SHORT COMMUNICATION

Isolation and Characterization of D-Ribose-positive Revertants of *Escherichia coli* B/r

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Escherichia coli B has been reported by Cohen & Raff (1951) to be D-ribose-negative (Rbs⁻). In an attempt to characterize this deficiency of *E. coli* B and to study some of the general properties of D-ribose utilization by this strain, a number of *N*-methyl-*N'*-nitrosoguanidine (NTG) induced D-ribose-positive (Rbs⁺) revertants of a prototrophic strain of *E. coli* B/r were isolated and characterized.

METHODS

For the isolation of Rbs⁺ revertants, *Escherichia coli* B/r Rbs⁻ culture was treated with NTG 100 µg./ml. in a Casamino acid medium, pH 6.0, for 15 min. at 37°, then centrifuged and washed with saline and resuspended in minimal medium containing 0.1 % D-ribose 0.05 % sodium citrate. The culture was then incubated for 24 h. at 37°, and 0.1 ml. aliquots were plated on to 0.1 % D-ribose minimal agar medium for Rbs⁺ revertants. D-Ribose-positive revertants were picked and purified after 48 to 72 h.

D-Ribokinase (Horecker, 1957) and D-ribose-5-phosphate isomerase (Axelrod, 1955) activities and differential rates of synthesis in the Rbs⁻ and Rbs⁺ strains were determined in cultures induced by D-ribose (0.1 %) and in non-induced cultures. Culture extracts were prepared by sonication.

Growth of the Rbs⁺ revertants on 0.1 % D-ribose; 0.1 % glucose and on 0.1 % D-ribose plus 0.1 % glucose media was measured turbidometrically on an electronic biophotometer.

Chromosomal location of the gene determining the Rbs⁺ phenotype was determined by P1 transduction using *ara leu* Rbs⁻ recipients (Gross & Englesberg, 1959).

RESULTS

D-Ribose revertants isolated were found to have a one and a half to fourfold increase in their growth rates on D-ribose over that of the Rbs⁻ parent strain. Growth on glucose, on the other hand, was reduced in all tested Rbs⁺ revertants. Mixtures of D-ribose and glucose supported a higher growth rate than was obtained on either sugar alone. The presence of glucose in the ribose growth media did not produce a diauxic growth response.

Specific activity determinations of D-ribokinase and D-ribose-5-phosphate isomerase in both the Rbs⁻ parent strain and Rbs⁺ revertants showed a partially active D-ribokinase in the Rbs⁻ strain which was restored with up to fourfold increase in the enzyme

activity in the Rbs⁺ revertants. Active D-ribose-5-phosphate isomerase enzyme, however, was found in both Rbs⁻ and Rbs⁺ strains. Synthesis of the D-ribokinase and the D-ribose-5-phosphate isomerase enzymes was found to occur constitutively as well as by D-ribose induction in both Rbs⁻ and Rbs⁺ strains (see Table 1).

Preliminary mapping of the mutation that restores the Rbs⁺ phenotype, by P1 transduction showed it to be closely linked to *leu* and *ara* genes and cotransduction frequencies indicate that it is closer to *leu* than to *ara*.

Table 1. *D-Ribokinase and D-ribose-5-phosphate isomerase activities in induced and non-induced Rbs⁻ parent strain and Rbs⁺ revertants of Escherichia coli*

Strain	D-Ribokinase*		D-Ribose-5-phosphate isomerase†	
	Constitutive	Induced	Constitutive	Induced
Rbs ⁻ B/r	0.48	0.65	11.1	11.8
Rbs ⁺ DRO 1	2.05	2.37	12.4	15.0
Rbs ⁺ DRO 6	1.8	1.6	16.5	17.6
Rbs ⁺ DRO 7	1.9	1.7	14.6	16.3
Rbs ⁺ DRO 17	2.5	2.3	15.9	17.6

* Specific activity determined as μ moles of D-ribose-5-phosphate formed/h./mg. protein.

† Specific activity determined as μ moles of D-ribulose-5-phosphate formed/h./mg. protein.

DISCUSSION

This preliminary analysis of the Rbs⁺ and Rbs⁻ *Escherichia coli* B/r mutants raises three questions: (1) What is the nature of the defect in *E. coli* B/r which makes it D-ribose-negative? (2) In what gene has the reversion occurred to make it D-ribose-positive? (3) How may this ribose gene relate to the previously mapped ribose locus (*rbs*) of *E. coli* K12 (Taylor & Trotter, 1967)? In view of the residual ribokinase activity in the Rbs⁻ B/r parent strain and the low level of activity restored in the Rbs⁺ revertants, several different explanations can account for the Rbs⁻ B/r deficiency: (a) a permease deficiency, (b) a mutation in a controlling element in a yet-to-be-discovered D-ribose operon, or (c) a mutation in the D-ribokinase gene causing only a partial impairment in the D-ribokinase activity.

Since we find that the ribokinase and ribose-5-phosphate isomerase are synthesized constitutively in both parent and revertant strains, the possibility of a permease deficiency is ruled out. Similarly, a promotor, regulator or operator mutation in the Rbs⁻ strain would be expected to affect both enzymes co-ordinately and this was not found. The simplest explanation, therefore, for the Rbs⁻ phenotype would be that *Escherichia coli* B/r has a partial defect in the ribokinase enzyme which prevents normal growth on D-ribose.

The question is thus raised whether the ribose gene studied here differs from that of *Escherichia coli* K12 since it maps at a different location and since the metabolic enzymes are constitutive in *E. coli* B/r and inducible in *E. coli* K12 (David & Weismeyer, 1968). A clear distinction cannot be made at this time since it is not known if the *rbs* locus of *E. coli* K12 specifies the ribokinase structural gene.

The Rbs⁺ strains of *Escherichia coli* B/r described above now permit selection to be made for ribose-negative mutants and their subsequent metabolic and genetic analysis.

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REFERENCES

- AXELROD, B. (1955). Pentose phosphate isomerase. In *Methods in Enzymology*, vol. 1, pp. 366-370. Edited by S. P. Colowick & N. O. Kaplan. New York: Academic Press.
- COHEN, S. & RAFF, R. (1951). Adaptive enzymes in the estimation of gluconate, D-arabinose and D-ribose. *Journal of Biological Chemistry* **188**, 501-508.
- DAVID, J. & WEISMAYER, H. (1968). The control of ribose metabolism in *E. coli*. *Genetics* **60**, 171-172 (Abstr.).
- HORECKER, B. (1957). Preparation and analysis of ribose-5-phosphate. In *Methods in Enzymology*, vol. 3, pp. 188-190. Edited by S. P. Colowick & N. O. Kaplan. New York: Academic Press.
- GROSS, J. & ENGBERG, E. (1959). Determination of the order of mutational sites governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage P1bt. *Virology* **9**, 314-331.
- TAYLOR, A. & TROTTER, C. (1967). Revised genetic map of *Escherichia coli* K12. *Bacteriological Reviews* **31**, 332-353.