Amersham/Searle Corp., Arlington Heights, Illinois, U.S.A.

Media. Cultures were maintained on nutrient agar slants containing 1.75 % (w/v) antibiotic medium no. 3 and 1.7 % (w/v) bacto-agar. The basal medium contained 0.034 M-NaH₂PO₄, 0.064 M-K₂HPO₄, 0.02 M-(NH₄)₂SO₄, 3×10^{-4} M-MgSO₄, and 1×10^{-6} M each of FeSO₄, ZnCl₂ and CaCl₂. The NaH₂PO₄, K₂HPO₄ and (NH₄)₂SO₄ were dissolved in deionized water at 10 times the required concentration, titrated with 1 M-NaOH to a final pH of 7.0, and sterilized at 121 °C for 20 min. The remaining salts were dissolved at 100 times the required concentration and sterilized separately. After cooling to about 40 °C, the two solutions were mixed with sterilized water. Supplements to basal medium were at the following concentrations: polyols and sugars, 0.2 %; casein acid hydrolysate, 1.0 %; amino acids, 20 µg ml⁻¹; streptomycin sulphate, 200 µg ml⁻¹; bacto-agar, 1.5 % (w/v). The medium for conjugation contained 1.75 % (w/v) antibiotic medium no. 3. The rich medium for bacteriophage infection contained 1.0 % bacto-peptone, 0.5 % yeast extract, 0.5 % NaCl, and 1 mM-MgCl₂ for λ_{vir}-phage or 1 mM-CaCl₂ for P1kc-phage.

Growth condition. Liquid media containing bacteria were swirled at 37 °C in Erlenmeyer flasks at 240 rev./min on a rotary shaker-incubator. For small samples, 25 ml liquid medium was incubated in a 250 ml Erlenmeyer flask; for enzyme extraction, 2 l Erlenmeyer flasks contained 1 l of liquid medium. Growth rates were measured as extinctions at 465 nm of samples in a Coleman 101 spectrophotometer.

Conjugation was carried out by mixing exponentially growing HfrC and female strains in a 1:10 ratio. After incubating at 37 °C without shaking for 1.5 h, bacteria were collected by centrifuging, washed with basal medium, serially diluted and plated on to selection medium (Skaar & Garen, 1956).

Transducing particles were prepared by mixing P1kc-phage with donor strain in soft agar (0.7 % bacto-agar) which was poured over a rich plate. After incubating overnight at 37 °C, the soft agar was scraped off and suspended in rich medium (10 ml) for 30 min. The soft agar and cell debris were removed by centrifuging and the supernatant liquid was used to infect the recipient strain (Lederberg, 1951).

Uptake of D-[1-¹⁴C]arabitol. The commercial D-[1-¹⁴C]arabitol was purified by the method of Wu *et al.* (1968) using strain 1 as the scavenger. This was necessary because the commercial product usually contained 1 to 2 % of impurities, most of which could be utilized by strain 1. The uptake of the purified D-[1-¹⁴C]arabitol was measured by the method of Hayashi, Koch & Lin (1964).

Enzyme assays. The NAD-linked dehydrogenase was assayed by the method of Lin (1961). The reaction mixture contained 0.67 mM-NAD, 50 mM-substrate and 100 mM-phosphate, glycine or carbonate/bicarbonate buffer at pH 9.6. The activity was independent of the nature of the buffer, within experimental errors (± 10 %); only that in glycine buffer is reported. Protein was measured by the biuret reagent (Gornall, Bardawill & David, 1949) and, for low concentrations, by the Lowry method (Lowry *et al.*, 1951). Lysozyme (Sigma, recrystallized three times) was used as the standard. One unit of activity represents 1 µmol NADH formed per minute.

D-Xylose isomerase (EC. 5.3.1.5) was assayed by the method of Anderson & Wood (1962). One unit of activity catalyses the formation of 1 µmol D-xylose/h. D-Xylulose kinase

Table 2. Purification of the new dehydrogenase from mutant strain 911

Preparation	Volume (ml)	Total protein (mg)	Specific activity* (units/mg protein)		Ratio of activities	Recovery (%)
			D-arabitol	D-galactose		
Extract	50	315	0.040	0.032	1.25	100
Streptomycin sulphate precipitation	50	215	0.056	0.040	1.40	95
40 to 50 % ammonium sulphate fractionation	12	104	0.072	0.052	1.38	59
DEAE-cellulose column chromatography (concentrated by 80 % ammonium sulphate)	2	4	1.64	1.23	1.33	52

* Enzyme activity on D-arabitol was measured with 500 mM D-arabitol and that on D-galactose with 50 mM D-galactose.

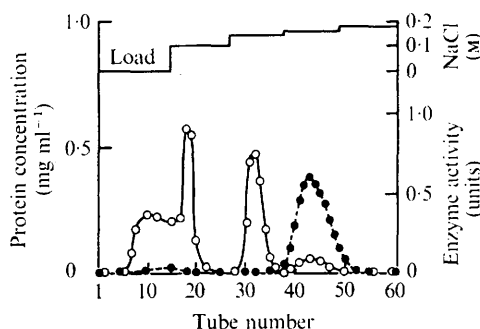


Fig. 1. Elution pattern of DEAE-cellulose column chromatography. The column size was 1.0 cm \times 10.0 cm. Protein concentration (○) and dehydrogenase activity (●) were measured in each tube of eluate (4 ml). NaCl concentrations are given at the top of the figure.

(EC. 2.7.1.17) activity was measured by the method of Wilson & Mortlock (1973). One unit of activity represents the oxidation of 1 μ mol of NADH/min.

Enzyme purification. All purification steps were carried out at 0 to 5 °C. Bacteria were grown at 37 °C in 1.0 % casein acid hydrolysate medium until early stationary phase. They were centrifuged at 7000 rev./min (8000g) in a Sorvall refrigerated centrifuge set at -4 °C (liquid medium temperature was about 2 °C in the type GSA rotor), and washed with basal medium. The bacteria were then suspended in standard buffer (0.01 M-sodium phosphate buffer pH 7.0, with 0.67 mM-NAD and 10 μ M each of MgSO₄, FeSO₄ and CaCl₂) and broken by sonication in a model W140 Sonifier Cell Disruptor (Heat System-Ultrasonics Inc., Plainview, New York, U.S.A.) used at full power for 3 min. The bacterial suspension was chilled to about 4 °C in a bath kept at -20 °C. Cell debris was removed by centrifuging in a Beckman no. 30 rotor at 29000 rev./min (99000g) for 1 h. The supernatant liquid (extract) was used as the source of enzyme for purification (Table 2).

Streptomycin sulphate was dissolved in the standard buffer to a final concentration of 20 % (w/v). This solution was added dropwise to the extract with constant stirring to attain a concentration of 4 % (w/v). After stirring for an additional 30 min, the precipitate was removed by centrifuging in a Sorvall type SS-34 rotor at 15000 rev./min (27000g) for 20 min.

A saturated solution of ammonium sulphate (with solid ammonium sulphate present)

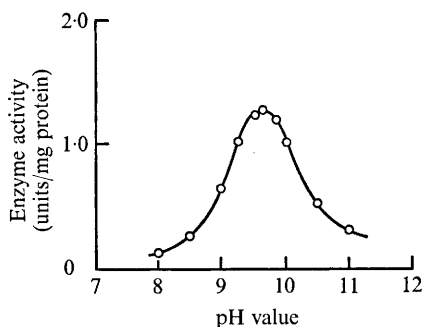


Fig. 2

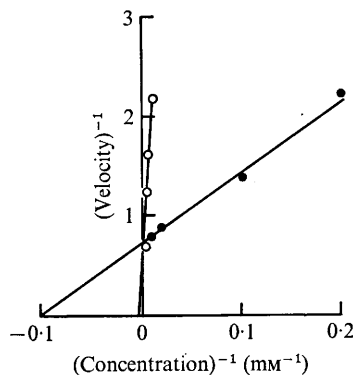


Fig. 3

Fig. 2. Effect of pH on the activity of the partially purified dehydrogenase, as measured in glycine buffer.

Fig. 3. Lineweaver-Burk plot of the partially-purified dehydrogenase activity. Activity (units/mg protein) was measured with D-arabitol (○) and D-galactose (●).

in standard buffer was prepared at 4 °C, and the pH value was adjusted to 7.0 by adding 1 M-NaOH. This solution was then used for ammonium sulphate fractionation as indicated in Table 2.

On adding the enzyme extract in standard buffer to a DEAE-cellulose column, and eluting with a linear gradient from 0.0 M to 1.0 M of NaCl solution in standard buffer, the enzyme activity coincided with a major protein peak. By trial and error, the best purification of the enzyme on DEAE-cellulose was obtained by addition of the ammonium sulphate fraction in standard buffer to the column, followed by steps of 0.10 M, 0.14 M, 0.16 M and 0.18 M NaCl solution in standard buffer. The enzyme activity peak then corresponded to a small protein peak (Fig. 1). The eluate with enzyme activity was concentrated by precipitating the protein with 80 % saturated ammonium sulphate and redissolved in the standard buffer.

Sephadex G-200 gel filtration was used to test the purity of the enzyme.

Thin-layer chromatography. In an attempt to identify the product of dehydrogenation of D-galactose by the partially purified enzyme, D-[6-³H]galactose was used in the enzyme-assay mixture. After various periods, the reaction was stopped by adding trichloroacetic acid. The supernatant solution was applied to an Eastman Kodak 6064 film for ascending thin-layer chromatography. D-[1-¹⁴C]Galactose and [2-¹⁴C]glycerol were used as standards.

RESULTS

Growth on D-arabitol

The second-stage mutant, strain 911, grew aerobically in 0.2 % D-arabitol as the sole source of carbon and energy, with a doubling time of about 4 h. This doubling time was the same in concentrations of D-arabitol as low as 0.025 %, suggesting that the saturation constant for growth on D-arabitol was less than 2 mM. The growth yields of strain 911 in various concentrations of D-arabitol were similar (i.e. within 10 %) to those obtained with the same concentrations of glucose; the yield was about 10⁹ bacteria/ml in 0.05 % carbon source. Thus, the entire molecule of D-arabitol was utilized.

Table 3. Uptake of D-[1-¹⁴C]arabitol by various strains of *E. coli* K12

Uptake was measured when *E. coli* strains were grown on casein acid hydrolysate in the presence of D-[1-¹⁴C]arabitol alone, and in the presence of this labelled arabitol plus unlabelled D-arabitol (10 mM) or unlabelled DL-1,2-propanediol (10 mM).

Strain	Uptake of D-[1- ¹⁴ C]arabitol [nmol (mg dry wt) ⁻¹ min ⁻¹] in the presence of:		
	No addition	Unlabelled D-arabitol	Unlabelled DL-1,2-propanediol
1	0.02	0.02	0.02
3	0.55	0.23	0.06
911	0.50	0.21	0.05

New enzyme activity

The crude extract of strain 911 contained a constitutive NAD-linked D-arabitol dehydrogenase which was not inducible in its parent strain 3 by either D-arabitol or D-galactose (see also Table 4). The activity was purified 41-fold (Table 2 and Fig. 1). Initial attempts to purify the enzyme in 0.01 M-sodium phosphate buffer resulted in 80 % loss of activity on DEAE-cellulose column chromatography. No re-activation was successful. It was further discovered that on diluting the cell-free extract with sodium phosphate buffer, a rapid loss of activity occurred. However, these losses of activity could be completely prevented in the presence of 0.67 mM-NAD and 10 μ M each of MgSO₄, FeSO₄ and CaCl₂. Further attempts at purification were unsuccessful because of instability of the enzyme in the absence of NAD. For example, polyacrylamide gel electrophoresis was tried, but most of the enzyme precipitated at the top of the gel. On Sephadex G-200 gel filtration, the partially-purified enzyme was eluted beyond the excluded volume, and the activity peak coincided with the protein peak. The specific activity was not changed.

The pH optimum was 9.6 (Fig. 2). The K_m for D-arabitol was 480 mM (Fig. 3), suggesting: that a transport system for D-arabitol was present in strain 911 since the saturation constant for growth on D-arabitol was about 250 times smaller; and that D-arabitol was not the native substrate since the K_m was unusually high. The product of D-arabitol dehydrogenation was suspected to be D-xylulose since it gave a very slow colour development at room temperature (Anderson & Wood, 1962) with the carbazole colour test (Dische & Borenfreund, 1951). The enzyme catalysed the reduction of authentic D-xylulose, but not D-ribulose, by NADH at pH 7.0 in 100 mM-phosphate buffer, and D-arabitol was assumed to be the product. The K_m for D-xylulose was about 50 mM.

Transport of D-arabitol

The uptake of D-[1-¹⁴C]arabitol was measured in strains 1, 3 and 911 (Table 3). Strain 1 could not accumulate this radioactive compound. The accumulation of radioactivity in strains 3 and 911 was inhibited by non-radioactive D-arabitol and, most effectively, by unlabelled DL-1,2-propanediol (Table 3), suggesting that the transport system for L-1,2-propanediol in strain 3 could fortuitously act on D-arabitol with a lower efficiency.

Native substrate for the new enzyme

The new dehydrogenase in strain 911 was also active on D-galactose with a K_m of about 10 mM (Fig. 3), i.e. about 50 times lower than that on D-arabitol. Various related polyols,

Table 4. Enzyme activities in various mutant strains of *E. coli* K12

Strain	Growth medium	Enzyme activity (units/mg protein)		
		D-arabitol dehydrogenase	D-xylose isomerase	D-xylulose kinase
1	Casein	0.00	0.00	0.00
1	D-xylose	0.00	0.11	0.36
3	Casein	0.00	0.00	0.00
3	D-xylose	0.00	0.10	0.32
3	Casein + D-galactose*	0.00	0.00	0.00
3	Casein + D-arabitol*	0.00	0.00	0.00
911	Casein	0.04	0.00	0.00
911	D-xylose	0.04	0.12	0.33
911	D-arabitol	0.04	0.05	0.12
921	Casein	0.00	0.00	0.00
921	D-xylose	0.00	0.11	0.36
951	Casein	0.04	0.00	0.00
951	D-xylose	0.04	0.10	0.31
952	Casein	0.04	0.00	0.00
952	Casein + D-xylose	0.04	0.00	0.00

* Other compounds which gave no induction of D-arabitol dehydrogenase were: L-arabitol, ribitol, xylitol, dulcitol, mannitol, sorbitol, glycerol, DL-1,2-propanediol, D-glucose, D-mannose, D-ribose, D- and L-arabinose, D- and L-lyxose, D- and L-xylose, D- and L-fucose, and D-fructose.

sugars and phosphorylated compounds (L-arabitol, ribitol, xylitol, dulcitol, mannitol, sorbitol, glycerol, DL-1,2-propanediol, D-glucose, D-mannose, D-ribose, D- and L-arabinose, D- and L-lyxose, D- and L-xylose, D- and L-fucose, D-fructose, D-galactose 1-phosphate, D-galactose 6-phosphate, mannitol 1-phosphate, D-sorbitol 6-phosphate, D-glucose 1-phosphate, D-glucose 6-phosphate, D-mannose 1-phosphate, D-mannose 6-phosphate, D-fructose 6-phosphate, and D-fructose 1,6-diphosphate) were inactive as substrates. On substituting NAD in the assay mixture with 0.67 mM-NADP, the dehydrogenase activities on D-arabitol and D-galactose were both decreased by about 50 %. It was therefore proposed that D-galactose might be the native substrate for this new enzyme. The product of D-galactose dehydrogenation gave a positive carbazole colour test (Dische & Borenfreund, 1951), indicating that it could not be γ -D-galactonolactone. Furthermore, it was unstable at pH 9.6. One of its degradation products co-chromatographed with authentic glycerol in three different solvent systems: acetone-acetic acid-water (4:1.2:1, by vol.); n-butanol saturated with water; and acetone-chloroform-5 M-NH₄OH (8:1:1, by vol.). With the finding that D-arabitol was converted to D-xylulose by the same enzyme, these results suggest that D-galactose was probably dehydrogenated at carbon-3 and the product was subsequently hydrolysed at pH 9.6 (Fig. 4).

Further catabolism

Since D-xylulose is a normal intermediate in the catabolism of D-xylose by *E. coli* K12 (David & Wiesmeyer, 1970), the activities of D-xylose isomerase and D-xylulose kinase were measured in the crude extract of strain 911 grown in 0.2 % D-arabitol. They were about 40 % of the fully induced levels in strains 1, 3 and 911 grown in 0.2 % D-xylose. These activities were absent from extracts of strains 1, 3 and 911 grown in 1.0 % casein acid hydrolysate (Table 4).

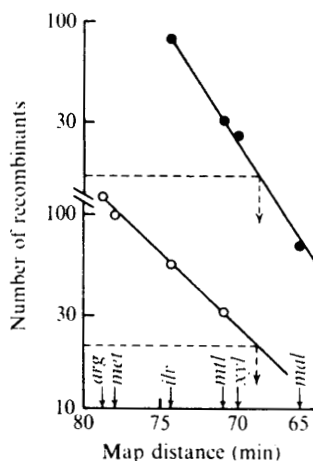


Fig. 5. Map location of the dehydrogenase gene (○) and the gene responsible for λ_{vir} -resistance (●) as determined by scoring unselected markers. For the lower curve (○—○), strain 163 was mated with strain 911, and Arg^+ and Str^R recombinants were selected. Among 121 such recombinants, 100 were Met^+ , 55 Ilv^+ , 32 Mtl^+ , and 21 were able to grow on D-arabitol. For the top curve (●—●), strain 162 was mated with strain 3, and Ilv^+ and Str^R recombinants were selected. Among 80 such recombinants, 31 were Mtl^+ , 26 Xyl^+ , 7 Mal^+ , and 16 were resistant to λ_{vir} -phage.

Abolishing the growth ability on D-arabitol

Various revertants of strain 911 were selected for their loss of ability to grow on D-arabitol. Three types were found (see also Table 4): (i) strain 921 which had lost the new dehydrogenase activity; (ii) strain 951 which could not grow on L-1,2-propanediol; and (iii) strain 952 which could not grow on D-xylose. Both strains 951 and 952 contained the new dehydrogenase. Strain 951 could grow on D-xylose, and its inability to utilize L-1,2-propanediol was assumed to indicate that the propanediol catabolic enzymes, including the transport system, were absent. Strain 952 could grow on L-1,2-propanediol, and its D-xylose catabolic enzymes were not inducible (Table 4).

Map location of the dehydrogenase gene

Since the genes responsible for growth on L-1,2-propanediol and D-xylose were required for the utilization of D-arabitol, strain 163 was mated with strain 911. Arg^+ and Str^R recombinants were selected. By scoring the unselected markers, we located the new dehydrogenase gene at 68.5 ± 1 min on the *E. coli* genetic map (Fig. 5).

Strain 162 was constructed by transducing the λ_{vir} -resistant gene of strain 911 into strain 152. All 17 transductants with this property had acquired the new dehydrogenase activity, suggesting that the gene responsible for λ_{vir} -resistance might be closely linked to the new dehydrogenase gene. This was supported by mating strain 162 with strain 3 and selecting Ilv^+ and Str^R recombinants. Again by scoring unselected markers, we located the λ_{vir} -resistant gene at the same region of the chromosome (Fig. 5). More refined localization was not possible due to the absence of other markers in this vicinity (Taylor & Trotter, 1972.).

DISCUSSION

Our study provides another example of constructing a new catabolic pathway by derepression of existing enzymes which are fortuitously active on the novel substrate and its metabolic intermediates (Hegeman & Rosenberg, 1970; Clarke, 1974). It also illustrates that the mechanism of derepression can be wasteful. Only the transport system of L-1,2-propanediol is useful in the catabolism of D-arabitol, while the propanediol dehydrogenase and subsequent enzymes of L-1,2-propanediol metabolism (Sridhara *et al.*, 1969; Sridhara & Wu, 1969; Cocks *et al.*, 1974) are not involved. An important question is whether we can now construct a mutant able to grow on D-arabitol without the gratuitous synthesis of propanediol dehydrogenase and D-xylose isomerase.

A novel feature of the present system is the NAD-linked dehydrogenase whose native substrate appears as D-galactose. However, D-galactose is normally phosphorylated to D-galactose 1-phosphate by a kinase in *E. coli* K12 with a much higher efficiency (Kalckar, Kurahashi & Jordan, 1959; Yamolinsky *et al.*, 1959), though it is dehydrogenated in *Pseudomonas saccharophila* (Doudoroff, 1962). Furthermore, the D-galactose dehydrogenase is not inducible in strain 3 by D-galactose, D-arabitol or other related compounds (Table 4). It is possible that this dehydrogenase may be an evolutionary remnant in *E. coli*. While there are many segments on the *E. coli* genetic map in which no functional gene has been located (Taylor & Trotter, 1972), such segments may direct the synthesis of ancient enzymes which are rendered useless by the emergence of more efficient enzymes. The only way to resolve this is to try to identify more such enzymes with similar properties.

Reiner (1975) has recently discovered that *E. coli* strain C can utilize D-arabitol, as can *K. aerogenes* (Charnetzky & Mortlock, 1974b). Furthermore, though *E. coli* K12 does not have the genes required for D-arabitol catabolism, they can be transduced in from *E. coli* C. Thus, two different approaches are now available for the construction of new metabolic pathways in *E. coli* K12: one involves the use of existing genetic capability; and the other brings in a new set of genes. The advantages and disadvantages of these two methods probably deserve careful study in other micro-organisms.

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