On the Mutations Responsible for the Rough Phenotype of Escherichia coli B

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Introduction

The O-antigenic lipopolysaccharide (LPS) of Enterobacteriaceae consists of an O-specific polysaccharide linked to a core oligosaccharide which, in turn, is bound to a lipid via 2-keto-3-deoxyoctonate (Lüderitz et al., 1971). The biosynthesis of the core and the O-specific polysaccharide is under the control of various rfa genes (Stocker & Mäkelä, 1971). The rfa genes, most of which are clustered in the cysE-pyrE region, determine the core synthesis, while the synthesis of O-specific polysaccharides is directed by the his-linked rfb genes. The ihv-linked rfe genes are involved in the synthesis of both the O-specific polysaccharides and the enterobacterial common antigen (Mäkelä et al., 1974). Mutation at any of the rfa loci leads to rough (R) mutants. In this paper we describe genetic studies which indicate that, in the rough strain Escherichia coli B, rfa as well as rfb genes are defective.

Methods

Bacteria. The E. coli B strains used are listed in Table 1. For genetic experiments, the donors were E. coli Hfr59, which has the O antigen 8 (Schmidt, 1969), and the rough E. coli K12 HfrC (Hayes, 1970). The recipient was the multiply-marked E. coli B strain F2540 (Prehm, 1974), a derivative of E. coli B Berkeley (F2499). For P1 transductions, E. coli B Berkeley was used as the donor and E. coli K12 strain F2578 as recipient.

Bacteriophages. The bacteriophages used were: Brio, FPI, C21, T3, T4, T7, Felix-01 (FO), 6SR, U3 and Q8. Phage U3 specifically lyses E. coli K12 strains, but not galE or galU mutants of these strains which have galactose- or glucose-deficient LPS (Watson & Paigen, 1971). Phage Q8 is specific for E. coli strains having the O8 antigen (Jann et al., 1971). The remainder are rough-specific phages (Schmidt, 1973; Lindberg, 1973; Rapin & Kalckar, 1971) used here for characterization of E. coli B strains. The phages were propagated (Adams 1959) on their respective hosts in beef extract broth. Phage sensitivity of strains was tested using phage suspensions containing about $10^8$ plaque forming units ml$^{-1}$. Drops were placed on dried bacterial lawns on agar plates and incubated overnight at 37°C.

Mating experiments. These were performed as previously described (Schmidt, Jann & Jann, 1970). Recombinants were first transferred to the media used for selection and then streaked on complete agar medium. Purification of recombinants was achieved by repeated single colony isolations.

Transductions. For transductions we used phage P1kc, a derivative of P1. The procedure was as described previously (Schmidt et al., 1976).

Determination of O-specific hapten. Bacteria were extracted with phenol/water at 65°C (Lüderitz et al., 1971) and the aqueous phase was centrifuged at 105000 g to sediment the lipopolysaccharide. The presence of hapten in the supernatant was detected by immunoelectrophoresis with the respective O antiserum (Schmidt et al., 1976).
Short communication

Table 1. Strains of bacteria used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Designation</th>
<th>Genetic markers</th>
<th>Reference/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli B</td>
<td>F2120</td>
<td>—</td>
<td>V. Braun, Tübingen, G.F.R.</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2154 (American)†</td>
<td>—</td>
<td>W. F. Goebel, New York, U.S.A.</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2156</td>
<td>—</td>
<td>J. Schlosshardt, Potsdam, G.D.R.</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2160</td>
<td>—</td>
<td>M. Bayer, Philadelphia, U.S.A.</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2468</td>
<td>—</td>
<td>H. Boman, Umeå, Sweden</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2499 (berkeley)‡</td>
<td>—</td>
<td>E. Kellenberger, Basel, Switzerland</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2500 (epstein)‡</td>
<td>—</td>
<td>F. Ørskov, Copenhagen, Denmark</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2502</td>
<td>—</td>
<td>J. P. Duguid, Dundee, Scotland</td>
</tr>
<tr>
<td>E. coli B</td>
<td>F2540</td>
<td>his trp xyl str⁺</td>
<td>derived from F2499</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>F2540</td>
<td>his trp str⁺</td>
<td>Schmidt, 1973</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>W1895</td>
<td>metB HfrC</td>
<td>Hayes, 1970</td>
</tr>
<tr>
<td>E. coli O8‡</td>
<td>F459</td>
<td>Hfr59</td>
<td>Schmidt, 1973</td>
</tr>
</tbody>
</table>

* his, Histidine; met, methionine; trp, tryptophan; xyl, xylose; str⁺, streptomycin resistance.
† Also designated E. coli BA, BB or BE, respectively.
‡ Acapsular mutant derived from E. coli F56b (O8:K27).

RESULTS AND DISCUSSION

Characterization of E. coli B strains

The E. coli B ‘wild-type’ strains listed in Table 1 were obtained from different laboratories. They all showed a rough colonial morphology and cells grown on complete medium were strongly agglutinated by 3.5% (w/v) saline or by 0.3% auramine solution (Schmidt, 1973). The E. coli B strains were sensitive to the rough-specific bacteriophages Br10, FP1, C21, T3, T4, T7 and resistant to FO and 6SR. All strains tested thus showed the typical traits of rough (R) mutants. The receptors for the phages used are assumed to be in the cell-wall lipopolysaccharide (Rapin & Kalckar, 1971; Lindberg, 1973). Therefore, the identical reaction pattern of the strains with these phages indicated identical LPS portions.

For the genetic study, the multiply-marked E. coli B strain F2540 was chosen. This auxotrophic mutant has the same phage pattern as E. coli B Berkeley (~2499) from which it is derived, indicating that the mutagenic treatment for the introduction of metabolic markers had not affected the LPS composition.

Introduction of the E. coli O8 rfb region into E. coli B

The rough phenotype of E. coli B may be due to mutations in one or more of the rfb genes. A defect in the rfb or in the rfe clusters would result in R mutants with the complete core oligosaccharide. An rfe defect can be excluded because E. coli B possesses the enterobacterial common antigen (unpublished observation), the synthesis of which is inhibited in strains with defective rfe genes (Schmidt, Mayer & Makela, unpublished results; Makela et al., 1974).

To test whether the rfa genes of E. coli B are intact, the rfb region of an E. coli O8 donor was introduced into E. coli B. Since the rfb genes are closely linked to the his operon (Stocker & Mäkelä, 1971), his⁺ str⁺ recombinants were selected from crosses of the E. coli donor Hfr59 (O8, his⁺ str⁺) with the E. coli B recipient F2540 (his str⁺) on suitably supplemented minimal agar. Thirty-eight his⁺ recombinants were obtained and tested serologically by slide agglutination with O8-antiserum and also with phages. None of the recombinants was agglutinated in O8 antiserum and none was sensitive to the O8-specific phage O8. All of them showed the phage reaction pattern of the parental E. coli B.

In some of the hybrids, an O8-specific hapten was detected. This indicated that the donor
rfb (O8) had been transferred in a functional state into E. coli B but the O8-specificity remained unexpressed presumably due to defective rfa gene(s).

**Genetic transfer of the E. coli K12 core into E. coli B**

Previous studies have shown that the LPS core of the rough E. coli K12 has the capacity to accept E. coli O-specific polysaccharides (Jones, Koeltzow & Stocker, 1972; Schmidt, 1973). Moreover the transfer of xyI-linked E. coli K12 rfa genes to a given E. coli serotype led to smooth hybrids with the E. coli K12 core (Schmidt, unpublished results). Therefore, it might be expected that the genetic transfer of the E. coli K12 core into E. coli B would result in smooth hybrids provided that the genes needed for O-specific chain synthesis were intact. To test this we selected xyI+ str' hybrids from a cross between the E. coli K12 donor w1895 and the E. coli B strain F2540. Forty-eight xyI+ hybrids which had retained the His^- Trp^- phenotype of the female parent were obtained. All recombinants were strongly agglutinated in 0.3% auramine or in 3.5% saline and thus behaved like R forms. The presence of the K12 core was inferred from the sensitivity of the hybrids to the K12-specific phage U3 and their resistance to C21. In this manner 27 of the 48 recombinants were found to have the E. coli K12 core. The failure to establish a smooth (S) phenotype together with the presence of the E. coli K12 core suggests that E. coli B additionally has a defect in the rfb cluster. This was corroborated by the fact that the introduction of rfb genes from an E. coli O8 donor into E. coli B recipients bearing the K12 core resulted in smooth hybrids expressing O8 specificity. It indicates that the transferred E. coli K12 core has the ability to accept O chains.

**Transduction of the his region from E. coli B to E. coli K12**

In a final experiment we transduced, with phage PiKc, the his region of E. coli B (F2499) into the E. coli K12 recipient F2578 (his) that has a rough phenotype. We expected that some of the his+ transductants would also have received his-linked rfb genes from the donor. This should lead to a smooth phenotype of the respective transductants, if E. coli B had intact rfb genes.

The 100 transductants were tested by slide agglutination in 3.5% saline and 0.3% auramine. All of them were agglutinated like typical rough (R) mutants. In a comparable experiment with an E. coli O8 donor, about 15% of the transductants were smooth forms expressing O8 specificity (Schmidt, unpublished results). The outcome of the transduction confirms the assumption that E. coli B has a defect in the rfb region.

The results described have shown that smooth hybrids could be generated only after introduction of intact rfa as well as of rfb genes into E. coli B. Thus the rough E. coli B has defects in the rfb region and in one or more of the rfa genes. Because of the rfa defect(s) it appears likely that the E. coli B core (Prehm et al., 1975) is incomplete. This conclusion, however, needs further proof since a possible defect of a gene equivalent to the Salmonella rfaL locus – responsible for O chain translocation to the complete core (Stocker & Mäkelä, 1971) – has not been excluded.

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REFERENCES


