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Letter to the Editors

Synthesis of the Enzyme Galactozymase during the Transduction by Coliphage-λ (1)

It has been reported by Morse, Lederberg and Lederberg (1956) that the coliphage λ obtained by the induction of heterogenote in *Escherichia coli* K12 can transduce the gal loci (ability or inability to metabolize galactose) with high frequency. With the intention of investigating the mechanisms of genetic transduction of gal-determinants mediated by coliphage λ, this preliminary communication deals with galactose metabolism in *Escherichia coli* K12 strains.

The organisms used were as follows: *E. coli* K12 (original) Gal^+, lysogenic for λ, prototroph; W3100 Gal^+, lysogenic for λ, prototroph; W3092 Gal_2^- (defect in galactokinase), lysogenic for λ, prototroph; W3094 Gal_4^- (defect in gal-1-P uridyl transferase), lysogenic for λ, prototroph; R3 Gal^-, non-lysogenic for λ which is a prototrophic revertant from Cll2 (H^-, C^-, Gal^-, $T^r_{1,5}$); R3B Gal^+ revertant of R3, non-lysogenic for λ. Some of the above strains were kindly supplied by E. Lederberg and H. Ozeki. Heterogenotic strains were prepared by the method of Morse *et al.* (1956). Cells were exponentially grown on a penassay broth (Difco), washed and resuspended in phosphate buffer. The galactozymase activity was determined by the manometric method.

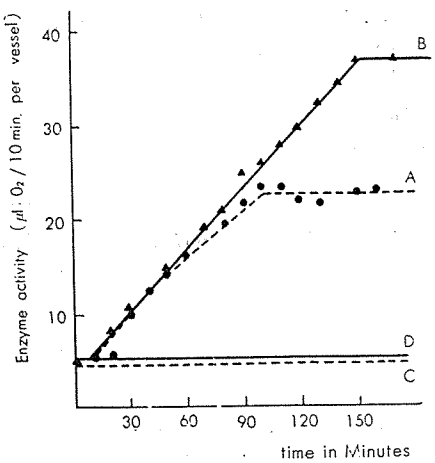
The enzyme, galactozymase, cannot be detected in significant amount in Gal^- strains. But it is adaptively synthesized by resting or growing cells of Gal^+ strains, as shown by Kurahashi (1957), though a little constitutive enzyme activity was also detected in the non-adapted state. The enzyme activity increases exponentially after a lag-time of about 5 minutes and subsequently, the activity reached a maximum after 90~150 minutes (Fig. 1. curve A and B).

The enzyme synthesis could be stopped by the addition of various inhibitors of protein synthesis at appropriate times. The most consistent result was obtained by the addition of 10^{-3} M oxine (8-hydroxyquinoline) which was used as a fixer of penicillinase-adaptation by Pollock (1952).

Fig. 1 Adaptation to galactose

The bacterial cells were grown in a penassay broth (Difco) with active aeration at 37°C, centrifuged, washed and resuspended in M/15 phosphate buffer (pH7.4). Reaction mixtures; bacterial cell suspension 1.0 ml, galactose 10^{-2}M; yeast extract 0.01 per cent. Reaction volumes 2.0 ml. Endogenous respiration was subtracted from the corresponding O_2 consumption.

Curve A; *E. coli* K 12 (original)
Curve B; W 3100
Curve C; Constitutive enzyme activity of E. coli K12 (10^{-3}M. oxine added at 0 time)
Curve D; Constitutive enzyme activity of W 3100 (10^{-3}M. oxine added at 0 time)



Various kinds of heterogenote can like ordinary Gal+ strains also adaptively metabolize galactose, though the maximal rate is slightly lower because they contain Gal- segregants.

Table 1. Comparison of enzyme activity of Gal+ strains of *E. coli* K 12

Strains	maximal enzyme actvity	
	μl./0_2.10 min. per vessel	μl 0_2/10 min. per cell
E. coli K 12 (original)	22.48	1.69×10^{-8}
R3B	18.00	1.35 〃
heterogenote of R3 (λ-gal from W 3100)	16.40	1.23 〃
W 3100	36.75	2.76 〃
heterogenote of W 3092 (λ-gal from K 12)	31.25	2.35 〃
heterogenote of W 3092 (λ-gal from W 3100)	32.80	2.46 〃
heterogenote of W 3094 (λ-gal from K 12)	33.35	2.43 〃
heterogenote of W 3094 (λ-gal from W 3100)	33.60	2.53 〃

Reaciton system is the same as Fig. 1.

As shown in Table 1, a maximal enzyme activity of W3100 and in heterogenotes of W3094 is about twice that of *E. coli* K12, R3B and the heterogenote of R3 at an equal optical density of cells (or equal cell count per ml). It seems however that this is not due to a difference in their galactose-fermenting enzyme bacause the glucose metabolism is also more active in the former bacteria than in the latter.

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