# Growth on D-Lyxose of a Mutant Strain of *Escherichia coli* K12 Using a Novel Isomerase and Enzymes Related to D-Xylose Metabolism

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## SUMMARY

*Escherichia coli* K12 cannot grow on D-lyxose, but a mutant was isolated which can utilize D-lyxose as sole source of carbon and energy for growth. D-Lyxose is transported into the bacteria by the D-xylose permease. The mutant constitutively synthesizes a new isomerase which is not inducible in the parent strain under any of the conditions tested. This enzyme, whose native substrate appears to be D-mannose, fortuitously converts D-lyxose into D-xylulose. Its structural gene is located at around 85 min on the *E. coli* genetic map, away from other known isomerase genes. D-Xylulose is subsequently catabolized by the enzymes of the normal D-xylose metabolic pathway. D-Mannose isomerase was partially purified and some of its properties were examined.

## INTRODUCTION

The evolution of pathways for the utilization of novel substrates has been extensively studied in a number of bacterial systems (Hegeman & Rosenberg, 1970; Clarke, 1974; Leisinger, 1975). In many instances, the basic mechanism involves the derepression of an inducible enzyme, normally active on another substrate, which can convert the novel substrate to a metabolizable compound. However, the derepressed enzyme may sometimes be nonfunctional under normal conditions. For example, in a mutant of *Escherichia coli* K12 selected for growth on D-arabitol (Wu, 1976), a D-galactose dehydrogenase is constitutively synthesized and can fortuitously convert D-arabitol to D-xylulose. But D-galactose is normally phosphorylated to D-galactose 1-phosphate in *E. coli* K12 (Kalckar, Kurahashi & Jordan, 1959; Yamolinsky *et al.*, 1959), suggesting that the D-galactose dehydrogenase may be an evolutionary remnant which became silent after the emergence of a more efficient pathway involving D-galactose kinase.

In this paper, a similar situation is described for a mutant of *E. coli* K12 isolated on the basis of ability to grow (unlike strain K12 itself) on D-lyxose. A novel isomerase, whose native substrate appears to be D-mannose, is derepressed and can fortuitously convert D-lyxose to D-xylulose.

## METHODS

*Bacteria.* Of the strains of *E. coli* K12 used in the present study (Table 1), strains 1, 3, 805, 812 and 851 are HfrC and the others are female. Strains 1, 3, 140 and 152 were kindly

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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 *et al.*, 1969) |
| 140 | Also known as JC411 (Bachmann, 1972). It is Lac⁻, Leu⁻, Met⁻, Mtl⁻, Xyl⁻, Mal⁻, Str$^R$, Arg⁻, His⁻ and Gal⁻ |
| 148 | Recombinant of strains 1 and 140 selected for Xyl⁺. It is Lac⁻, Leu⁻, Met⁻, Mtl⁻, Mal⁻, Str$^R$, Arg⁻, His⁻ and Gal⁻ |
| 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⁻ |
| 161 | Spontaneous mutant of strain 152 selected for Xyl⁺. It is Lac⁻, Thi⁻, Arg⁻, Met⁻, Ilv⁻, Mtl⁻, Mal⁻, Str$^R$, His⁻ and Gal⁻ |
| 805 | Selected from strain 3 for growth on D-lyxose |
| 812 | Revertant of strain 805 selected for loss of ability to grow on D-lyxose. It has also lost the ability to grow on D-xylose |
| 851 | Revertant of strain 805 selected for loss of ability to grow on D-lyxose. It has also lost the isomerase activity |

provided by Professor E. C. C. Lin, Harvard Medical School, Boston, Massachusetts 02115, U.S.A. Strain 805 is a mutant of strain 3 selected for growth on D-lyxose after treatment with N-methyl-N'-nitro-N-nitrosoguanidine (Adelberg, Mandel & Chen, 1965). This sort of mutant arose with a frequency of about one in $10^7$. Strain 3 can grow on L-1,2-propanediol (Sridhara *et al.*, 1969) and this property was used to eliminate possible contamination. Strains 148 and 161 were constructed from strains 140 and 152 respectively for Xyl⁺ characteristics; all the other markers were retained. Revertants of strain 805 which had lost the ability to grow on D-lyxose were isolated by plating strain 805 on D-lyxose medium with a drop of ethyl methanesulphonate at the centre of the plate. After incubating for 2 days, the plate contained a lawn of growing bacteria around a clear zone surrounding the mutagen. Cells from the clear zone were transferred to glucose medium, and the resulting colonies were tested for loss of the ability to utilize D-lyxose. Among about 100 colonies, five were unable to grow on D-lyxose; two of these had also lost the ability to grow on D-xylose. One of the two was designated strain 812. The remaining three had also lost D-mannose isomerase activity; one was designated strain 851.

*Bacteriophages.* Dr L. B. Dumas, Northwestern University, Evanston, Illinois 60201, U.S.A., provided the P1kc-phage.

*Chemicals.* D-Lyxose, D-xylulose, D-xylose, D-mannose, D-fructose, ATP, phosphoenolpyruvate, NADH, β-mercaptoethanol, lysozyme (crystallized three times), glucose-6-phosphate dehydrogenase and lactic acid dehydrogenase with pyruvate kinase were purchased from Sigma; DEAE-cellulose from Biorad; antibiotic medium no. 3, Bacto-agar, casein acid hydrolysate, Bacto-peptone and yeast extract from Difco; Sephadex G-200 and Blue-dextran 2000 from Pharmacia; ammonium sulphate (enzyme grade) from Schwarz/Mann, Orangeburg, New Jersey, U.S.A.; reduced glutathione and EDTA from Fischer Scientific Co., Fairlawn, New Jersey, U.S.A.; D-[U-$^{14}$C]xylose from The Radiochemical Centre, Amersham; and D-[$^3$H]lyxose (randomly labelled) was custom-synthesized by New England Nuclear.

*Media, growth conditions and uptake of radioactively labelled compounds.* These were as previously described (Wu, 1976).

*Enzyme assays.* D-Mannose (or D-lyxose) isomerase was assayed by the method of Anderson (1966). The reaction mixture contained 100 mM D-mannose (or D-lyxose), 0·5 mM-MnSO$_4$, 10 mM-Na$_2$HPO$_4$/NaH$_2$PO$_4$, and 100 mM-Na$_2$SO$_4$ at pH 7·0. The keto-sugar

Table 2. *Purification of the new isomerase from mutant strain* 805

| Preparation | Volume (ml) | Total protein (mg) | Specific activity [u. (mg protein)$^{-1}$] | | Ratio of activities | Recovery (%) |
| --- | --- | --- | --- | --- | --- | --- |
| | | | D-lyxose | D-mannose | | |
| Extract | 50 | 1335 | 0·88 | 3·67 | 0·24 | 100 |
| Streptomycin sulphate precipitation | 61 | 1335* | 0·85 | 3·52 | 0·24 | 96 |
| pH 5·8 treatment | 59 | 1150* | 1·09 | 4·21 | 0·26 | 76 |
| 0 to 35 % ammonium sulphate fractionation at pH 5·2 | 15·5 | 193 | 2·53 | 10·1 | 0·25 | 41 |
| DEAE-cellulose column chromatography | 14·4 | 18·6 | 12·4 | 44·3 | 0·28 | 17 |

* These protein concentration determinations may be in error because of the presence of streptomycin sulphate.
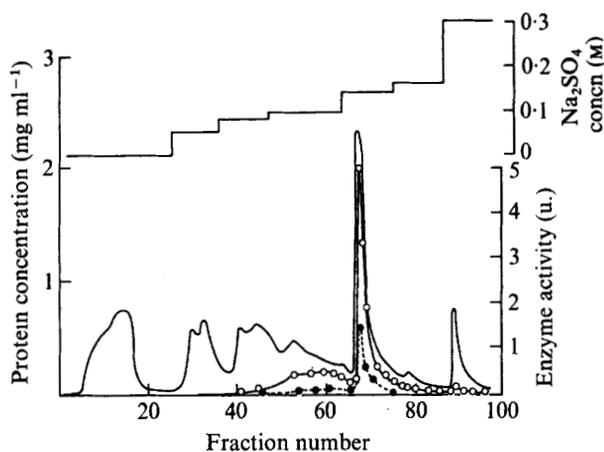


Fig. 1. Elution pattern of DEAE-cellulose column chromatography. The column size was 1·0 × 10·0 cm. Protein concentration (——), D-mannose isomerase activity (○) and D-lyxose isomerase activity (●) were measured in each tube of eluate (4 ml). The concentration of Na₂SO₄ is given at the top of the figure.

formed was measured by the carbazole colour test (Dische & Borenfreund, 1951). Authentic D-fructose (or D-xylulose) was used for calibration. One unit of activity (u.) represents the formation of 1 $\mu$mol D-fructose (or D-xylulose) min$^{-1}$. Protein was measured by the biuret reagent (Gornall, Bardawill & David, 1949), or by the Lowry method (Lowry *et al.*, 1951) for low concentrations, using lysozyme as the standard.

D-Xylose isomerase (EC. 5.3.1.5) was assayed by the method of Anderson & Wood (1962). One unit of activity (u.) catalyses the formation of 1 $\mu$mol D-xylulose h$^{-1}$. D-Xylulose kinase (EC. 2.7.1.17) activity was measured by the method of Wilson & Mortlock (1973). One unit of activity (u.) represents the oxidation of 1 $\mu$mol NADH min$^{-1}$.

*Enzyme purification.* All purification steps were carried out at 0 to 5 °C. Growth of bacteria, the method of obtaining cell 'extract', and streptomycin sulphate precipitation (Table 2) were as previously described (Wu, 1976). The standard buffer was 10 mM-Na₂HPO₄/NaH₂PO₄, pH 7·0, with 0·1 mM-MnSO₄.

The pH value of the fraction after streptomycin sulphate precipitation was lowered to 5·8
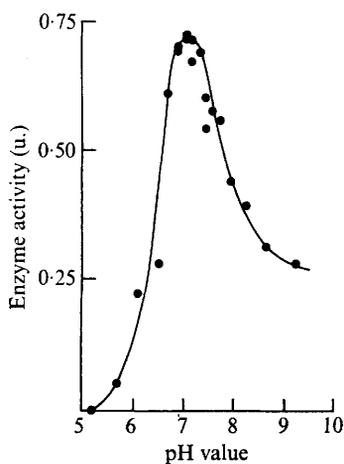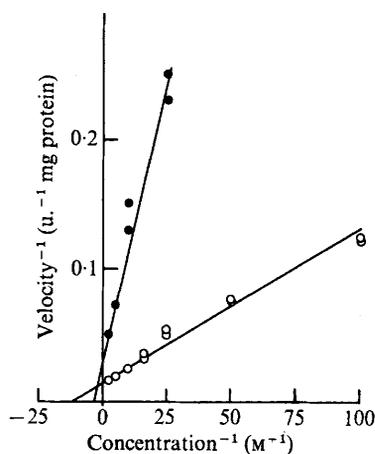
Fig. 2. Effect of pH value on the activity of the partially purified D-mannose isomerase measured in 0·01 M-sodium phosphate buffer. Various amounts of NaH$_2$PO$_4$ and Na$_2$HPO$_4$ were used to adjust the pH values.

Fig. 3. Lineweaver–Burk plot for the partially purified isomerase activity. Activity was measured with D-mannose (O) or D-lyxose (●) as substrate.

by adding 1·0 M-sodium acetate buffer, pH 5·0, dropwise with constant stirring. After storing at 4 °C for 15 h, the precipitated protein was removed by centrifuging at 27000 g for 20 min. Solid ammonium sulphate was slowly added to the supernate to 35 % saturation (209 mg ml⁻¹) and the precipitated protein was removed. The pH was lowered further to 5·2. After stirring for another 30 min, the precipitate was collected by centrifuging and dissolved in standard buffer. This enzyme solution was then applied to a DEAE-cellulose column (Table 2). The best purification using the DEAE-cellulose column was obtained by eluting with standard buffer alone then Na$_2$SO$_4$ in standard buffer in steps of 0·05, 0·08, 0·095, 0·14, 0·16 and 0·3 M (Fig. 1). The enzyme activity peak was eluted at 0·14 M and corresponded to a protein peak of very similar shape.

Sephadex G-200 gel filtration was used to test the purity of the partially purified enzyme. Since the enzyme had a tendency to stick to the G-200 gel, standard buffer with 0·1 M-Na$_2$SO$_4$ was used for elution. The molecular weight of the enzyme was estimated by the method of Andrews (1965) using Blue-dextran 2000 and glucose-6-phosphate dehydrogenase mol. wt 104000) as standards.

## RESULTS

### Growth on D-lyxose

Strain 805 grew aerobically on 0·2 % D-lyxose as the sole source of carbon and energy, with a doubling time of 3·1 h; the parental strain 3 could not grow on D-lyxose. The doubling times were 3·6, 4·3 and 7 h at 0·1, 0·05 and 0·02 % D-lyxose respectively, suggesting that the saturation constant for growth on D-lyxose was about 2 mM. The growth yields of strain 805 in various concentrations of D-lyxose were similar (within 10 %) to those obtained with the same concentrations of glucose; the yield was about 10⁹ bacteria ml⁻¹ in 0·05 % of carbon source. The entire molecule of D-lyxose must be utilized.

Table 3. *Uptake of* D-[³H]*lyxose and* D-[U-¹⁴C]*xylose by washed suspensions of E. coli* K12 *strains* 3 *and* 805 *after growth in various media*

| | | Uptake of D-[³H]lyxose [nmol (mg dry wt)$^{-1}$ min$^{-1}$] in the presence of: | | | |
| --- | --- | --- | --- | --- | --- |
| Strain | Growth medium | No further addition | Unlabelled D-lyxose (10 mM) | Unlabelled D-xylose (10 mM) | Uptake of D-[U-¹⁴C]xylose [nmol (mg dry wt)$^{-1}$ min$^{-1}$] |
| 3 | Casein | 0·00 | 0·00 | 0·00 | 0·01 |
| 3 | Casein + D-lyxose | 0·00 | 0·00 | 0·00 | 0·00 |
| 3 | Casein + D-xylose | 0·67 | 0·22 | 0·01 | 7·10 |
| 805 | Casein | 0·00 | 0·00 | 0·00 | 0·00 |
| 805 | Casein + D-lyxose | 0·33 | 0·10 | 0·00 | 3·53 |
| 805 | Casein + D-xylose | 0·70 | 0·25 | 0·02 | 7·21 |

## New enzyme activity

Compared with its parent strain 3, the extract of strain 805 contained a new constitutive D-lyxose isomerase activity which was not inducible in strain 3 by D-lyxose, D-mannose or D-fructose (see Table 4). This activity was purified 14-fold (Table 2 and Fig. 1). Sephadex G-200 gel filtration showed a major protein peak which coincided with a peak of activity and a very small trailing peak which was also active. The molecular weight of the partially purified isomerase was estimated to be about 160000. An identical purification procedure carried out with strain 3 resulted in a very small protein peak on the DEAE-cellulose column at the position where the activity peak of strain 805 occurred.

The pH optimum of the partially purified isomerase was between 7·0 and 7·2 (Fig. 2).

The product of D-lyxose isomerization was suspected to be D-xylulose, since the reverse reaction could be demonstrated with D-xylulose. The equilibrium constant between D-lyxose and D-xylulose was approximately [D-xylulose]/[D-lyxose] = 0·3/0·7 = 0·43.

The $K_m$ for D-lyxose was around 300 mM (Fig. 3), suggesting (i) that a transport system for D-lyxose was present in strain 805 since the saturation constant for growth on D-lyxose was 150 times smaller, and (ii) that D-lyxose was not the native substrate since the $K_m$ was unusually high. The $V_{max}$ on D-lyxose was about 30 u. (mg protein)$^{-1}$.

The D-mannose isomerase was stable in the presence of 1 mM-$\beta$-mercaptoethanol or EDTA, but was almost completely inhibited by 5 mM reduced glutathione. About 50 % of the isomerase activity was inhibited in 0·2 M-KCl or NaCl, or in 0·05 M-sodium phosphate buffer, pH 7·0. $Cu^{2+}$ or $Hg^{2+}$ at 0·1 mM completely inhibited the enzyme activity; $Cd^{2+}$ or $Zn^{2+}$ at the same concentration gave about 50 % inhibition; and other metal ions ($Ba^{2+}$, $Ca^{2+}$, $Cr^{3+}$, $Cs^+$, $Fe^{2+}$, $Fe^{3+}$, $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$, $Pb^{2+}$, $Sn^{2+}$, and $Sr^{2+}$) had no effect.

## Transport of D-lyxose

Uptake of D-[³H]lyxose was measured in strains 3 and 805 grown in various media (Table 3). The accumulation of radioactivity was inhibited by non-radioactive D-lyxose and, more effectively, by unlabelled D-xylose. The uptake of D-[³H]lyxose paralleled that of D-[U-¹⁴C] xylose, except that it was less rapid. These results suggested that the D-xylose permease (David & Wiesmeyer, 1970) could be induced in strain 805 by growth on D-lyxose, and that the D-xylose permease could fortuitously transport D-lyxose into the bacteria.

Table 4. *Enzyme activities in extracts of E. coli* K12 *strains* 3 *and* 805 *after growth in various media*

| | | Enzyme activity [u. (mg protein)$^{-1}$] | | |
|---|---|---|---|---|
| Strain | Growth medium | D-Lyxose isomerase | D-Xylose isomerase | D-Xylulose kinase |
| 3 | Casein | 0·00 | 0·00 | 0·00 |
| 3 | D-Xylose | 0·00 | 0·10 | 0·32 |
| 3 | Casein + D-lyxose | 0·00 | 0·00 | 0·00 |
| 3 | D-Mannose | 0·00 | 0·00 | 0·00 |
| 3 | D-Fructose | 0·00 | 0·00 | 0·00 |
| 805 | Casein | 0·88 | 0·00 | 0·00 |
| 805 | D-Xylose | 0·81 | 0·11 | 0·35 |
| 805 | D-Lyxose | 0·90 | 0·06 | 0·16 |

### Native substrate for the new isomerase

The new isomerase in strain 805 was also active on D-mannose with a $K_m$ of about 80 mM (Fig. 3). The product of D-mannose isomerization was presumably D-fructose, since the reverse reaction could be measured with D-fructose as the substrate. The equilibrium constant in this case was [D-fructose]/[D-mannose] = 0·7/0·3 = 2·33. These results suggested that D-mannose might be the native substrate of this isomerase. Various other related sugars and phosphorylated compounds (D- and L-arabinose, D- and L-fucose, D-galactose, D-galacturonate, D- and L-glucose, D-glucuronate, L-lyxose, *N*-acetylmannosamine, L-mannose, L-rhamnose, D-ribose, D-talose, D- and L-xylose, D-glucose 6-phosphate, D-mannose 1-phosphate and D-mannose 6-phosphate) were inactive as substrates.

### 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 extracts of strains 3 and 805 grown in various media (Table 4). For strain 805 grown in 0·2 % D-lyxose, the enzymes were present at about 50 % of the fully induced activities of strains 3 and 805 grown in 0·2 % D-xylose. These activities were absent from extracts of strains 3 and 805 grown in 1·0 % casein acid hydrolysate.

### Abolishing the growth ability on D-lyxose

Two classes of revertants of strain 805 selected for their loss of ability to grow on D-lyxose were obtained and they were typified by: (i) strain 812 which was also unable to grow on D-xylose, and (ii) strain 851 which had lost the new isomerase activity. Thus, the pathway of D-lyxose catabolism (Fig. 4) probably involves the D-xylose permease which by chance transports D-lyxose into the bacteria, the D-mannose isomerase which fortuitously converts D-lyxose to D-xylulose and the D-xylulose kinase and subsequent enzymes for the normal D-xylose metabolic pathway (David & Wiesmeyer, 1970).

### Map location of the isomerase gene

Since the genes responsible for growth on D-xylose were required for the utilization of D-lyxose, strain 148 was constructed by mating strains 1 and 140 and selecting for Xyl$^+$ recombinants which retained all other markers (Table 1). Strain 161 was a spontaneous revertant of strain 152 on D-xylose (Table 1). Strain 805 was then mated with strain 148. In
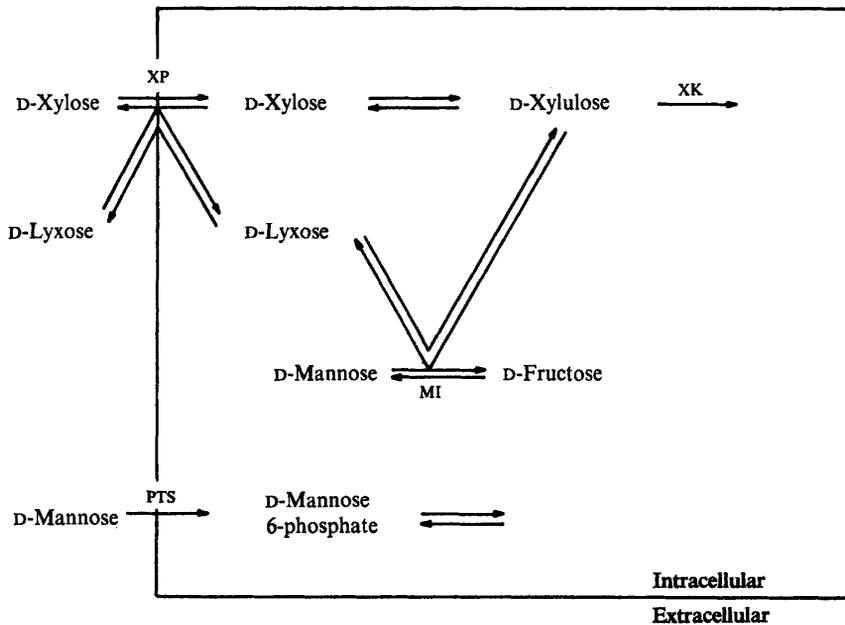
Fig. 4. Proposed metabolic pathway for the utilization of D-lyxose. D-Lyxose is transported into the cell by D-xylose permease (XP), converted to D-xylulose by the new D-mannose isomerase (MI), and then catabolized by D-xylulose kinase (XK) and subsequent enzymes of the normal D-xylose metabolic pathway. Under normal circumstances, D-mannose is transported into the cell by the phosphotransferase system (PTS) as D-mannose 6-phosphate, and very little, if any, free D-mannose is present inside the cell.

one experiment, 160 Leu$^+$ and Str$^R$ recombinants were isolated. Among them, two were Met$^+$ and one could grow on D-lyxose, suggesting that the new isomerase gene was probably between the *met* and *str* markers. In a second experiment, 175 Met$^+$ and Str$^R$ recombinants were isolated; 82 were also able to utilize D-lyxose, 14 were Mtl$^+$ and 5 Mal$^+$. In a third experiment, strain 805 was mated with strain 161, and 130 Arg$^+$ and Str$^R$ recombinants were isolated. Of these, 86 were Met$^+$, 39 could grow on D-lyxose, 20 were Ilv$^+$, 7 Mtl$^+$ and 1 Mal$^+$. These results were used to locate the isomerase gene, *mni*, at around 85 min on the *E. coli* genetic map (Bachmann, Low & Taylor, 1976).

The cotransduction frequencies of the isomerase gene with the *arg*, *met*, and *ilv* markers were all less than 5 %. This was consistent with the location of the isomerase gene being at around 85 min (Wu, 1966), away from all known isomerase genes (Bachmann *et al.*, 1976).

## DISCUSSION

The present study provides another example of the acquisition of a new growth ability in *E. coli* K12 by the derepression of an enzyme (in this case D-mannose isomerase) which appears to be non-functional under normal conditions. The specific activity of the purified enzyme from *E. coli* K12 appears to be at least as high as that of the corresponding enzymes from *Pseudomonas saccharophila* (Palleroni & Doudoroff, 1956), *Klebsiella (Aerobacter) aerogenes* (Anderson & Allison, 1965), *Mycobacterium smegmatis* (Hey-Fergusen & Elbein 1970), *Xanthomonas rubrilineans* (Takasaki, Takano & Tanake, 1964) and *Streptomyces aerocologenes* (Takasaki, 1967); though its $K_m$ is much higher than the others. The enzyme

from *K. aerogenes* (Anderson & Allison, 1965) is more active on D-lyxose, and has a molecular weight of about 40000. The molecular weight of the *E. coli* D-mannose isomerase is similar to that of D-xylose isomerase from *Streptomyces albus* (Hogue-Angeletti, 1975).

Assuming that kinases are more efficient enzymes than isomerases, with affinities possibly several orders of magnitude less, we may speculate that in *E. coli* K12 the D-mannose isomerase could have evolved first and interconverted D-mannose and D-fructose. As soon as the phosphotransferase system (Kundig *et al.*, 1966) emerged, D-mannose could be phosphorylated to D-mannose 6-phosphate and subsequently converted to D-fructose 6-phosphate (Markovitz, Sydiskis & Lieberman, 1967). The micro-organism would thus have acquired the ability to grow on low concentrations of D-mannose, and the isomerase would become inefficient or even useless. The induction of isomerase under such conditions could be wasteful, and might be abolished. However, the isomerase gene may not be immediately deleted but become silent. What we have found in strain 805 is that a possible evolutionary remnant, the D-mannose isomerase, can provide the necessary enzyme to catabolize D-lyxose; a similar situation was found in strain 911 where another possible evolutionary remnant, D-galactose dehydrogenase, plays a vital role in metabolizing D-arabitol (Wu, 1976).

If the above assumption of evolving enzymes is correct, the study of evolution of pathways for the utilization of novel substrates (Hegeman & Rosenberg, 1970; Clarke, 1974; Leisinger, 1975) provides a valuable tool for analysing the silent genes of *E. coli* and other micro-organisms, and these genes deserve careful investigation not only for the understanding of the reserved genetic capacities of micro-organisms but also for the knowledge of their evolutionary past.

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# CORRIGENDUM

## In T. T. WU (1976)

### *Journal of General Microbiology* **94**, 246–256

Page 251, Table 3. The legend should read:

*Escherichia coli* strains were grown on casein hydrolysate and the uptake of D-[1-$^{14}$C]arabitol was measured without any addition and in the presence of unlabelled D-arabitol (10 mM) or DL-1,2-propanediol (10 mM).