Presence of Different O Antigen Forms in Three Isolates of One Clone of 
Escherichia coli

Dan Liu and Peter R. Reeves

Department of Microbiology, The University of Sydney, Sydney, New South Wales 2006, Australia

Manuscript received January 3, 1994
Accepted for publication May 24, 1994

ABSTRACT

Escherichia coli strains ECOR2, ECOR3 and K-12 are very closely related in genotype as indicated by multilocus enzyme electrophoresis. We show that they have very different rfb regions indicating that recombination has occurred in this region, and we suggest that it may be associated with niche adaptation.

Bacterial populations have a clonal structure with long lived clones showing little variation (ACHTMAN et al. 1986; SELANDER et al. 1987). A set of 72 Escherichia coli isolates (OCHMAN and SELANDER 1984) is broadly representative of the genetic diversity in the species as detected by multilocus enzyme electrophoresis (MLEE) and ECOR2 and ECOR3 from this set have a close relationship with E. coli K-12 (R. K. SELANDER, personal communication). ECOR2 and E. coli K-12 have identical mobilities for the 35 enzymes used, and ECOR3 differed from E. coli K-12 only in the mobility of threonine dehydrogenase. In this study we present a comparison of the rfb regions of E. coli K-12, ECOR2 and ECOR3, and show that, despite their general similarity, their rfb regions are very different.

MATERIALS AND METHODS

Bacterial strains, plasmids and bacteriophage: The bacterial strains ECOR2 and ECOR3 are gifts of R. K. SELANDER: strain C600 (APPLEYORD 1953) was used as the K-12 strain. The allozyme profiles of the three strains were checked by electrophoretic typing of the enzymes 6-phosphogluconate dehydrogenase, malate dehydrogenase, acid phosphatase 1, acid phosphatase 2 and alcohol dehydrogenase, which allow ECOR2 and ECOR3 to be easily distinguished from most strains in the ECOR set (SELANDER et al. 1986). All three strains had the pattern expected for ECOR2 and ECOR3. A set of λ phage clones (KOHARA et al. 1987) covers the whole E. coli K-12 chromosome and five of the clones, K350, K351, K352 and K353, with insert from the 44-minute region, were used in this study (see Fig. 1). Phage were prepared as described by LIU and REEVES (1994). Bacterial strains and plasmids used in this study are listed in Table 1.

Enzymes and reagents: All enzymes and biochemicals were from Boehringer Mannheim or Pharmacia LKB. A λ packaging kit was from Promega, and [α-32P]dCTP and [α-35S]dATP were from Bresatec. Sequencing was done using a kit from U.S. Biochemical Corp., Cleveland, Ohio.

DNA methods and computer analysis: The preparations of chromosomal and plasmid DNA were as described by BASTIN et al. (1991), and Southern hybridization was as described by SAMBROOK et al. (1989). The strategies employed for cosmid cloning and restriction mapping were as described by TARTOF and HOAS (1988). The dyeoxy chain termination method (SANGER et al. 1977) was used for DNA sequencing. The DNA sequence was analyzed using the Australian National Genomic Information Service (ANGIS) (Reissner et al. 1993). The programs SEQH and SEQA (KANEISHI 1982) were used for comparing the DNA sequences.

RESULTS AND DISCUSSION

Relationship between E. coli K-12, ECOR2 and ECOR3 in the rfb regions: The three E. coli strains K-12, ECOR2 and ECOR3 are identical or near identical on MLEE (R. K. SELANDER, personal communication) and were expected to be members of a single clone and have the same O antigen, but surprisingly the O serotype of ECOR3 is O1, while that of ECOR2 is of a different unknown type (T. WHITAM, personal communication). All extant strains of K-12 lack O antigen due to mutations in the rfb region, but it has recently been shown (LIU and REEVES 1994) that the parent K-12 strain had O16 antigen.

BamHI, HindIII and EcoRI digestion of ECOR2, ECOR3 and C600 chromosomal DNA was probed with fragments from the Kohara clones covering the region from positions 2093 to 2147 (Figure 1). The regions from positions 2093 to 2103.7, and from positions 2124.2 to 2147 of E. coli K-12 were present in ECOR2. The regions from positions 2103.7 to 2111, and from positions 2116.2 to 2124.2 of K-12, hybridized to ECOR2 DNA, but the restriction fragment sizes were very different from those found with DNA from K-12 (data not shown). The plasmid pPR1168 (Table 1), which carries rhamnose pathway genes of K-12 (LIU and REEVES 1994), hybridized with ECOR2 and ECOR3 DNA, suggesting that the rhamnose pathway also exists in these strains. Probes A, B and C covering positions from 2111 to 2116.2, from the rfb region of K-12, did not hybridize with DNA from ECOR2 or ECOR3 (Figure 1). Southern hybridization of ECOR2 and ECOR3 DNA allowed us to determine the extent of similarity. The restriction maps

Genetics 138: 7-10 (September, 1994)
### TABLE 1

**Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strains and plasmids</th>
<th>Laboratory stock no.</th>
<th>Characteristic</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C600</td>
<td>P334</td>
<td>F^- thr-I, leuB6, tonA1, lacY1, tonA21, supE44, λ^- rfbD</td>
<td>APPLEVARD (1953)</td>
</tr>
<tr>
<td>JM109</td>
<td>P3584</td>
<td>supE44, nala96, recA1, relA1, endA1, thi, hisD17, Δ(pro-lac)</td>
<td>YANICH-PERRON et al. (1985)</td>
</tr>
<tr>
<td>X8619</td>
<td>P3551</td>
<td>F^- lacY1, glnV44, galT22, Δhya57, recA56, melB1, hisD2, lysogenized with λc1857, b2, red β5β7</td>
<td>JACOBS et al. (1986)</td>
</tr>
<tr>
<td>SO874</td>
<td>P4052</td>
<td>lacZ4503, try-355, upp-12, relA1, tpsL150, Δ(sbcB-38)</td>
<td>NEUHARD and THOMASSEN (1976)</td>
</tr>
<tr>
<td>ECOR2</td>
<td>M134</td>
<td>Natural isolate</td>
<td>OCHMAN and SELANDER (1984)</td>
</tr>
<tr>
<td>ECOR3</td>
<td>M135</td>
<td>Natural isolate</td>
<td>OCHMAN and SELANDER (1984)</td>
</tr>
<tr>
<td>pPR1168</td>
<td></td>
<td>4.1-kb EcoRI-HindIII fragment from K352 in pUC18</td>
<td>This study</td>
</tr>
<tr>
<td>pPR1218</td>
<td></td>
<td>Functional rfb clone of ECOR2 with 40-kb insert in KT1</td>
<td>This study</td>
</tr>
<tr>
<td>pPR1249</td>
<td></td>
<td>9.4-kb HindIII fragment from pPR1218 in pUC18</td>
<td>This study</td>
</tr>
<tr>
<td>pPR1372</td>
<td></td>
<td>Sall deletion derivative of pPR1218, with 12.7-kb insert</td>
<td>This study</td>
</tr>
<tr>
<td>pPR1376</td>
<td></td>
<td>HindIII deletion derivative of pPR1218, with 2.6-kb insert</td>
<td>This study</td>
</tr>
<tr>
<td>pPR1377</td>
<td></td>
<td>HindIII deletion derivative of pPR1218, with 5.3-kb insert</td>
<td>This study</td>
</tr>
<tr>
<td>KT1</td>
<td></td>
<td>High copy number cosmid</td>
<td>TARTOF and Hobbs (1988)</td>
</tr>
</tbody>
</table>

#### FIGURE 1

Restriction maps of the rfb regions and comparison of *E. coli* K-12 with ECOR2 and ECOR3. The restriction map and scale are from the genomic physical map (KOHARA et al. 1987) as modified by K. RUDD et al. (BOUFFARD et al. 1992; RUDD et al., 1992). The scale is from EcoMap6 produced by the program Genescape. The restriction sites with asterisk are not present in the physical map as published, but were found in Kohara clones in this study. The restriction maps of ECOR2 and ECOR3 were constructed by hybridization, and show only those restriction sites which are also in K-12. The regions in ECOR2 and ECOR3 which have DNA homology but differ in restriction sites compared with K-12 are indicated by dashed line, while the regions which have no homology with K-12 are shown as break. Kohara clones and probes are displayed above the restriction maps. Probe A was a polymerase chain reaction product of the ORF between the IS5 and the gnd gene (LIU and REEVES 1994) which is part of the last gene in rfb gene cluster of strain K-12, probes B and C were 2.2- and 1.6-kb HindIII fragments isolated from K351. Restriction sites: B, BamHI; E, EcoRI and H, HindIII.

#### FIGURE 2

Silver staining of LPS. The membrane extracts were electrophoresed in a 18% SDS-PAGE and silver-stained. Extracts are 1, *E. coli* K-12; 2, ECOR2; 3, pPR1218 in JM109; 4, pPR1372 in JM109; 5, pPR1372 in Sf874 (Δrfb K-12 strain); 6, ECOR3, indicating that the rfb regions of these three strains are substantially different.

**Cloning the rfb region of ECOR2:** The level of divergence in the rfb regions of the three closely related strains was surprising and to facilitate further restriction mapping and comparison we cloned the rfb region from strain ECOR2.

Chromosomal DNA from strain ECOR2 was partially digested with Sau3A and ligated into the BamHI site in high copy number cosmid KT1 (TARTOF and HOBBS...
A bank of 2000 clones was made using *E. coli* strain χ2819 as host strain. Clones carrying rfb DNA were identified by Southern blotting using pPR1168 as the probe. The clones which hybridized to the probe were then tested for expression of LPS on SDS-PAGE. One showed smooth type LPS (Figure 2), and the plasmid was named pPR1218. The restriction map of pPR1218 (Figure 3) was determined by the method of TARTOF and HOBBS (1988) and confirmed by double digestion of derivatives of pPR1218 (Figure 3). Plasmid pPR1372, which is a *SalI* deletion derivative of pPR1218, has a 12.7-kb insert, conferred expression of LPS on both *E. coli* and *Klebsiella pneumoniae*. The position and direction of the spot sequencing of the gnd gene is indicated by the arrow line. Restriction sites: B, *BamHI*; E, *EcoRI*; H, *HindIII*; N, *NotI*; P, *PstI*; R, *Rv*; *EcoRV*; and *SalI*. The vector of pPR1218 is indicated by a heavy line.

**Figure 3.**—The restriction map of ECOR2 clone pPR1218 and its derivatives. The detailed restriction map of the rfb region is also displayed. Part of the DNA from pPR1218 has a different restriction map compared to that constructed from Southern hybridization of chromosome of ECOR2 and is indicated by dash line. Plasmids pPR1372, pPR1376 and pPR1377 are deletion derivatives of pPR1218. Plasmid pPR1249 is in pUC18. Probes 1, 2 and 3 were isolated from pPR1376, pPR1377 and pPR1249, respectively. The position and direction of the spot sequencing of the gnd gene is indicated by the arrow line. Restriction sites: B, *BamHI*; E, *EcoRI*; H, *HindIII*; N, *NotI*; P, *PstI*; R, *Rv*; *EcoRV*; and *SalI*. The vector of pPR1218 is indicated by a heavy line.

1988). A bank of 2000 clones was made using *E. coli* strain χ2819 as host strain. Clones carrying rfb DNA were identified by Southern blotting using pPR1168 as the probe. The clones which hybridized to the probe were then tested for expression of LPS on SDS-PAGE. One showed smooth type LPS (Figure 2), and the plasmid was named pPR1218. The restriction map of pPR1218 (Figure 3) was determined by the method of TARTOF and HOBBS (1988) and confirmed by double digestion of derivatives of pPR1218 (Figure 3). Plasmid pPR1372, which is a *SalI* deletion derivative of pPR1218, has a 12.7-kb insert, conferred expression of LPS on both *E. coli* and *Klebsiella pneumoniae*. The position and direction of the spot sequencing of the gnd gene is indicated by the arrow line. Restriction sites: B, *BamHI*; E, *EcoRI*; H, *HindIII*; N, *NotI*; P, *PstI*; R, *Rv*; *EcoRV*; and *SalI*. The vector of pPR1218 is indicated by a heavy line.

**Figure 3.**—The restriction map of ECOR2 clone pPR1218 and its derivatives. The detailed restriction map of the rfb region is also displayed. Part of the DNA from pPR1218 has a different restriction map compared to that constructed from Southern hybridization of chromosome of ECOR2 and is indicated by dash line. Plasmids pPR1372, pPR1376 and pPR1377 are deletion derivatives of pPR1218. Plasmid pPR1249 is in pUC18. Probes 1, 2 and 3 were isolated from pPR1376, pPR1377 and pPR1249, respectively. The position and direction of the spot sequencing of the gnd gene is indicated by the arrow line. Restriction sites: B, *BamHI*; E, *EcoRI*; H, *HindIII*; N, *NotI*; P, *PstI*; R, *Rv*; *EcoRV*; and *SalI*. The vector of pPR1218 is indicated by a heavy line.

Location of the gnd gene of ECOR2: The gnd gene is located very close to the downstream end of the rfb gene clusters in *S. enterica* groups B (JANG et al. 1991), C1 (LEE et al. 1992) and E (WANG et al. 1992) and in *E. coli* K-12 (LIU and REEVES 1994) and *E. coli* O7 (MAROLDA and VALVANO 1993), and marks the end of the rfb gene cluster. The gnd gene of ECOR2 was located precisely by sequencing (Figure 3). A 1.6-kb *EcoRI* fragment from pPR1218 (position 0 to 1.6) was cloned into M13mp19, and the sequencing was carried out using universal primer. A 252-bp sequence was obtained (data not shown) and was homologous to the gnd sequence of *E. coli* K-12 from positions 1263 to 1514 (NASOFF et al. 1984). There is 5.1% divergence in the gnd sequence between strains ECOR2 and *E. coli* K-12. This level of divergence is high for these closely related strains, indicating that the recombination event involved gnd and at least part of the rfb region in one of these two strains.

**Hybridization using ECOR2 probe:** Three probes derived from pPR1218 (Figure 3) were hybridized with ECOR2, ECOR3 and K-12 chromosomal DNA by Southern blotting. Probe 1 was a 1.7-kb *EcoRI* fragment carrying the gnd gene of ECOR2 (Figure 3): it hybridized to ECOR3 and *E. coli* K-12 chromosomal DNA. Probe 2 was a 1.6-kb *EcoRI* fragment carrying DNA from the rfb gene cluster and did not hybridize to ECOR3 or *E. coli* K-12 chromosomal DNA. Probe 3 carries DNA upstream of rfb and hybridized to the two strains (data not shown). These results showed again that the three strains from a clone of *E. coli* have three different rfb gene clusters and the regions flanking rfb appear to be similar within the three strains.

**Conclusion:** There have been previous reports of clones identified by lack of variation at many genes nonetheless having more than one O antigen type: ACHTMAN et al. (1986) observed O1, O18 and O2 O antigens in strains otherwise identical except at the gnd locus which is closely linked to rfb, and KAPUR et al. (1992) observed clones identical on similar criteria with both O2 and O78 represented. The three strains *E. coli* K-12, ECOR2 and ECOR3 have almost identical genotype as shown by MLEE of 35 enzymes (R. K. SELANDER, personal communication). However, not only do they have different O antigens, but the differences in their rfb gene clusters are extreme. It is clear that the differences cannot be due to mutation and that at least two of the three rfb regions must have been substituted by recombination during the life of the ECOR2/ECOR3/K-12 clone. Our observation of three different O antigens encoded by quite different rfb regions in three essentially randomly chosen isolates of a single widespread clone, together with the other observations discussed above, suggests that interclonal transfer of the rfb genes is much more frequent than transfer of most other genes.
The high level of polymorphism at the rfb locus with over 160 known forms of O antigen in \textit{E. coli} (Ewing 1986) indicates that some form of balanced selection operates. Both Ørskov and Ørskov (1976) and Achtman and Pluschke (1986), who observed that some O antigen forms are commonly present in \textit{E. coli} clones with specific modes of pathogenesis, concluded that O antigen specificity is important in determining pathogenicity of clones; and one of us (Reeves 1992) has proposed a model for a form of balanced selection under which niche-specific selection for alleles of genes or gene clusters of significance in determining niche adaptation can maintain polymorphisms such as that found at the rfb locus.

The high frequency of transfer of rfb genes between clones may perhaps be due to selection for specific O antigen forms for adaptation to different niches, as O antigen variation in otherwise closely related strains can be associated with differences in disease specificity (Achtman and Pluschke 1986); however, in the absence of any specific details of the selection involved this remains speculative. Explanations for the high frequency of transfer based on higher intrinsic levels of recombination at the rfb locus do not seem plausible as the frequency appears to be much above that for other genes, but given that the G + C content of rfb regions is often atypical [see Reeves (1994) for review], it cannot be ruled out that this may affect recombination.

**LITERATURE CITED**


Communicating editor: M. M. Howe.