PLASMID-MEDIATED CARBENICILLIN HYDROLYZING BETA-LACTAMASES OF *PROTEUS MIRABILIS*

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Since ABRAHAM and CHAIN[1] reported the production of beta(β)-lactamase by *Escherichia coli*, penicillinases have been shown to play an important role in the resistance of organisms against β-lactam antibiotics[2,3]. In many cases, the β-lactamases of Gram-negative bacteria have been shown to be mediated by R plasmids[4], in contrast to chromosomally encoded drug resistance[5]. β-Lactamases of *Proteus mirabilis* are known to be encoded both by plasmids and by the host chromosome. These type enzymes, except the β-lactamase mediated by the plasmid R22K[6], have previously been classified according to their substrate specificity[2,7~10]. This report describes the substrate profiles, isoelectric points and immunological specificities of β-lactamases mediated by R plasmids obtained from *P. mirabilis*.

The R plasmids used in this study were isolated from 7 different ampicillin resistant strains of *P. mirabilis*. The resistance patterns and incompatibility groups of these plasmids are shown in Table 1. All the R plasmids conferred resistance to multiple drugs and were conjugally selftransmissible. Incompatibility grouping of R plasmids was conducted as described by COETZEE *et al.*[11] and by DENNISON[12]. Crude β-lactamase extracts were prepared from *E. coli* C transconjugants carrying each R plasmid. β-Lactamase activity was determined either spectrophotometrically[13] by measuring the change in absorption maximum for the β-lactam ring at a constant temperature of 30°C (Beckman model 24) or by a modification of the NOVICK microiodometric method[14]. One unit of enzyme activity was defined as the amount of enzyme which hydrolyzed 1 μmole of a substrate per minute at 30°C in 0.05 M phosphate buffer (pH 7.0). The concentration of protein was estimated by the method of LOWRY *et al.*[15] using bovine serum albumin as the standard. The isoelectric point of the enzyme was determined by the isoelectric focusing chromatography column (model LKB8100). The immunological identification of a β-lactamase was done by neutralization test of the hydrolyzing activity of the enzyme[16], as follows: Enzyme preparations were incubated with antiserum for 1 hour at 37°C and then stored for 18 hour at 4°C before the addition of a substrate.

Table 1. Resistance patterns and incompatibility group of R plasmids from *Proteus mirabilis*.

R plasmid	Incompatibility group	Resistance pattern
Rms426	—[a]	APC TC CM SM SA KM
Rms427	—	APC TC CM SM SA KM
Rms428	V	APC SM SA
Rms429	P	APC TC CM SM SA
Rms430	T	APC SM SA
Rms431	V	APC SM SA KM
Rms432	M	APC SM SA KM

[a] Not determined.

APC, ampicillin; TC, tetracycline; CM, chloramphenicol; SM, streptomycin; SA, sulfonamide; KM, kanamycin.

The substrate profiles of R plasmid-mediated β-lactamases from *P. mirabilis* are listed in Table 2. Comparisons were made with the corresponding profiles of the four β-lactamase standards. Plasmids Rms212, Rms213, Rte16, and Rms139, respectively, encode type I (or TEM type[17]), type II[18], type III (oxacillin-hydrolyzing or OXA type[19]), and type IV (carbenicillin-hydrolyzing or PSE type[20]) β-lactamase.

Based on the relative rate of carbenicillin hydrolysis, these *P. mirabilis* β-lactamases can be classified into two groups. The β-lactamases mediated by plasmids Rms428 and Rms429 gave high rates of hydrolysis of carbenicillin and showed very similar substrate profiles to the type IV enzyme of plasmid Rms139. The isoelectric points of the β-lactamases of Rms428 and Rms 429 were pH 5.7 which was identical with that of the type IV (Rms139) enzyme. Further studies revealed that the β-lactamases of Rms428 and Rms429 were inhibited by antiserum to the type IV (Rms139) enzyme (unpublished data, KATSU *et al.*). Therefore, it appears that these enzymes are type IV β-lactamases. In contrast, β-lacta-

Table 2. Substrate profiles of R plasmid-mediated β-lactamases from *Proteus mirabilis*.

Plasmid designation	β-Lactamase type	Specific[a] activity (unit/mg of protein)	Relative rate of hydrolysis[b]				
			PCG	APC	CPC	CLX	CER
Rms426	type I	5.37	100	101	16	<5	14
Rms427	type I	6.60	100	100	20	<5	16
Rms428	type IV	1.47	100	90	123	<5	4
Rms429	type IV	0.58	100	95	145	<5	5
Rms430	type I	0.33	100	110	10	<10	15
Rms431	type I	0.32	100	96	10	<10	15
Rms432	type I	6.28	100	115	12	<5	14
Rms212	type I	1.98	100	108	11	<5	15
Rms213	type II	0.09	100	472	44	130	31
Rte16	type III	0.29	100	110	45	300	50
Rms139	type IV	1.15	100	94	117	<5	5

[a] Specific activity was determined with 100 μM PCG as the substrate.
[b] Rates of hydrolysis of substrates were expressed in percent of hydrolysis of PCG.
PCG, benzylpenicillin; CPC, carbenicillin; CLX, cloxacillin; CER, cephaloridine.

mase mediated by the remaining plasmids Rms 426, Rms427, Rms430, Rms431, and Rms432, showed substrate profiles similar to the type I enzyme mediated by plasmid Rms212.

Several investigators have reported various types of R plasmid-mediated β-lactamases derived from *P. mirabilis*[7,8,9], but there is no report of a type IV β-lactamase from *P. mirabilis*. The type IV β-lactamase is normally specified by plasmids occurring only in *Pseudomonas*[21]. Therefore, the identification of type IV β-lactamases mediated by R plasmids in *P. mirabilis* strains is of evolutionary significance.

HEDGES and JACOB[22] reported that the structural gene encoding the TEM type β-lactamase is located on transposable DNA units which can move between different replicons. The transposability of type I β-lactamase genes is reflected in the variety of bacterial species in which these enzymes have been found, and in the range of incompatibility groups to which the plasmids specifying the enzymes belong. On the other hand, the type IV β-lactamase determinants have been detected only on plasmids of incompatibility group P[23]. In this study, however, plasmids Rms428 and Rms429 belong to incompatibility group V and P, respectively. In addition, two R plasmids, Rms298 and Rms433, that mediate type IV β-lactamases have recently been obtained from clinical isolates of *E. coli*[24] and *Enterobacter cloacae* (unpublished data; this laboratory). Plasmid Rms433 belongs to incompatibility group W, but plasmid Rms298 could not be identified. MEDEIROS *et al.*[25] also reported the type IV β-lactamase specified by plasmids of new incompatibility group in *E. coli*. Recently, we have obtained evidence that the structural gene for the type IV β-lactamase, like that of the type I (or TEM type) enzyme, is located on a transposon (manuscript in preparation). These results suggested that the type IV β-lactamase may be disseminated among various species of Gram-negative bacteria. Detailed studies on the enzymological and physicochemical properties of these latter β-lactamases will be described elsewhere.

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