Plasmid-Determined $\beta$-Lactamase Indistinguishable from the Chromosomal $\beta$-Lactamase of *Escherichia coli*

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Received for publication 8 July 1975

A plasmid, derived from a naturally occurring strain of *Proteus mirabilis*, conferred resistance to cephalosporins, apparently mediated by a $\beta$-lactamase indistinguishable from that determined by the chromosomal gene of *Escherichia coli* K-12. There was evidence for a recombination event between the wild-type plasmid and a defective F factor (Fsp) in the *Escherichia coli* K-12 culture in which it was stored.

A plasmid, R22K, determining the production of a $\beta$-lactamase that hydrolyzed cephalosporins more rapidly than penicillins was described by Kontomichalou, Papachristou, and Levis (18). The specificity of this enzyme was unique among R factor-determined $\beta$-lactamases so far described. Most such enzymes fall into two classes, neither of which shows preferential activity against cephalosporins (17).

R22K was lost from its original host strain, *Proteus mirabilis* 22, during storage. The resistance genes, transferred by conjugation from *P. mirabilis* 22, were retained in a strain of *Escherichia coli* K-12, but with altered genetic linkage. The $\beta$-lactamase gene was carried on a nonconjugative plasmid, designated R22Ka, whose genetic properties and possible derivation are described here. The properties of the $\beta$-lactamase, which resembled those of the chromosomally determined $\beta$-lactamases of *E. coli* and Shigella sonnei (9, 19, 27, 29), are also described.

Detailed studies of these $\beta$-lactamases showed that the enzyme mediated by the plasmid was indistinguishable from that of one strain, *E. coli* K-12 D3 (4).

**MATERIALS AND METHODS**

**Bacterial strains.** The following *E. coli* K-12 sublines were used: 58-161sp met, which carries a defective F factor (22); J62-2 pro his trp lac rifc $F^{-}$ (7); J53 pro met (6); W3110 Thy" (1); H1t4 pro thr leu lac ampa1, ampc1, a transductant of H1t3 with TP1 as donor (5); D3 pro his trp lac amp(A1B4); and D31, the streptomycin-resistant mutant of D3 (4). *E. coli* strain 214T (27) and S. sonnei strain 2 (29) were used for $\beta$-lactamases.

**Plasmids.** The plasmids R22Ka and R22Kb were derived from *E. coli* K-12 strain 58-161sp which had acted as recipient in conjugation, in 1964, with *P. mirabilis* strain 22 (18). At that time it appeared that a single plasmid, designated R22K, had been transferred, since resistance to $\beta$-lactam antibiotics and streptomycin was transferred at low frequency and always together. Of 200 transconjugant colonies tested, all were resistant to both antibiotics, though 100 had been ampicillin-selected and 100 streptomycin-selected. *E. coli* 58-161sp (R22K) transferred linked resistance to $\beta$-lactam antibiotics and streptomycin to *Salmonella typhi* strain 152 (P. Kontomichalou, Thesis, University of Athens, 1971). The original *P. mirabilis* 22 and *E. coli* K-12 58-161sp (R22K) strains were stored on Dorset egg slopes at room temperature for several years by two of us (P. K. and N. D.). When retested, all stocks of *P. mirabilis* 22 had lost resistance to $\beta$-lactam antibiotics and streptomycin. Thus, R22K was lost from its original host, as sometimes occurs during storage on Dorset egg. Another strain, 58-161sp (R22K), retained resistance to $\beta$-lactam antibiotics and to streptomycin, but the two resistances were no longer linked (see Results).

Standard plasmids of all known incompatibility groups came from laboratory stocks (12).

**Defective F factor.** The defective F factor, Fsp, of strain 58-161sp is described under Results.

**Acridine orange curing.** The method used for acridine orange curing was that described by Salisbury et al. (26).

**Transfer of R factors.** Direct transfer of conjugative plasmids was as described by Coetzee et al. (7). Indirect transfer, by the introduction of a second
plasmid as a mobilizing agent, as was described by Ozeki, Stocker, and Smith (23).

**Plasmid compatibility.** Tests for compatibility with plasmids of known incompatibility groups were made by methods previously described (17).

**Isolation and molecular weight analysis of plasmid DNA.** Plasmid deoxyribonucleic acid (DNA) was labeled with [3H] or [14C] thymine and isolated by ethidium bromide-cesium chloride equilibrium centrifugation as previously described (2). Molecular weight estimations of purified plasmid DNA samples were made by velocity sedimentation through neutral 5 to 20% sucrose gradients. [14C]-labeled R1 DNA was co-sedimented as a molecular weight marker (60 × 10^9) with each sample. Methods and calculations were as described by Barth and Grinter (2).

DNA-DNA hybridization. Samples of sheared [3H]-labeled plasmid DNA were mixed with sheared unlabeled DNA from H14(R22K) isolated according to Marmur (20) in a final volume of 0.4 ml. The DNA was in 0.42 M NaCl at a final concentration of 10 ng/ml and 150 μg/ml for the labeled and unlabeled DNA, respectively. These mixtures were heated to 101.5 °C for 10 min and then incubated at 75 °C for 24 h. Annealed DNA was assayed with S1 endonuclease by the method of Barth and Grinter (3).

**Thin-layer isoelectric focusing.** Isoelectric focusing was done on sheets on polyacrylamide gel. The chromogenic cephalosporin 87/312 was used as previously described (21) to stain for β-lactamase activity.

**Immunoisoelectric focusing.** With the use of a perspex former, troughs were set into a sheet of polyacrylamide gel on which the samples were focused. Antiserum, raised in rabbits to E. coli D31 β-lactamase and purified by the method used for P99 β-lactamase (24), was added to the troughs. The gel was incubated at 4 °C in a humid atmosphere for 7 days to allow precipitin arcs to form. These were detected with the use of cephalosporin 87/312 (21).

**Estimation and characterization of β-lactamases.** The bacteria were cultivated and β-lactamase preparations were made as described previously (17). The β-lactamase activities were estimated by the hydroxylamine assay (8) with benzylpenicillin, ampicillin, cephaloridine, and oxacillin as substrates. Absolute levels of β-lactamase activity were determined by using as units of activity that amount of enzyme catalyzing the hydrolysis of 1 μmol of substrate per min at 30 C in 25 mM of phosphate buffer, pH 7.4. In practice, milliunits were used where 1 μU = 0.001 U. The effects of 0.5 mM parachloromercuribenzoate and 100 mM sodium chloride were tested as described previously (17).

Molecular weights of β-lactamases were determined by gel filtration on Sephadex G-100 with bovine serum albumin, ovalbumin, chymotrypsinogen, and cytochrome c used as markers as described before (8).

Km values were determined by using Novick's microiodometric method (8), and starch gel electrophoretic mobilities were measured as described by Dale and Smith (11).

**RESULTS**

**Antibiotic resistance and transfer.** The levels of resistance of the original strain of P. mirabilis 22 and E. coli K-12 58-161sp with and without R22K are shown in Table 1. In both hosts, R22K conferred a high level of resistance to cephalothin. As mentioned above, P. mirabilis strain 22 lost its R factor during storage. The resistance to β-lactam antibiotics, derived from P. mirabilis strain 22, was retained in E. coli 58-161sp; however, when this strain was used as donor to E. coli K-12 J62-2, only streptomycin resistance was transferred (not resistance to β-lactam antibiotics). The streptomycin-resistance plasmid, now designated R22Kb, proved to be incompatible with N3T (13), and therefore it belonged to compatibility group N.

To mobilize resistance to β-lactam antibiotics in the stored 58-161sp(R22K), R144rd3 was introduced, and this strain was mated with E. coli K-12 J62-2 as recipient, with ampicillin used for selection. Resistance was successfully transferred, usually together with transfer of R144rd3. A minority of J62-2 transciipients received resistance to β-lactam antibiotics only; the plasmid in these transciipients was non-transmissible and was termed R22Ka. R22Ka was also transferred to E. coli K-12 H114 by means of R144rd3.

Repeated attempts were made, without success, to transfer R22Ka, by means of mobilizing

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>P. mirabilis strain 22</th>
<th>E. coli K-12 58-161sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>R*</td>
<td>R*</td>
<td>R*</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>500</td>
<td>4</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>NT</td>
<td>0.5</td>
</tr>
<tr>
<td>Chephaloridine</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>125</td>
<td>8</td>
</tr>
<tr>
<td>Cephalaxine</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

* The figures given are the minimal concentrations, in micrograms per milliliter of nutrient agar, required to prevent visible growth from surface inocula of approximately 100 viable cells. The figures for E. coli K-12 R* and R* were obtained in 1964 and confirmed in 1973 (18). The figures for P. mirabilis 22 R* and R* were obtained before and after loss of R22K on storage.

* NT, Not tested.

Table 1. Levels of resistance to the β-lactam drugs of Proteus mirabilis 22 and E. coli K-12 58-161sp with and without R22K

* Downloaded from http://jb.asm.org on July 6, 2017 by guest
agents, into its original host, *P. mirabilis*, 22, or other strains of *P. mirabilis*, *P. morganii*, *P. rettgeri*, or *Enterobacter cloacae*.

**Compatibility properties of R22Ka.** We considered that R22Ka could have originated by recombination of another plasmid with the defective F factor of 58-161sp (see below). Assuming that this defective F factor had retained the compatibility properties of F, R22Ka was tested for incompatibility with F' lac and R386 (both of IncFI [12]). Introduction of these plasmids did not eliminate resistance to β-lactam antibiotics in any of 100 clones tested, nor was β-lactam antibiotic resistance eliminated by the introduction of plasmids of any of the other known incompatibility groups (12). All the doubles were stable, and from each the introduced plasmid was transmissible, unlinked to resistance to β-lactam antibiotics. In some cases, however, mobilization of R22Ka was observed. Thus, R22Ka was compatible with all known plasmid groups.

When F' lac was transferred to J62-2 and to J62-2(R22Ka) in parallel, in a timed mating experiment, the frequency of transfer to both recipients was $5 \times 10^{-2}$ per donor cell; there was no exclusion of F' lac by R22Ka.

**Resistance of R22Ka to acridine orange curing.** The sensitivity of J62-2(R22Ka) and H1t4(R22Ka) to acridine orange curing was compared with that of C600(F' lac). After exposure to acridine orange, 27 of 294 colonies had lost F' lac (no lac- colony was found in over 100 untreated controls). No loss of R22Ka was observed in 150 colonies after acridine orange treatment, or in controls.

**Plasmid molecular weights.** When extracts of 58-161sp were examined by ethidium bromide-cesium chloride equilibrium centrifugation, a plasmid DNA species which we term Fsp was detected. This plasmid is not seen in true F- strains of *E. coli* K-12, and we conclude that it is the defective F factor known to be present in this strain (22).

The molecular weights of the plasmid from strain H1t4(R22Ka) and of Fsp were compared by velocity sedimentation through sucrose gradients. The results are shown in Fig. 1a and 1b. Each plasmid sedimanted as two peaks, in the positions (left to right) expected for covalently closed circular and open circular forms, respectively. R22Ka and Fsp can be seen to have very similar molecular weights of about $61 \times 10^6$ (Table 2).

J62-2(R22Ka) was also examined, and plasmid DNA of similar molecular weight was observed (results not shown).

**Coexistence of F' lac and R22Ka.** A plasmid is defined as belonging to the FI compatibility group if it is incompatible with the F factor or F' factors such as F' lac (16). F' lac and the antibiotic resistance of R22Ka were stably inherited in a single host (see above: Compatibility properties of R22Ka). To test for their coexistence as separate plasmids, covalently closed circular DNA was isolated from H1t4(F' lac)(R22Ka). Its sedimentation analysis (Fig. 1c) showed the presence of covalently closed circular and open circular forms of two plasmids, with molecular weights of $91.9 \times 10^6$ and $60.8 \times 10^6$. These closely match the molecular weights of F' lac (Fig. 1d) and R22Ka (Fig. 1a), respectively. Since the double contained both plasmids separately, R22Ka does not belong to group FI.

**Molecular relationship of R22Ka to Fsp and F' lac.** The nonconjugative plasmid, R22Ka, appeared spontaneously in a strain of *E. coli* K-12 shown in this paper to contain the defective F factor, Fsp. The question therefore arose, whether the plasmid transferred from *P. mirabilis* 22 had recombined with Fsp. To test this hypothesis, the DNA homology of R22Ka from strain H1t4 with Fsp, and with F' lac, was measured. The results (Table 2) showed that R22Ka included DNA equivalent to about $20 \times 10^6$ daltons, homologous with each of these plasmids, and presumably derived from Fsp.

**Properties of the β-lactamase of R22Ka in E. coli J62-2.** The pH activity curve of the enzyme (Fig. 2) was determined over a range of pH values from 4.4 to 9.0 using fourfold-diluted McIlvaine's citrate-phosphate or 0.05 M borate buffer mixture. The optimum pH was 7.6, with the 90% limits of activity being 7.1 to 8.3. The activity decreased more sharply at the acidic pH values, and practically no activity could be detected at pH 4.4. These properties agree with those described (19) for the *E. coli* K-12 chromosomal enzyme.

The temperature-activity dependence of the R22Ka enzyme (Fig. 3) showed a diffuse optimum at about 40 C and a steep decline of activity at temperatures above 50 C.

The rate of heat inactivation of the enzyme was then examined by measuring the activity of enzyme solutions heated in a water bath at 60 C for various time periods and assayed at 30 C. As shown in Fig. 4, treatment for 2.5 min resulted in over 50% loss of the original activity, and heating for 20 min was sufficient to inactivate the enzyme almost completely. Thus the R22Ka β-lactamase was more thermolabile than the TEM-like β-lactamases studied by Kontomichalou et al. (18) and by Yamagishi et al. (31),
but was not as susceptible to heat denaturation as the oxacillin-hydrolyzing \( \beta \)-lactamases of RGN88 or R1818 (10).

**Comparison of the \( \beta \)-lactamase of R22Ka with that of E. coli K-12.** The organisms used in these studies were *E. coli* J62-2(R22Ka) and *E. coli* K-12 strain D3. The substrate specificity results (Table 3) show that the two \( \beta \)-lactamases were indistinguishable in their ability to hydrolyze cephaloridine and ampicillin in relation to their ability to hydrolyze benzylpenicillin. Neither enzyme hydrolyzed oxacillin or was inhibited by parachlormercuribenzoate or by sodium chloride. These results are in complete accord with those reported for the chromosomal \( \beta \)-lactamases of *S. sonnei* strains and *E. coli* strain 214T (29) which are also shown in Table 3. The characteristics of these four enzymes contrast markedly with those of the two main types of \( \beta \)-lactamase that until now have been found to be mediated by R factors (17). Included in Table 3 for comparison are the properties of the \( \beta \)-lactamases mediated by R6K and R46, which represent the characteristics of the TEM and oxacillin-hydrolyzing types of R factor-mediated \( \beta \)-lactamases, respectively.

The molecular weights of the \( \beta \)-lactamases from *E. coli* strains D3 and J62-2(R22Ka) were identical and similar to those of the enzymes from *E. coli* strain 214T and *S. sonnei* strain 2 (Table 3). However, the starch gel electrophoretic mobility results, while supporting the identity of the \( \beta \)-lactamases from *E. coli* strains D3 and J62-2(R22Ka), showed that the enzymes from *E. coli* strain 214T and *S. sonnei* differed from them and from each other. Bearing in mind the close similarity of these four enzymes in other respects, the different electrophoretic mobilities could reflect variation in a small number of non-essential amino acid residues in the enzyme proteins.

The *E. coli* strain D3 and R22Ka \( \beta \)-lactamases were also similar in their \( K_m \) values for benzylpenicillin, penicillin V, and cephalo-

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**Fig. 1. Neutral sucrose gradient analyses of \( ^{3}H \)-labeled plasmid DNA.** Plasmid DNA was isolated from (a) H1t4(R22Ka), (b) 58-161sp, (c) J62(Flac)(R22Ka) and (d) W3110Thy- (Flac). Each of these DNA samples (20 to 40 \( \mu \)l) was layered, together with 10 \( \mu \)l of 14C-labeled R1 DNA, onto a 4.6-ml 5 to 20%1o sucrose gradient and centrifuged for 90 min at 35,000 rpm in an MSE 65 rotor (3 by 5) at 20 C. Sedimentation is from right to left.
rin C (Table 3). There was a slight difference in the $K_m$ values for cephaloridine, but this could result from the technical difficulties of using the microiodometric assay with this substrate at comparatively high concentrations. These values were therefore redetermined using the spectrophotometric assay at 255 nm (25). By this method, the $K_m$ values were found to be 128 and 140 µM, respectively, for the enzymes from strain D3 and R factor R22Ka.

One possibility could explain the finding that the $\beta$-lactamase of R22Ka was the same as the enzyme mediated by the known chromosomal gene in E. coli strain D3: the plasmid might not carry the structural gene for $\beta$-lactamase, but instead have a gene which merely derepresses the low level of E. coli chromosomally determined $\beta$-lactamase. To test this hypothesis, the activities of E. coli strains H1t4 (which has a mutation affecting its level of $\beta$-lactamase production) and J62-2, with and without R22Ka, were tested against benzylpenicillin. In this test, strain J62-2 hydrolyzed 0.2 nmol of benzylpenicillin per min per $10^9$ bacteria, and strain H1t4 had no detectable activity when tested by the hydroxylamine assay technique. With the R$^+$ strains, H1t4(R22Ka) and J62-(R22Ka) hydrolyzed 16.1 and 15.7 nmol of benzylpenicillin per min per $10^9$ bacteria, respectively. Thus, as the plasmid mediated a similar level of $\beta$-lactamase activity in both hosts, it cannot have acted by derepressing the synthesis of the chromosomal $\beta$-lactamase of E. coli.

**Isoelectric focusing.** Analytical isoelectric focusing clearly separates different $\beta$-lactamases present in a single extract (21). Only a single $\beta$-lactamase band could be seen after focusing an extract of E. coli H1t4(R22Ka). This appeared to be identical with the $\beta$-lactamase bands observed with extracts of E. coli H1t4 and E. coli D31. However, when the specific activities of the $\beta$-lactamase extracts were estimated by using cephalosporin 87/312 as substrate and reading the protein concentration from the optical density at 280 nm, there was about 1,500-fold more activity in the extract from strain H1t4(R22Ka) than from the R$^-$ strain of H1t4. Hence, the great majority of
However, precipitin similar characteristic enzyme immunoisoelectric focusing from mases believed to the enzyme precipitin arc, in can be attributed from mase the p-lactamase mid.

\[ \text{\(E. coli\)} \]

The \(\beta\)-lacta-mase activity of strain H1t4(R22Ka) can be attributed to the presence of the plasmid.

**Immunooisoelectric focusing.** The \(\beta\)-lactamase from strain H1t4(R22Ka) gave a single precipitin arc, in the position characteristic of the enzyme from \(E. coli\) D31, after immunooisoelectric focusing with antiserum prepared against the \(\beta\)-lactamase of strain D31. In other immunooisoelectric focusing tests using \(\beta\)-lactamases from a variety of other strains of \(E. coli\), believed to possess solely the chromosomal enzyme characteristic of this species (9, 27), a similar precipitin arc was given with this antiserum. However, no serological reactions occurred when the antiserum was tested against \(\beta\)-lactamases from \(E. cloacae\), \(Pseudomonas aeruginosa\), \(Citrobacter freundii\), \(Hafnia\), \(Pro-tetus mirabilis\), or \(Klebsiella aerogenes\), or mediated by a variety of R factors other than R22Ka (unpublished data).

**DISCUSSION**

Gram-negative bacilli carry chromosomal genes determining \(\beta\)-lactamases characteristic

\[ \text{\(F. coli\)} \]

**TABLE 2. Physical properties of plasmid DNA**

<table>
<thead>
<tr>
<th>H-labeled plasmid</th>
<th>Mol wt (^a)</th>
<th>Homology with R22Ka (%) (^b)</th>
<th>Mol wt homologous with R22Ka</th>
</tr>
</thead>
<tbody>
<tr>
<td>R22Ka</td>
<td>(6.0 \times 10^6)</td>
<td>100</td>
<td>23.7 \times 10^4</td>
</tr>
<tr>
<td>Fsp(^c)</td>
<td>(6.4 \times 10^6)</td>
<td>38</td>
<td>18.3 \times 10^4</td>
</tr>
<tr>
<td>Flac</td>
<td>(9.1 \times 10^6)</td>
<td>20</td>
<td>23.7 \times 10^4</td>
</tr>
</tbody>
</table>

\(^a\)Calculated from the sucrose gradient analyses (Fig. 1).

\(^b\)Annealed and assayed at 75 C as described. The results have been normalized for the homologous reaction (82.5%).

\(^c\)Fsp is the defective (nonfertile) F plasmid found in \(E. coli\) 58-161sp.

**FIG. 2. Effect of pH on the activity of R22Ka \(\beta\)-lactamase.** Crude enzyme extracts were tested for \(\beta\)-lactamase activity in the presence of MacIlvaine’s buffer for the range pH 4.4 to 8.0 and 0.05 M borate buffer for the range pH 8.0 to 9.0 at 30 C. The results are expressed as a percentage of that obtained at pH 7.6.

**FIG. 3. Effect of temperature on the activity of R22Ka \(\beta\)-lactamase.** Crude enzyme extracts in 25 mM sodium phosphate buffer, pH 7.0, were tested for \(\beta\)-lactamase activity at various temperatures. The results are expressed as a percentage of that obtained at 40 C.

**FIG. 4. Rate of heat inactivation of R22Ka \(\beta\)-lactamase at 60 C.** Samples of crude enzyme extracts in 25 mM sodium phosphate buffer, pH 7.0, were heated at 60 C for various times. The samples were cooled in ice-water, and then the \(\beta\)-lactamase activity was measured at 30 C and compared with that observed in unheated controls.
TABLE 3. Enzymatic and molecular properties of β-lactamase

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>E. coli K-12</th>
<th>E. coli J62 (R22Ka)</th>
<th>E. coli strain 214T</th>
<th>Shigella sonnei strain 2</th>
<th>E. coli J62 (R46) (= R1818)</th>
<th>E. coli J62 (R6K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactamase activity (nmol of benzylpenicillin hydrolyzed per min/10^8 bacteria)</td>
<td>13.2</td>
<td>15.9</td>
<td>18.8</td>
<td>49.8</td>
<td>25.2</td>
<td>629.8</td>
</tr>
<tr>
<td>Activity relative to benzylpenicillin: †</td>
<td>Cephaloridine</td>
<td>324</td>
<td>325</td>
<td>332</td>
<td>323</td>
<td>37</td>
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<tr>
<td>Ampicillin</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>179</td>
<td>106</td>
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<tr>
<td>Oxacillin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>646</td>
<td>5</td>
</tr>
<tr>
<td>Sensitivity to 100 mM NaCl</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Sensitivity to 0.5 mM PCMB ‡</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>31,800</td>
<td>31,800</td>
<td>31,150</td>
<td>32,900</td>
<td>44,600</td>
<td>21,110</td>
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<tr>
<td>Starch gel electrophoretic mobility (mm/h towards the cathode)</td>
<td>+10.0</td>
<td>+10.0</td>
<td>+14.0</td>
<td>+11.5</td>
<td>+5.1</td>
<td>-17.0</td>
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<tr>
<td>Kₘ values (μM)</td>
<td>benzylpenicillin</td>
<td>5.5</td>
<td>3.5</td>
<td>4.0</td>
<td>14.0</td>
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<td>Penicillin V</td>
<td>8.6</td>
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<td>Cephalosporin C</td>
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<tr>
<td>Cephaloridine</td>
<td>134</td>
<td>202</td>
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<td></td>
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<td>75.4</td>
</tr>
</tbody>
</table>

* Micromoles of substrate hydrolyzed per minute, expressed as a percentage of the activity against benzylpenicillin.
† R, Resistant to inhibition; 15 mM = concentration required for 50% inhibition; PCMB, parachloromercuribenzoate.
‡ Data from Smith, Bremner and Datta (29).
§ Data from Dale and Smith (9).
¶ Data from Hedges et al. (17).
∥ Data from Dale and Smith (8).
¶¶ Data from Dale and Smith (11).
¶¶¶ Data from J. W. Dale, unpublished.

for each genus (30). However, even within one species, variations in types of β-lactamase are known (15). Plasmids determining β-lactamases are also widespread among gram-negative bacteria. The plasmid β-lactamases so far described belong to two main types, referred to by us as TEM-type and oxacillin-hydrolyzing (17). The evolutionary history of the plasmid-determined enzymes is unknown. One possibility is that the genes determining them originated as bacterial chromosomal genes which became incorporated into plasmids by recombinational steps analogous to those involved in the formation of F-prime factors. For example, it has been suggested that the gene for TEM-like β-lactamase originated as a chromosomal gene in the genus Klebsiella (14). The TEM β-lactamase resembles, but is not identical with, β-lactamases from K. aerogenes (9); indeed, they are quite different when tested by immunoisoelectric focusing (unpublished data). The R22Ka enzyme described here was, in our tests, identical with the β-lactamase determined by a chromosomal gene of E. coli, though further studies would be required to prove beyond dispute their true identity. Kontomichalou et al. (18) reported that the enzyme, unlike that of E. coli K-12, was sensitive to parachloromercuribenzoate, but subsequent experiments indicated that the reported parachloromercuribenzoate inhibition was an artifact resulting from the thermolability and general instability of the R22Ka β-lactamase.

R22K was originally found in P. mirabilis, a species whose strains either produce no detectable β-lactamase (28) or enzymes unrelated to those of E. coli (unpublished data). During storage, the R factor underwent genetic interactions. Nevertheless, the β-lactamase was still plasmid-determined, since acquisition of resistance to β-lactam antibiotics by strain H114 or by strain J62-2 was accompanied by the acquisition of a new plasmid DNA species.

The genetic events leading to the formation of
R22Ka cannot now be elucidated. It belongs to none of the known incompatibility groups, but we have no grounds for suggesting that its compatibility specificity arose de novo during storage of the R+ culture. The original R22K, now lost, may have exhibited the same specificity: it may not have been a single plasmid. It would seem that in E. coli K-12 58-161sp, a recombinant event between the resident Fsp plasmid and R22K resulted in the formation of R22Ka. The DNA hybridization studies showed that R22Ka contains a DNA segment equivalent to about one-third of the Fsp genome. Thus, two-thirds of the Fsp plasmid have been deleted, and this DNA would seem to have included at least the genes necessary for the F exclusion properties of Fsp (22). As R22Ka has a similar molecular weight to Fsp, two-thirds of its DNA must have come from the original P. mirabilis strain 22. All that is known about this stretch of DNA is that it contains the gene(s) necessary for resistance to β-lactam antibiotics. Although the β-lactam antibiotic resistance gene(s) were derived from P. mirabilis, the enzyme studies show that R22Ka determines a β-lactamase indistinguishable from that determined by the chromosome of E. coli strain K-12. In the course of these experiments, we failed to transfer R22Ka to any bacterial host other than E. coli and we could not, therefore, study its β-lactamase without a background of the E. coli enzyme. An approach was made with strain H1t4, a mutant of E. coli K-12 reported to produce only traces of β-lactamase activity (H. G. Boman, personal communication) and that has a lesion in its β-lactamase structural gene (5). The results showed that the R22Ka plasmid conferred a level of β-lactamase activity as efficiently in this mutant as that produced in E. coli strain J62-2, which is wild type with respect to chromosomal β-lactamase synthesis. Hence, the R22Ka plasmid seems to possess the structural gene for the synthesis of a β-lactamase that could have been acquired by the plasmid from the chromosome of an E. coli ancestor.

ACKNOWLEDGMENTS

We thank the Medical Research Council for grants to Naomi Datta and J. T. Smith. We thank Hans G. Boman for supplying strain H1t4 and J. Surridge for technical assistance.

LITERATURE CITED


