Analytical Isoelectric Focusing of R Factor-Determined $\beta$-Lactamases: Correlation with Plasmid Compatibility

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R factor-determined $\beta$-lactamases have been investigated by analytical isoelectric focusing. The enzymes such as those specified by the R6K and RP4 plasmids (TEM-type enzymes) are notably homogenous in biochemical tests (Hedges et al., 1974), but two subclasses can be distinguished by isoelectric focusing. Three subclasses can be distinguished among the oxacillin-hydrolyzing enzymes, in good agreement with the classification based upon biochemical characteristics (Dale and Smith, 1974). The TEM-type $\beta$-lactamases are promiscuously distributed among plasmids of a wide variety of compatibility groups, whereas the various oxacillin-hydrolyzing enzymes show some degree of correlation with compatibility.

Proteins that appear to be identical in biochemical and immunological tests can be separated by analytical isoelectric focusing (13). This method has been applied to $\beta$-lactamases (EC 3.5.2.6) (11).

$\beta$-Lactamases determined by R factors were previously characterized biochemically and fell into two main types (7). One type was exemplified by the enzyme specified by plasmid R6K (TEM enzyme); the other type was characterized by its ability to hydrolyze oxacillin. The oxacillin-hydrolyzing (OXA) enzymes were later subdivided on biochemical criteria (3).

In this paper we classify, by isoelectric focusing, the $\beta$-lactamases of those R factors that have been examined previously and those of numerous other plasmids.

MATERIALS AND METHODS

Bacterial strains. The hosts for the R factors were Escherichia coli K-12 strains J53, J53-1, and J53-2 (2). Another strain of E. coli K-12, D31 (1), was used as a source of chromosomally mediated $\beta$-lactamase.

Plasmids. The R factors tested are shown in Table 1.

Enzyme preparation and isoelectric focusing. The methods used for preparation of the enzymes and for isoelectric focusing were as described previously (11). Each $\beta$-lactamase focuses as a group usually consisting of one main band and some satellite bands. However, plasmid R46 specifies a $\beta$-lactamase that focuses as a doublet of main bands accompanied by satellites.

RESULTS

$\beta$-Lactamase cultures of E. coli K-12 carrying 107 different R factors determining ampicillin resistance were examined by isoelectric focusing (Table 1). Of the plasmids tested, 100 were introduced into E. coli K-12 from naturally occurring bacterial isolates and 7 were laboratory constructs, the $\beta$-lactamase genes being part of a transposon; this is a sequence of deoxyribonucleic acid (DNA), transposable between replicons (8).

Two groups of $\beta$-lactamase bands were observed in each instance, one representing the chromosomally determined enzyme (Fig. 1, I), and the other representing the R-factor determined enzyme (Fig. 1, II to VI).

All of the plasmid-determined $\beta$-lactamases fell into two main classes, as described previously (7), but the isoelectric focusing technique showed subdivisions within each class.

TEM-type $\beta$-lactamases. The enzymes previously described as TEM type (4, 7), although uniform in their biochemical and serological reactions, were seen to be of two, and only two, types by isoelectric focusing (Fig. 1, II and III). One type, exemplified by the enzyme of plasmid R6K, predominated and was specified by R factors of nearly all compatibility groups. We refer to this $\beta$-lactamase as type TEM-1 (Fig. 1, II). The other, exemplified by the enzyme of the plasmid RP4, was less common. It was determined by a minority of plasmids within groups FI, C, and P. We call this type of enzyme TEM-2 (Fig. 1, III). It is identical to the $\beta$-lactamase RP1 (11).

Of the 100 naturally occurring R factors examined, 77 specified $\beta$-lactamases of type TEM; of these, 70 were TEM-1 and 7 were TEM-2. Where plasmids had acquired their
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ampicillin resistance by transposition, the β-lactamase was either TEM-1 or TEM-2, according to that of the donor plasmid.

**OXA β-lactamases.** These enzymes were divisible into two main classes (by biochemical tests), one of which contained two subclasses (3). Isoelectric focusing confirmed this classification. We designate the three types OXA-1, OXA-2, and OXA-3 (Fig. 1, IV to VI).

Of the 107 R factors examined, 23 determined OXA β-lactamases.

**DISCUSSION**

All of the naturally occurring R factors that confer resistance to penicillins determine production of a β-lactamase. On biochemical criteria these penicillinases fall into two classes, the TEM-like enzymes and the OXA enzymes (4, 7). The TEM-like β-lactamases are strikingly uniform in biochemical properties (7), but two types (TEM-1 and TEM-2) are distinguishable by isoelectric focusing. Because of the many similarities between TEM-1 and TEM-2 enzymes, we believe that these enzymes are closely related. This conclusion is supported by studies of the DNA that encodes the enzymes.

The β-lactamase of RP4 is determined by a gene that forms part of transposon A, a $3 \times 10^4$-dalton sequence of DNA (8). Homologous sequences (for the whole transposon, not merely the β-lactamase gene) are carried by R factors that determine TEM-1 or TEM-2 (10). Thus, the gene for TEM-1 must be highly homologous with that for TEM-2, and the origin of the transposon must predate the divergence of the TEM enzymes.

The high proportion of ampicillin R factors that determine TEM enzymes and the promiscuity with which the genes for these enzymes are distributed among plasmids of so many different compatibility groups is evidence for the efficiency of the transposon mechanism.

Sawai et al. (12) showed that one R factor (R(INs23)0-lactamase by Sawai et al. [12]) had an isoelectric point of 6.9 and is therefore unlikely to be identical to TEM-1 and TEM-2 β-lactamases, which have isoelectric points of 5.4 and 5.6 (11), respectively.

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<td>R775</td>
<td>+</td>
<td>TEM-2 + OXA-2</td>
<td>Canada</td>
</tr>
<tr>
<td>R781</td>
<td>+</td>
<td>TEM-1 + OXA-1</td>
<td>U.K.</td>
</tr>
<tr>
<td>R784</td>
<td>+</td>
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<td>U.K.</td>
</tr>
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<td>R787</td>
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<tr>
<td>R799a</td>
<td>+</td>
<td>TEM-2 + OXA-2</td>
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</tr>
<tr>
<td>R799b</td>
<td>+</td>
<td>TEM-1 + OXA-1</td>
<td>Canada</td>
</tr>
<tr>
<td>R811r</td>
<td>+</td>
<td>TEM-2 + OXA-2</td>
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<tr>
<td>R840</td>
<td>+</td>
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<tr>
<td>R959</td>
<td>+</td>
<td>TEM-2 + OXA-2</td>
<td>U.K.</td>
</tr>
<tr>
<td>R963A</td>
<td>Non-self-</td>
<td>+</td>
<td>U.S.A.</td>
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<tr>
<td>ROX176</td>
<td>transmissible</td>
<td>+</td>
<td>E. coli</td>
</tr>
<tr>
<td>R645</td>
<td>+</td>
<td>Laboratory construct</td>
<td>U.K.</td>
</tr>
<tr>
<td>R870</td>
<td>+</td>
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<td>Poland</td>
</tr>
<tr>
<td>AP110</td>
<td>+</td>
<td>Laboratory construct</td>
<td>Laboratory construct*</td>
</tr>
<tr>
<td>AP201</td>
<td>+</td>
<td>Laboratory construct*</td>
<td>Laboratory construct*</td>
</tr>
</tbody>
</table>

*a Plasmid known to carry the transposon A polynucleotide sequence (10).

* Plasmid carrying transposon A, derived from RP4 (8).

* R factor previously assigned to an established compatibility group but, subsequently, strains carrying this factor were found to carry two distinct species of DNA supercoil. Hence, the compatibility properties are not well established.

* A fusion plasmid (9). The ampicillin resistance was originally on an R factor transmissible between strains of *P. aeruginosa* but not to enterobacteria. The compatibility properties of this plasmid are under investigation.

* Plasmid carrying transposon A, derived from RI (F. Heffron, personal communication).
Fig. 1. β-Lactamases determined by R factors. Preparations from the following strains of E. coli were examined by isoelectric focusing (the type of enzyme determined by each R factor is shown in brackets): I, D31; II, K-12 R6K (TEM-1); III, K-12 RP4 (TEM-2); IV, K-12 R_{6239} (OXA-1); V, K-12 R46 (OXA-2); VI, K-12 R55 (OXA-3). The positions of the comparatively weak chromosomal β-lactamases from strains II through VI are marked with a series of slashes because they are seen as bands only in later serial photographs.
The OXA enzymes are much more heterogeneous than the TEM enzymes, by biochemical criteria. Analytical isoelectric focusing serves to distinguish three classes, and these correspond with the biochemical classification of Dale and Smith (3).

The relative rarity of the OXA β-lactamases makes it more difficult to investigate correlations between enzyme type and R factor compatibility; nevertheless, some relationships may be significant. The OXA-1 enzymes are determined only by plasmids of groups Fl, Iα, and V and an unclassified plasmid, R775. The OXA-2 genes have been detected on plasmids of groups N, O, and P and on R62, a plasmid of group I that is known to include extensive polynucleotide sequences homologous with (and presumably derived from) N group DNA. There is evidence that the β-lactamase determinant of R62 may be part of the DNA from the N plasmid (5). The OXA-3 enzymes are determined only by plasmids of the A-C complex (6). Other A-C complex members are known which determine TEM enzymes and, in at least one case, R746 (10), the transposon A DNA sequence is present.

We have not observed any significant correlation between β-lactamase type and the wild host species, the country in which the wild host was isolated, or the date of the isolation.

ACKNOWLEDGMENTS

We thank Naomi Datta for generously providing some of the strains, Janet E. Horne for preparing the enzymes, and S. R. Jones for taking the photographs.

ADDENDUM

After this manuscript was submitted for publication, Sawai kindly sent us plasmid R_{ONSA}. We have shown that this plasmid is a member of compatibility group P and specifies a single β-lactamase, TEM-2. Sawai et al. (12) used zone electrophoresis to determine the isoelectric point of the type Ib β-lactamase specified by R_{ONSA} and thus differentiate it from the classical TEM-type Ia enzyme specified by R_{ON}. We confirm this distinction, although the apparent isoelectric points of the two TEM enzymes, determined by isoelectric focusing (11), differ from the values given by Sawai et al. (12). R_{ONSA} belongs to compatibility group D.

LITERATURE CITED