SHORT COMMUNICATION

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

By M. ABOU-SABE'

Department of Bacteriology, Rutgers University, New Brunswick, New Jersey 08903, U.S.A.

(Accepted for publication 13 January 1971)

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°C, 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°C, 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 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 Escherichiu coli

<table>
<thead>
<tr>
<th>Strain</th>
<th>D-Ribokinase*</th>
<th>D-Ribokinase+</th>
<th>D-ribose-5-phosphate isomerase*</th>
<th>D-ribose-5-phosphate isomerase+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constitutive</td>
<td>Induced</td>
<td>Constitutive</td>
<td>Induced</td>
</tr>
<tr>
<td>Rbs- B/r</td>
<td>0.48</td>
<td>0.65</td>
<td>11.1</td>
<td>11.8</td>
</tr>
<tr>
<td>Rbs+ DRO</td>
<td>2.05</td>
<td>2.37</td>
<td>12.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Rbs+ DRO6</td>
<td>1.8</td>
<td>1.6</td>
<td>16.5</td>
<td>17.6</td>
</tr>
<tr>
<td>Rbs+ DRO7</td>
<td>1.9</td>
<td>1.7</td>
<td>14.6</td>
<td>16.3</td>
</tr>
<tr>
<td>Rbs+ DRO17</td>
<td>2.5</td>
<td>2.3</td>
<td>15.9</td>
<td>17.6</td>
</tr>
</tbody>
</table>

* Specific activity determined as μmoles of D-ribose-5-phosphate formed/h./mg. protein.
† Specific activity determined as μmoles of D-ribose-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 promoter, 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.

Work supported by Rutgers University Research Council grant no. 072134.
REFERENCES


