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 *rf* 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 *ilv*-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 *rf* 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 (Pehm, 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: Br10, FP1, C21, T3, T4, T7, Felix-O1 (FO), 6SR, U3 and Ω 8. 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 Ω 8 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).

Table 1. *Strains of bacteria used*

Strain	Designation	Genetic markers*	Reference/source
<i>E. coli</i> B	F2120	—	V. Braun, Tübingen, G.F.R.
<i>E. coli</i> B	F2154 (American)†	—	W. F. Goebel, New York, U.S.A.
<i>E. coli</i> B	F2156	—	J. Schlosshardt, Potsdam, G.D.R.
<i>E. coli</i> B	F2160	—	M. Bayer, Philadelphia, U.S.A.
<i>E. coli</i> B	F2468	—	H. Boman, Umeå, Sweden
<i>E. coli</i> B	F2499 (Berkeley)†	—	E. Kellenberger, Basel, Switzerland
<i>E. coli</i> B	F2500 (Epstein)†	—	F. Ørskov, Copenhagen, Denmark
<i>E. coli</i> B	F2502	—	J. P. Duguid, Dundee, Scotland
<i>E. coli</i> B	F2540	<i>his trp xyl str^r</i>	derived from F2499
<i>E. coli</i> K12	F2578	<i>his trp str^r</i>	Schmidt, 1973
<i>E. coli</i> K12	w1895	<i>metB</i> HfrC	Hayes, 1970
<i>E. coli</i> O8‡	F459	Hfr59	Schmidt, 1973

* *his*, Histidine; *met*, methionine; *trp*, tryptophan; *xyl*, xylose; *str^r*, streptomycin resistance.

† Also designated *E. coli* BA, BB or BE, respectively.

‡ Acapsular mutant derived from *E. coli* E56b (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, FPI, 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 (F2499) 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 *rf* 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 & Mäkelä, unpublished results; Mäkelä *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^r* recombinants were selected from crosses of the *E. coli* donor Hfr59 (O8, *his⁺ str^r*) with the *E. coli* B recipient F2540 (*his str^r*) 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 Ø28. 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 *xyl*-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 *xyl*⁺ *str*^r hybrids from a cross between the *E. coli* K12 donor w1895 and the *E. coli* B strain F2540. Forty-eight *xyl*⁺ 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 P1kc, 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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