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Properties of PSE-2 β-Lactamase and Genetic Basis for Its Production in *Pseudomonas aeruginosa*

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The properties of PSE-2 β-lactamase have been examined by using two new PSE-2-producing plasmids, pMG33 and pMG74, as well as plasmid R151, found in *Pseudomonas aeruginosa*. PSE-2 β-lactamase resembled other PSE enzymes in activity against carbenicillin, but it also resembled OXA enzymes, such as OXA-1, in rapid hydrolysis of oxacillin, cloxacillin, and methicillin and in inhibition by sodium chloride but not by cloxacillin. Antisera that inactivated TEM-1, TEM-2, OXA-1, or PSE-1 and PSE-4 β-lactamase failed to cross-react with PSE-2, which thus appears to be immunologically distinct. The plasmids determining PSE-2 varied in geographical origin, size, transfer proficiency, and incompatibility specificity, but all determined resistance to carbenicillin, gentamicin, kanamycin, streptomycin, spectinomycin, sulfonamide, and tobramycin. From a pUZ8-R151 recombinant plasmid in *Escherichia coli*, the PSE-2 β-lactamase gene could be transposed to a second plasmid in a 6.4-megadalton unit together with resistance to gentamicin, kanamycin, streptomycin, spectinomycin, sulfonamide, and tobramycin. Transposition was *recA* independent. We propose the designation Tn*1404* for this unit, which, like transposons carrying OXA-1, PSE-1, PSE-4, and some transposons determining TEM-1, includes genes for β-lactam, aminoglycoside, and sulfonamide resistance.

Of the β-lactamases determined by plasmids in gram-negative bacteria, four have been termed *Pseudomonas*-specific enzymes (PSE) since they were initially found only in strains of *Pseudomonas aeruginosa* (10, 31). As a group, the PSE β-lactamases have higher activities against carbenicillin than other plasmid-determined enzymes, but one, PSE-2, resembles the OXA β-lactamase group in substrate range as well (30). PSE-2 was characterized by Matthew by using plasmid R151, the sole *Pseudomonas* plasmid then known to produce this β-lactamase (30). Although readily transmissible between *Pseudomonas* strains, R151 is not transferable to *Escherichia coli* (3), thus apparently accounting for the distribution of the enzyme (14).

Recently, the PSE designation has been questioned because of the discovery of *E. coli* and other gram-negative enteric organisms that elaborate PSE-1 β-lactamase (18, 33). In these *Enterobacteriaceae*, a variety of plasmids are responsible for PSE-1 production, and the gene for PSE-1 has been shown to be carried on a transposon linked to resistance to streptomycin, spectinomycin, sulfonamide, and mercuric ion (19, 33). As additional β-lactamase-producing *P. aeruginosa* strains have been characterized, two new plasmids have been found that produce PSE-2. This paper describes the characteristics of the enzyme and the properties of the *Pseudomonas* plasmids responsible for its production. In addition, evidence is presented that the gene for PSE-2, like that for PSE-1, is linked to genes for aminoglycoside and sulfonamide resistance on a transposon.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. The bacterial strains and plasmids used are listed in Table 1. In addition, plasmid pMG33 was detected in *P. aeruginosa* McCl isolated by S. Cohen in Chicago and provided by G. Miller (Schering Corp., Bloomfield, N.J.). Plasmid pMG74 originated in *P. aeruginosa* 1978–81 provided by Y. Tselentis from the urine of a patient hospitalized in Athens, Greece. The sources of phages B3, B39, D3, E79, F116, G101, M6, PB1, and PR4 have been given (12).

Media. For liquid cultures, *P. aeruginosa* strains were grown in nutrient broth (Difco Laboratories, Detroit, Mich.) containing 4 mg of potassium nitrate per ml (NNB) (12). *E. coli* strains were grown in Luria (L) broth (28). Selection plates contained minimal medium A (7), supplemental growth factors as required, 2% agar (Difco), and 0.5% glucose. Supplements were added at the following concentrations (in micrograms per milliliter): L-arginine, 100; L-isoleucine, 70; L-leucine, 80; L-methionine, 30; L-proline, 50; thiamine, 0.34; L-tryptophan, 25; and L-valine, 117. Antibiotics were added at the following concentrations for *P. aeruginosa* (in micrograms per ml): carbenicil-

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or phenotype[a]	Reference
P. aeruginosa		
PAO38 Rif^r	*leu-38 rif*	16
PAO303	*argB18 chl-2*	16
PU21	*ilvB112 leu-1 rif str-1*	12
E. coli		
E5-2	*trpE5 rif*	10
J53-1	*metF63 pro-22 nal*	5
KL16-99	Hfr *recA1 thi-1*	29
C Ilv$^-$	*ilv res mod*	12
Plasmid		
R1	Ap Cm Km Sm Su IncFII	11
RP1-1	Cb IncP-11	14
pMG5	Ak Km Su Tm Hg Pma Ter IncP-2	15
R6K	Ap Sm IncX	11
RP8	Cb IncP-11	15
pUZ8	Km Tc Hg IncP-1	10
pUZ8-R151	Ap Gm Km Sm Sp Su Tc Tm Hg IncP	10
RPL11	Cb Cm Gm Sm Su Tc Hg Ter IncP-2	15
pMG19	Cb Sm Sp Su	10
R56Be	Cb IncP-11	14
RIP64	Cb Cm Gm Su Tm Hg IncP-3	15
R151	Cb Gm Km Sm Sp Su Tm IncP-11	3
RGN238	Ap Cm Sm Su Tc Hg IncFI	11
R388	Su Tp IncW	11
pUB5573	Tp IncW	41
CAM	Cam Ter Uv IncP-2	15
P*lac*	Lac Su IncC	11
S-a	Cm Gm Km Sm Su Tm IncW	11

[a] Genotype symbols: *arg*, arginine; *chl*, chloramphenicol resistance; *ilv*, isoleucine plus valine; *leu*, leucine; *met*, methionine; *mod*, modification; *nal*, nalidixic acid resistance; *pro*, proline; *rec*, recombination; *res*, restriction; *rif*, rifampin resistance; *str*, streptomycin resistance; *thi*, thiamine; *trp*, tryptophan. Plasmid resistance phenotype symbols are as follows: Ak, amikacin; Ap, ampicillin; Cb, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim; Hg, mercuric chloride; Pma, phenylmercuric acetate; Ter, potassium tellurite; Uv, ultraviolet irradiation. Others: Cam, ability to utilize camphor: Lac, ability to utilize lactose; Inc, incompatibility group.

lin, 200 or 2,000; gentamicin, 20; kanamycin, 500; rifampin, 100; streptomycin, 100; sulfadiazine, 5,000; and tobramycin, 10. For *E. coli*, lower concentrations of antibiotics were employed (in micrograms per ml): carbenicillin, 25; chloramphenicol, 25; kanamycin, 25; nalidixic acid, 100; spectinomycin, 50; streptomycin, 25; sulfadiazine, 100; tetracycline, 25; and trimethoprim, 100. For selecting or scoring Hg^{2+} resistance, 0.1 mM $HgCl_2$ was added to appropriately supplemented minimal plates. Tellurite resistance was scored on brain heart infusion agar (Difco) plates containing 0.5 mM K_2TeO_3 (39). Spectinomycin resistance was determined with a 200-μg disk (A/S Rosco, Taastrup, Denmark).

Phage was propagated with ZC agar overlays on Z agar plates, and it was harvested and diluted in Z broth (12). Lysates were sterilized with chloroform, except for chloroform-susceptible F116 and PR4, which were passed through membrane filters (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.).

Matings. Matings were performed in NNB for *P. aeruginosa* and in L medium for *E. coli* by mixing equal volumes of exponentially growing cultures of the donor at 10^8 cells per ml and the recipient at 5×10^8 cells per ml at 37°C, usually for 2 h but occasionally overnight (18 h) (12). The frequency of transfer was calculated with respect to the number of donor cells at the end of mating. Compatibility tests were performed as described previously (12).

Plasmid detection and characterization. Plasmids pMG33 and pMG74 were detected in 18-h crosses between multiresistant *P. aeruginosa* clinical isolates and strain PAO38 Rif^r by plating on antibiotic-containing media with rifampin for counterselection. PAO38 R$^+$ derivatives were scored for unselected markers by spotting on plates containing antibiotic, Hg^{2+}, or K_2TeO_3, tested for UV light resistance as described (12), crossed with PAO303 to establish transfer proficiency, and spotted with phage lysates to screen for inhibition of phage propagation (12). Their plasmid contents were determined by agarose gel electrophoresis.

Gel electrophoresis. Agarose gel electrophoresis was performed as described by Birnboim and Doly (1).

Plasmid molecular weight was estimated from the mobilities in the same gels of standard plasmids of known sizes.

β-Lactamase assay. Cultures were grown in the presence or absence of 0.5 mg of benzylpenicillin per ml, and sonic extracts were prepared (26, 27). Values of V_{max} for various substrates were measured by a computerized microacidimetric method (20) and are expressed relative to the V_{max} for benzylpenicillin as 100. One unit of β-lactamase was defined as the amount of enzyme that hydrolyzed 1 μmol of benzylpenicillin per min at pH 7 and 30°C. Specific activities were expressed as milliunits of β-lactamase per milligram of protein, estimated by measuring absorbance at 280 nm.

Analytical isoelectric focusing. Analytical isoelectric focusing on polyacrylamide gels was performed as described by Matthew et al. (32), except that an iodometric procedure was used to localize enzyme activity (24). Differential isoelectric focusing was carried out with samples applied at the cathode as well as the anode (36).

Inhibition of β-lactamase activity. The concentration of Cl^- required to cause 50% inhibition of activity was determined by adding NaCl in concentrations between 1 and 100 mM before the addition of benzylpenicillin (9). Inhibition by cloxacillin was determined from the rate of benzylpenicillin hydrolysis in the presence of 0.1 mM inhibitor. Clavulanic acid and sulbactam (40 μM) were preincubated with the enzyme for 10 min at 30°C before testing the rate of benzylpenicillin hydrolysis.

Purification of PSE-2 β-lactamase. PU21 (R151) was grown for 18 h at 30°C in 20 liters of NNB, harvested by centrifugation, suspended in 0.05 M phosphate buffer (pH 7), and disrupted by sonication (27). The broken-cell suspension was centrifuged at 49,000 × *g*, and β-lactamase was purified from the supernatant by DEAE-cellulose chromatography (DE52, Whatman Ltd.) and gel filtration on 5% acrylamide and 4% agarose (Ultrogel AcA 54, Industrie Biologique Française) (34). Active β-lactamase fractions were concentrated by ultrafiltration with a Micro-ProDiCon apparatus (Bio-Molecular Dynamics, Beaverton, Oreg.) with a 10,000-molecular-weight cutoff. The enzyme was purified 56-fold with 26% yield.

Preparation of antisera. Antisera to β-lactamase TEM-1 (purified 450-fold from *E. coli* P111 [23]), CARB-3 (purified 150-fold from *P. aeruginosa* Cilote [25]), and OXA-1 (purified over 1,000-fold from *P. aeruginosa* PU21 pMG90 [A. Philippon, G. Paul, and G. A. Jacoby, Abstr. 13th Int. Cong. Microbiol. 1982, P42:1, p. 121]) were raised in rabbits by five intramuscular injections of enzyme emulsified in Freund complete adjuvant. The rabbits were bled 2 weeks after the last booster injection.

Neutralization of β-lactamase activity. Antiserum (50 μl) was mixed with an equal volume of β-lactamase preparation containing 0.5 U/ml for 20 min at 4°C and centrifuged at 10,000 rpm in capillary tubes with a microhematocrit centrifuge. Specific inhibition was assessed from residual enzyme activity determined by a zymogram technique (22). Supernatant (10 μl) was placed in wells made in an iodine-iodide-starch-benzylpenicillin agar gel. After 6 h at 4°C, the zone of discoloration (proportional to the β-lactamase activity) was compared with the zone produced by the same β-lactamase preparation in normal rabbit serum undiluted and diluted serially in twofold steps.

RESULTS

The properties of the three PSE-2 β-lactamase-producing plasmids from *P. aeruginosa* are summarized in Table 2.

Plasmid R151. Plasmid R151 was detected by Bryan et al. (3) in *P. aeruginosa* POW (17) from Chicago that transferred the resistance phenotype Cb Gm Km Sm Sp Su Tm to *P. aeruginosa* recipients (the following abbreviations for resistance will be used: Ap, ampicillin; Cb, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim; and Hg, mercuric chloride). Although assigned initially to the P-3 incompatibility (Inc) group (3), crosses 1 and 2 in Table 3 showed that R151 was compatible with IncP-3 plasmid RIP64 as either the entering or resident plasmid. Crosses 3 to 5 demonstrated that R151 as the resident plasmid was eliminated by entry of plasmid RP1-1, RP8, or R56Be, suggesting that these four plasmids belonged to the same incompatibility group. Crosses 6 to 8, however, showed that although R151 as the entering plasmid was subject to entry exclusion by RP1-1, RP8, or R56Be, these plasmids were eliminated in less than half of the R151 transconjugants. The remainder carried both plasmids stably, and a strain carrying both R151 and RP1-1 could outcross each plasmid independently with the same incompatibility relationships as the parental plasmids when transferred into RP1-1- or R151-containing recipients. Nonreciprocal results in incompatibility tests have been observed previously in other incompatibility groups (6) and also between RP1-1 or R56Be and the fertility plasmid FP110 that appears to belong to the same incompatibility group (37). We propose IncP-11 for this group that includes R151, RP1-1, RP8, R56Be, and FP110. Like RP1-1, R56Be, and FP110 (16, 37), R151 inhibited the propagation of phage B39. R151 could not be transferred from *P. aeruginosa* to *E. coli*, confirming the findings of Bryan et al. (3). Its molecular weight was about 22 × 10^6 (Fig. 1).

Plasmid pMG33. Plasmid pMG33 was detected in a cross between a multiply resistant *P. aeruginosa* isolate, also from Chicago, and strain PAO38 Rif^r that at a low frequency (ca. 10^{-7} per donor after an 18-h mating) yielded Cb Gm Km Sm Sp Su Tm transconjugants. Plasmid pMG33 could not be outcrossed to strain PAO303 and thus appeared to be transfer deficient (Tra^-). It had a molecular weight of 26 × 10^6 (Fig. 1) and inhibited the propagation of phages B3, D3, F116, and G101 by determining the *Pae*R7 DNA restriction-modification system

TABLE 2. Properties of plasmids producing PSE-2 β-lactamase

Plasmid	Origin	Resistance phenotype[a]	Transfer proficiency	Bacteriophage inhibition	Mol wt ($\times 10^6$)	Incompatibility group
R151	Chicago	Cb Gm Km Sm Sp Su Tm	+	B39	22	P-11
pMG33	Chicago	Cb Gm Km Sm Sp Su Tm	−	B3 D3 F116 G101	26	Unclassified
pMG74	Athens	Cb Gm Km Sm Sp Su Tm Hg Ter Uv	+	B3 D3 E79 G101 M6 PB1	>200	P-2

[a] For abbreviations, see Table 1, footnote *a*.

(16). It thus resembled other Tra⁻ plasmids that produce this system for phage restriction and that appear to belong to a single, as yet unnamed, incompatibility group distinct from IncP-11 (16).

Plasmid pMG74. Plasmid pMG74 was detected in a cross between a multiply resistant *P. aeruginosa* isolate from Athens, Greece, and strain PAO38 Rif^r that at a frequency of 10^{-3} in a 2-h mating yielded Cb Gm Km Sm Sp Su Tm transconjugants. The plasmid also determined resistance to mercuric ions, potassium tellurite, and UV irradiation and inhibited the propagation of phages B3, D3, E79, G101, M6, and PB1. The property of tellurite resistance (39) and this pattern of phage inhibition (12) suggested the presence of an IncP-2 plasmid. Plasmid pMG74 indeed proved to be incompatible with the IncP-2 metabolic plasmid CAM (Table 3, cross 9). By agarose gel electrophoresis (Fig. 1), its molecular weight was found to be in excess of 200×10^6 and similar to that of IncP-2 plasmid pMG5, which has been sized at a molecular weight of 280×10^6 by electron microscopy (8). Like other IncP-2 plasmids (12), pMG74 could not be transferred to *E. coli* by conjugation.

Properties of the β-lactamase produced by R151, pMG33, and pMG74. The β-lactamase produced by plasmids R151, pMG33, and pMG74 focused at the identical isoelectric point (pI 6.1) when applied in the usual manner at the anode (Fig. 2 and 3). When applied at the cathode (36), the apparent pIs were also identical (Fig. 2 and 3, tracks F and H). Growth of plasmid-containing strains in benzylpenicillin produced derepression of the chromosomal β-lactamase (pI > 8) also present in these strains, but the plasmid-determined enzymes were produced constitutively (Fig. 2 and 3, tracks A and D).

Table 4 shows the substrate profiles for these β-lactamases and the OXA-1 enzyme produced by plasmid RGN238 for comparison. The isoxazoyl penicillins oxacillin, methicillin, and especially cloxacillin were readily hydrolyzed, with rates at least as rapid as with ampicillin. Carbenicillin hydrolysis was appreciable but more variable. Cephaloridine was an active substrate, but cephalothin was a poor one. The substrate profiles of the β-lactamases produced by R151, pMG33, and pMG74 were similar but differed from that of RGN238 (OXA-1) in relatively greater activity toward cloxacillin and lack of activity with cefotaxime (35).

Clavulanic acid and sulbactam at a concentration of 40 μM produced equivalent inhibition of the PSE-2 β-lactamases, but cloxacillin was not inhibitory. Chloride ion was also inhibitory, but not as effective an inhibitor with the PSE-2 β-lactamases as with the OXA-1 enzyme.

TABLE 3. Incompatibility crosses

Cross	Plasmids		Selection[a]	Relative transfer frequency[b]	Properties of transconjugants[b]
	Entering	Resident			
1	RIP64	R151	Hg	1	20 Sm^r, 20 Hg^r
2	R151	RIP64	Sm	4×10^{-2}	20 Hg^r, 20 Sm^r
3	RP1-1	R151	Cb	3×10^{-1}	0 Tm^r, 20 Cb^r
4	RP8	R151	Cb	5×10^{-2}	0 Tm^r, 20 Cb^r
5	R56Be	R151	Cb	8×10^{-2}	0 Tm^r, 20 Cb^r
6	R151	RP1-1	Tm	3×10^{-4}	11 Cb^r, 20 Tm^r
7	R151	RP8	Tm	3×10^{-3}	11 Cb^r, 20 Tm^r
8	R151	R56Be	Tm	4×10^{-3}	14 Cb^r, 20 Tm^r
9	pMG74	CAM	Gm	8×10^{-4}	2 Cam^{+d}, 20 Gm^r

[a] For abbreviations, see Table 1, footnote *a*. Carbenicillin at 2,000 μg/ml was used since RP1-1, RP8, and R56Be provide resistance to this concentration but R151 does not.

[b] Relative to that into an R⁻ recipient.

[c] Purified transconjugants were tested for retention of the resident plasmid marker.

[d] Cam$^+$, Ability to grow on camphor as sole carbon source.

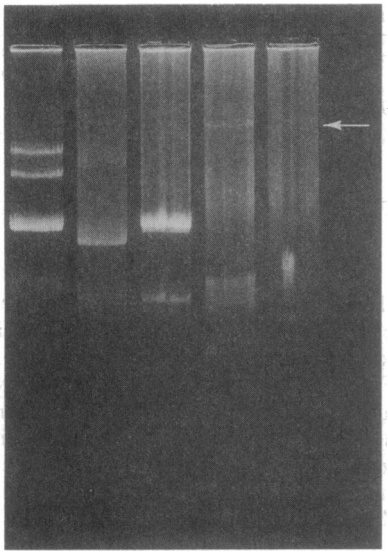

FIG. 1. Agarose gel electrophoresis of plasmids present in: (track 1) *E. coli* J53-1 containing plasmid size standards *Plac* (molecular weight, 101×10^6), R1 (62×10^6), and R6K (26×10^6); (track 2) *P. aeruginosa* PU21(R151); (track 3) *P. aeruginosa* PAO38 Rif^r(pMG33); (track 4) PAO38 Rif^r(pMG33); and (track 5) PU21(pMG5) (molecular weight, 280×10^6). The arrow indicates the plasmid band visible in track five in the original photograph.

The β-lactamase produced by plasmids R151, pMG33, and pMG74 was not inhibited by antisera to TEM-1, CARB-3, or OXA-1 that inhibited homologous β-lactamases (TEM-1 and TEM-2 for TEM-1 antiserum and PSE-1 and PSE-4 in the case of CARB-3 antiserum) 75% or more (Table 5).

Transposition of PSE-2 β-lactamase production. The distribution of PSE-2 β-lactamase production on plasmids belonging to different incompatibility groups suggested that it might be determined by a transposon.

Hedges and Matthew have reported the formation of recombinant plasmids containing the PSE-2 gene by crossing a *P. aeruginosa* strain carrying plasmid R151 and the broad-host-range plasmid pUZ8 (Km Tc Hg IncP-1) with *E. coli* selecting for ampicillin resistance (10). *E. coli* E5-2(pUZ8-R151) in addition to the resistances of pUZ8 carries the resistance phenotype Ap Gm Sm Sp Su Tm. The pUZ8-R151 recombinant has IncP specificity and confers susceptibility to donor-specific phage PR4 that is active on strains containing IncN, P, or W plasmids (2). A Tra$^-$ derivative of strain E5-2(pUZ8-R151) was selected with PR4 on plates containing carbenicillin to prevent loss of the entire plasmid, and IncW plasmid R388 (Su Tp) was introduced. In a 4-h mating between strain E5-2(pUZ8-R151 Tra$^-$) (R388) and *E. coli* J53-1, ampicillin-resistant transconjugants were obtained at a frequency of 10^{-6} per donor. Of 10 transconjugants analyzed, 8 were tetracycline susceptible and hence lacked at least part of pUZ8-R151. All eight had the resistance phenotype Ap Gm Km Sm Sp Su Tm but were trimethoprim susceptible. These resistances were transferred together on outcross and were eliminated together on selecting for PR4-resistant colonies in the absence of antibiotic. Furthermore, introduction of IncW plasmid S-a (Cm Gm Km Sm Su Tm) by selecting for chloramphenicol-resistant transconjugants resulted in loss of ampicillin resistance. These results suggest that PSE-2 β-lactamase production together with Gm Km Sm Sp (Su) Tm resistance was transposed from pUZ8-R151 to R388 with loss or inactivation of trimethoprim resistance. Sulfonamide resistance is indicated in parentheses since this marker is already present on R388.

To demonstrate that transposition was *recA* independent, *E. coli recA* strain KL16-99(pUZ8-R151 Tra$^-$) (R388) was constructed and mated for 18 h with *E. coli* J53-1. Ampicillin-resistant or streptomycin-resistant transconjugants were selected at a frequency of 6×10^{-6} on plates containing nalidixic acid for counterselection. Of 40 transconjugants analyzed, 39 were tetracycline and trimethoprim susceptible but carried the resistance pattern Ap Gm Km Sm Sp Su Tm. Hence, transposition of resistance from pUZ8-R151 to R388 is *recA* independent. The designation Tn*1404* is proposed for the unit carrying the PSE-2 β-lactamase gene and the resistance phenotype Gm Km Sm Sp (Su) Tm.

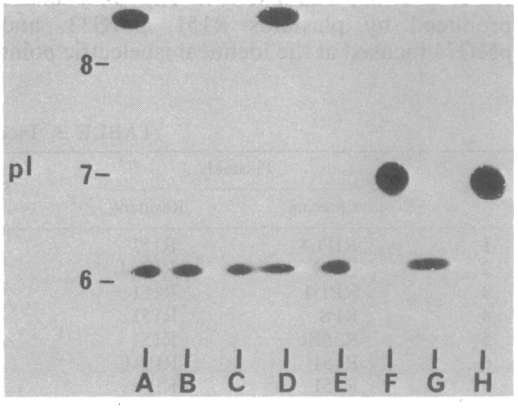

FIG. 2. Analytical isoelectric focusing of β-lactamases produced by strains PU21(R151) (tracks A, B, E, F) and PAO38 Rif^r(pMG33) (tracks C, D, G, H). In tracks A and D, strains were induced with 0.5 mg of benzylpenicillin per ml, and in tracks F and H, the β-lactamase extracts were applied at the cathode rather than the anode.

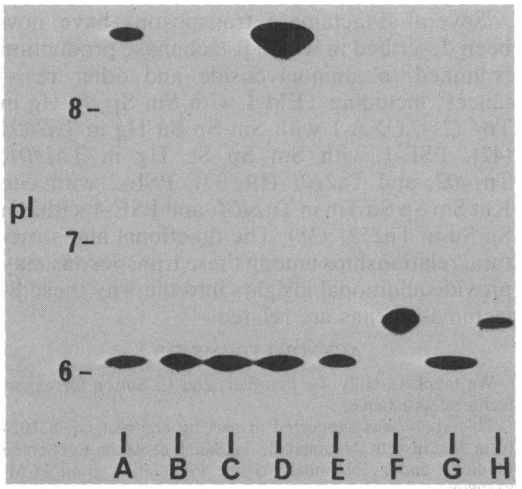

FIG. 3. Analytical isoelectric focusing of β-lactamases produced by strains PU21(R151) (tracks A, B, E, F) and PU21(pMG74) (tracks C, D, G, H). In tracks A and D, strains were induced with 0.5 mg of benzylpenicillin per ml, and in tracks F and H, β-lactamase extracts were applied at the cathode rather than the anode.

When the molecular weights of R388 and R388::Tn*1404* were compared by agarose gel electrophoresis, however, both plasmids appeared to have the same size, as though deletion of R388 DNA accompanied acquisition of Tn*1404*. To overcome this difficulty, IncW plasmid pUB5573 was used as an alternate transposon acceptor. Plasmid pUB5573 is an R388 derivative in which a portion of the sulfonamide resistance gene has been removed by deletion (41). On mating *E. coli* E5-2(pUZ8-R151 Tra$^-$) (pUB5573) with *E. coli* J53-1, ampicillin-resistant transconjugants were obtained at a frequency of 10^{-8} that were tetracycline susceptible, resistant to gentamicin, kanamycin, streptomycin, spectinomycin, sulfonamide, and tobramycin and resistant to trimethoprim. Hence, sulfonamide resistance was carried by Tn*1404*. Introduction of IncW plasmid S-a into such strains resulted in loss of ampicillin and trimethoprim resistance, confirming the formation of pUB5573::Tn*1404* derivatives. By agarose gel electrophoresis, such plasmids were 6.4 megadaltons larger than pUB5573, indicating that Tn*1404* had a molecular weight of about 6.4 × 10^6.

DISCUSSION

PSE-2 β-lactamase production is relatively uncommon among the *Pseudomonas* plasmids that we have studied. Of about 60 carbenicillin resistance plasmids analyzed by isoelectric focusing (13, 14; A. A. Medeiros, A. M. Philippon, and G. A. Jacoby, unpublished observations), only three produced PSE-2. Two of these plasmids were detected in isolates from Chicago, but the third was obtained from a strain originating in Athens, Greece. PSE-2-producing plasmids varied in size, transfer proficiency, and incompatibility specificity, but all also determined the resistance phenotype Gm Km Sm Sp Su Tm.

PSE-2 β-lactamase functions efficiently in *E. coli* if incorporated into a broad-host-range plasmid such as pUZ8 (10). From a pUZ8-R151 recombinant plasmid, the PSE-2 gene could be transposed to a second plasmid along with the resistance phenotype Gm Km Sm Sp Su Tm. Transposition was *recA* independent, and for plasmid pUB5573 it was associated with an increase in molecular weight of 6.4 × 10^6. The designation Tn*1404* is proposed for this unit. Transposition of Tn*1404* to plasmid R388 was unusual in that trimethoprim resistance was invariably lost, apparently by deletion since the size of R388::Tn*1404* did not increase. Transposition of Tn*2521* (38) and other multiresistance PSE β-lactamase transposons to R388 has also been found to produce trimethoprim-susceptible deletion derivatives (G. A. Jacoby, unpublished observations). Recombination between homologous segments carried by R388 and certain transposons, such as common sulfonamide resistance genes, might be involved. In support of this

TABLE 4. Properties of β-lactamases produced by R151, pMG33, pMG74, and RGN238 (OXA-1)

Plasmid specifying β-lactamase	Relative rate of hydrolysis[a]									Inhibition by[b]:			
	Penicillin	Ampicillin	Carbenicillin	Oxacillin	Methicillin	Cloxacillin	Cephaloridine	Cephalothin	Cefotaxime	Cloxacillin (0.1 mM)	Clavulanic acid (40 μM)	Sulbactam (40 μM)	Cl^- (mM)
R151	100	173	87	273	286	902	50	11	1	0	69	60	19
pMG33	100	202	130	265	279	>1,500	60	3	1	0	70	64	20
pMG74	100	233	62	243	262	>1,500	49	15	1	0	67	49	16
RGN238	100	419	64	271	343	119	112	34	24	0	47	98	3.1

[a] Expressed as V_{max} relative to that of benzylpenicillin (100).

[b] Percentage of activity at the indicated concentration of inhibitor, except for Cl^-, for which the concentration for 50% inhibition is shown.

TABLE 5. Neutralization of β-lactamase with antiserum

β-Lactamase	Plasmid	Residual activity (%) after treatment with antiserum to:		
		TEM-1	CARB-3	OXA-1
TEM-1	p111	<10	100	100
TEM-2	RP4	<10	100	100
PSE-1	RPL11	100	<10	100
PSE-4	pMG19	100	<10	100
OXA-1	RGN238	100	100	<25
PSE-2	R151	100	100	100
PSE-2	pMG33	100	100	100
PSE-2	pMG74	100	100	100

hypothesis, transposition of Tn*1404* to pUB5573, derivative of R388 in which a portion of the sulfonamide resistance gene has been deleted, was not associated with loss of trimethoprim resistance, but against it is the observation that the formation of trimethoprim-susceptible R388::Tn*1404* derivatives was *rec*A-independent.

The PSE-2-producing plasmids are either Tra^- (pMG33) or unable to transfer to *E. coli* by conjugation (R151 and pMG74), but there is no reason to believe that PSE-2 production will remain specific to the genus *Pseudomonas*. Indeed, D. M. Livermore, J. P. Maskell, and J. D. Williams have detected isolates of *E. coli, Klebsiella pneumoniae, Providencia stuartii*, and *Enterobacter cloacae* in London that determine PSE-2 and that contain plasmids transmissible to *E. coli* or *P. aeruginosa* (personal communication). Hence, both PSE-1 (18, 33) and PSE-2 can no longer be considered β-lactamases specific for the genus *Pseudomonas*.

Although PSE-2 resembles PSE-1, PSE-3, and PSE-4 β-lactamases in efficient hydrolysis of carbenicillin (30, 31), it also resembles the OXA group in activity against oxacillin and cloxacillin, and it resembles OXA-1 in activity against methicillin. Like the OXA group, PSE-2 was inhibited by Cl^- ions but not by cloxacillin (in contrast to the findings of Matthew [30]). Although PSE-2 β-lactamase was inhibited in vitro by clavulanic acid or sulbactam, these β-lactamase inhibitors failed to show synergy against a PSE-2-producing strain of *P. aeruginosa* in combination with the β-lactamase-susceptible antibiotic azlocillin, as was also found for strains producing enzymes of the OXA group (4). Immunological tests indicated that antisera neutralizing TEM-1, TEM-2, OXA-1, or PSE-1 and PSE-4 failed to inactivate PSE-2. Sykes and Matthew also found that antiserum to PSE-2 did not cross-react with other plasmid-mediated enzymes (40). Hence, PSE-2 has properties resembling the OXA group of enzymes but appears immunologically distinct.

Several β-lactamase transposons have now been described in which β-lactamase production is linked to aminoglycoside and other resistances, including TEM-1 with Sm Sp Su Hg in Tn*4* (21), OXA-1 with Sm Sp Su Hg in Tn*2603* (42), PSE-1 with Sm Sp Su Hg in Tn*1401*, Tn*1402*, and Tn*2101* (19, 33), PSE-2 with Gm Km Sm Sp Su Tm in Tn*1404*, and PSE-4 with Sm Sp Su in Tn*2521* (38). The functional and structural relationships among these transposons may provide additional insights into the way these β-lactamase genes are related.

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