

A NEW ENZYMIC DEFECT OF GALACTOSE METABOLISM

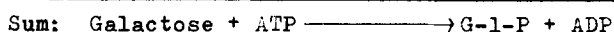
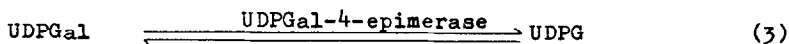
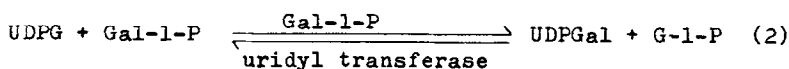
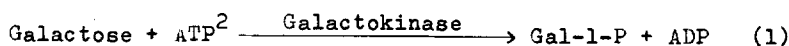
IN ESCHERICHIA COLI K-12 MUTANTS¹

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Escherichia coli K-12 metabolizes galactose by way of the Leloir pathway as follows (Kurahashi, 1957; Kalckar et al., 1959):



The catalytic amount of UDPG required for the above conversion of galactose to a glycolytic intermediate can be formed by the action of UDPG pyrophosphorylase as follows (Munch-Petersen et al., 1955):



E.M. and J. Lederberg and their associates obtained a number of mutants of E. coli K-12 which cannot ferment galactose. These mutants were classified genetically into five groups, A, B, C, D and E (Morse et al., 1956; Lederberg, 1960). The first four groups correspond to kinase-less, transferase-less, the one which lacks all three enzymes (triple-less) and epimerase-less, respectively (Kalckar et al., 1959; Lederberg, 1960; Yarmolinsky et al., 1961).

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2. Abbreviations: ATP and ADP, Adenosine tri- and di-phosphate; UDPG and UDPGal, uridine diphospho-glucose and -galactose; G-1-P and Gal-1-P, α -glucose- and α -galactose-1-phosphate; kinase, galactokinase; transferase, Gal-1-P uridyl transferase; epimerase, UDPGal-4-epimerase; UTP, uridine triphosphate; pyrophosphorylase, UDPG pyrophosphorylase; PP, inorganic pyrophosphate.

This paper deals with the finding of a new type of enzymic defect in the *E. coli* K-12 mutants of group E.

EXPERIMENTAL RESULTS

Three strains of *E. coli* K-12, all of which are F⁻ and Lp^S, were kindly supplied by Drs. E.M. and J. Lederberg. They are a galactose positive (wild type) strain, W3110 and two galactose negative strains of group E, W3142 and W4597. W4597 appeared to produce a trace amount of acid on bromthymol blue-nutrient agar containing 2 % galactose. W3142 did not produce any detectable amount of acid on the same medium. The activities of the enzymes which catalyze reactions 1, 2, 3 and 4 were determined as shown in Table 1.

Table 1

Levels of the enzymes required for galactose fermentation
in K-12 strains¹

Strain	Specific Activity (micromoles/mg protein/hr.)							
	Galactokinase		Gal-1-P uridyl transferase		UDPGal-4-epi- merase		UDPG-pyro- phosphorylase	
	ni ²	i	ni	i	ni	i	ni	i
W3110	3.3	27.8	3.0	30.5	7.78	62.4	2.37	2.54
W3142	<0.2	<0.2	12.2	22.2	18.3	13.7	0.17	0.19
W4597	1.5	6.0	1.7	10.0	0.62	19.0	0.18	0.27

1. Enzymes were determined on sonicates of the cells which had been grown in broth with vigorous shaking (Kurahashi, 1957; Kalckar *et al.*, 1959). Induced cells were harvested after 2 hours growth in the presence of 0.1 % D-galactose.

2. ni = non-induced cells; i = induced cells.

It is seen that both galactose negative strains, W3142 and W4597, had abnormally low activity of pyrophosphorylase in contrast to the level of this enzyme in W3110, the wild type strain. W4597 had practically normal activities of three enzymes in steps 1, 2 and 3. In addition, it had a normal galactose transport system as measured by the

galactose-1-C¹⁴ uptake method of Horecker et al. (1960). Therefore it can be concluded that non-fermentation (strictly speaking, weak-fermentation) of galactose in this particular strain must be due to a defect in pyrophosphorylase. W3142 was already reported to be a kinase-less mutant (Kurahashi 1957). In the present study it was further found that this strain also has a defect in pyrophosphorylase.

Since pyrophosphorylase is responsible for the synthesis of UDPG, the levels of UDPG in these strains were determined by the specific enzymic method (Kalckar et al., 1956). The amount of UDPG accumulated in the cells of both galactose non-fermenting strains was found to be significantly smaller than that in wild type cells as shown in Table 2.

Table 2

Intracellular level of UDPG in K-12 strains¹

Strain	W3110	W3142	W4597
UDPG (micromole/ 100 mg dry weight cells)	0.022	0.0022	0.0041

1. Cells were grown in tryptone broth. Exponentially growing cells were harvested and extracted with 70 % ethanol at 70°C for 5 minutes. UDPG was determined on the supernatant according to Kalckar et al. (1956).

In addition, the cells of W4597 were found to accumulate 0.24 μ mole per 100 mg. dry weight of cells of Gal-1-P intracellularly during their growth in tryptone broth containing 0.1 % galactose. The cells of W3110 and W3142 accumulated only one sixth of the amount found in the cells of W4597. This accumulation of Gal-1-P in the cells of W4597 may reflect the low level of UDPG which is required for the efficient conversion of Gal-1-P to UDPGal as shown in reaction 2.

Discussion

As reported by Lederberg (1960), groups A, B, C and D map in the same cluster, Gal-region, which can be incorporated as a segment into lambda genome. The last group, E, differs in that it contains mutants

which are susceptible to lambda but are not transformed to galactose fermenters by lambda lysates (Lederberg, 1960). In addition, we found that these strains are not transformed by a type of F-gal (Lederberg, 1960) which is known to be able to transform any other galactose non-fermenters, including members of groups A, B, C and D, to galactose fermenters.¹ These findings suggest the existence of a locus which, although located apart from the Gal-region, participates in the control of galactose fermentation.

While the three enzymes of steps 1, 2 and 3 in Leloir pathway are inducible by galactose, the activity of pyrophosphorylase in the wild type cells was not increased by addition of galactose (Table 1). We also found that mutants of group C (triple-less), which according to Jacob *et al.* (1960) are operator mutants, have normal activity of pyrophosphorylase (unpublished observation). These two findings may imply that the structural gene of pyrophosphorylase, unlike those of the three enzymes in Leloir pathway, does not belong to Gal-operon (Buttin *et al.*, 1961; Fukasawa *et al.*, 1961). A locus apart from the Gal-region appears to control the deficiency of pyrophosphorylase in the strains of group E that we examined. We suggest from these considerations that the mutants of group E are characterized by mutation in the structural gene of pyrophosphorylase.

The present studies have furnished the first demonstration of a mutation which affects specifically the activity of pyrophosphorylase. In view of the functions of this enzyme, this mutation would be expected to have profound effects on the organisms other than those mentioned above. According to other studies we have conducted, both mutant strains of the present work contain no detectable amount of hexoses in their cell walls² in contrast to the wild type which contains glucose

1. Personal communication from Dr. Y. Hirota of Osaka University.

2. Heptoses and hexosamines are the major polysaccharide components of the cell walls of these mutant strains.

and galactose. Moreover they are resistant to some phages which attack the wild type strain.¹ Since UDPG, synthesized by the catalytic action of this enzyme, is considered to act as an important glycoside donor for the synthesis of polysaccharides, these changes may reflect some abnormalities in function and structure of their cell wall polysaccharides. We are investigating these relationships at the present time.

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1. Similar observation was made by Dr. J. Adler of University of Wisconsin. (Personal communication through Dr. M. Yarmolinsky.)