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# RPL11, an R Factor of *Pseudomonas aeruginosa* Determining Carbenicillin and Gentamicin Resistance

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R factor RPL11 from *Pseudomonas aeruginosa* determines multiple drug resistance including resistance to gentamicin and carbenicillin. The host range and incompatibility properties of RPL11 are those of incompatibility group P-2. Strains harboring the factor are not altered with respect to the major immunotypes 1 through 7 of Parke-Davis, or with respect to pyocin type by using the 18 indicators of Jones and co-workers. Analytical ultracentrifugation of crude extracts of R factor-containing strains shows a band of satellite DNA with a buoyant density of 1.717 g/cm$^3$.

R factors from *Pseudomonas aeruginosa* determining resistance to either carbenicillin or to gentamicin have been described (2, 4, 8, 9, 13-15, 17-19). Certain of these R factors are transferrable to pseudomonas species but not to *Escherichia coli* or *Proteus mirabilis*, and are classified as members of incompatibility group P-2 (2, 3, 9).

In an accompanying report, we describe a population of R factors showing joint transfer of resistance to gentamicin, carbenicillin, and other antibiotics from hospital isolates of *P. aeruginosa* (13). The clinical significance of such factors (6) led us to further characterize RPL11, the R factor of strain PL11. Strain PL11 is a clinical isolate of *P. aeruginosa* showing resistance by the Bauer-Kirby method (1) to gentamicin, carbenicillin, streptomycin, tetracycline, sulfadiazine, chloramphenicol, kanamycin, and mercury, but not to tobramycin or colistin. It is representative of the hospital strains which show transfer of resistance to gentamicin, carbenicillin, streptomycin, tetracycline, sulfa, chloramphenicol, and to mercury (13). Properties of RPL11 relating to host range and incompatibility group, pyocin and immunotyping, and banding of satellite deoxyribonucleic acid (DNA) are presented in this report.

## MATERIALS AND METHODS

**Bacterial strains and growth procedures.** Table 1 lists the pertinent characteristics and sources of strains used in mating experiments. Additionally, a set of type strains for the major seven immunotypes of *P. aeruginosa* was used as recipients in mating and for immunotyping. These strains and the typing procedures are described elsewhere (13). Pyocin indicator strains ALA 1 through 18 of Jones et al. (10) were obtained from B. H. Minshew. Cell culture, mating procedures, chemicals, and media are described elsewhere (13).

**Pyocin typing.** Strains to be tested for pyocin production were inoculated from overnight cultures across the center of a tryptic soy agar plate. After 14 to 18 h at 31 C, growth was removed with a glass microscope slide and the plates were stored inverted over $CHCl_3$ for 1 h to kill remaining bacteria.

Indicator strains ALA 1 through 18 were prepared from overnight tryptic soy broth cultures, diluted 1/200 into 2 ml of fresh tryptic soy broth and inoculated with shaking at 37 C for 4 h. These cell suspensions were then cross-streaked with a sterile cotton swab perpendicular to the zone of previous growth (and possible pyocin production) of the test strain. The plates were incubated overnight at 37 C. Inhibition of growth of an indicator strain was scored as (+).

**Efficiency of transfer and plasmid compatibility studies.** The capacity of PL11 to transfer RPL11 to strains harboring R factors of different incompatibility groups was measured. *P. aeruginosa* PL1 strains containing factors of incompatibility groups P, C, W, or P-2 were prepared by overnight matings with PU21 containing RP1, R64, S-a, or pMG1, respectively. Factor R931 was transferred to *280 met* by using Ps. 931 as donor. Resistance markers transferred by each of these factors are indicated in Table 1. Selection of transcipients was by conferred resistance as appropriate to the R factor involved; counterselection was by nutritional deficiency (PU21) or by sensitivity to rifampin (Ps. 931). Transcipients were purified by single-colony isolation on recipient plate medium, repeated once; they were then scored for acquisition of the resistance pattern of the particular R factor. These strains, together with PL1, were then used as recipients in transfer efficiency studies. PL11 was used as donor of RPL11. Log phase cells of both recipient and donor were combined in 2-h matings at $10^8$ cells/ml. Selection was for RPL11 transfer; counterselection of PL11 was by sensitivity to rifampin.

TABLE 1. *Bacterial stains*

| Strain | Chromosomal and plasmid markers[a] | Source |
|---|---|---|
| *Pseudomonas aeruginosa* | | |
| PL1 | *met, leu, rif* | Mutant isolate of 280 (13) |
| PL11 | RPL11 (Gm,Cb,Sm,Tc,Su,Cm) | Clinical isolate (13) |
| Ps.931 | R931 (Sm,Tc) | L. E. Bryan (4) |
| 280 *met rif* | *met, rif* | L. E. Bryan (4) |
| PU21 | FP⁻*ilvB112, leu-1, str-1, rif* | G. A. Jacoby (9) |
| PU21 (RPL11) | see above | Transcripient selected using PL11 as donor |
| PU21 (RP1) | see above, RP1 (Cb,Nm/Km,Tc) | G. A. Jacoby (9) |
| PU21 (R64) | see above, R64 (Cb,Km,Gm,Su, Cm,Tb) | G. A. Jacoby (9) |
| PU21 (S-a) | see above, S-a (Gm,Km,Sm,Su) | G. A. Jacoby (9) |
| PU21 (pMG1) | see above, pMG1 (Gm,Sm,Su) | G. A. Jacoby (9) |
| *Pseudomonas putida* | | |
| AC20 | *trp ilv*, R⁻ | A. M. Chakrabarty (5) |
| *P. fluorescens* | prototroph, R⁻ | ATCC 11251 |
| *E. coli* | | |
| PL353 | K12, *his-750 rif* | lab culture collection |
| PL358 | C, *rif* | lab culture collection |
| *Proteus mirabilis* | | |
| PM17 | *thy ura nic lac trp* | R. H. Olsen (16) |

[a] Gm, gentamicin; Cb, carbenicillin; Sm, streptomycin; Tc, tetracycline; Su, sulfa; Cm, chloramphenicol; Km, kanamycin; and Tb, tobramycin.

Frequency of transfer was based on number of donors at the end of the mating period.

Compatibility was tested by incubating a purified colony containing pairs of R factors in antibiotic-free tryptic soy broth for 30 generations. Strains were then titered on tryptic soy agar without antibiotics and on tryptic soy agar containing pooled antibiotics to score simultaneously for maintenance of markers of both R factors.

**Analytical CsCl ultracentrifugation.** Ten milliliters of overnight cultures at $3 \times 10^9$ cells per ml was collected by centrifugation. Pellets were washed with 0.9% NaCl, drained, frozen, and then thawed. Lysis was completed by adding 1 ml of 1% sodium dodecyl sulfate. Solutions were brought to 10 ml with 0.9% NaCl and vortexed vigorously to shear DNA. A 0.7-ml amount of aqueous CsCl saturated at 100 C was cooled to room temperature and mixed with 0.165 ml each of test and reference DNA. A 0.7-ml sample of the resulting mixture was centrifuged at 44,700 rpm for 20 h at 25 C in a Beckman Model E analytical ultracentrifuge equipped with a standard ultraviolet light source.

## RESULTS

**Host range and frequency of transfer.** With PL11 as donor, RPL11 is transferred to PL1 at a frequency of $8 \times 10^{-1}$, and to PU21 at a lower frequency, $3 \times 10^{-3}$. With PL1 (RPL11) as donor, frequencies of transfer to other *Pseudomonas* species were *P. putida* AC20, $7 \times 10^{-4}$; and *P. fluorescens*, $8 \times 10^{-5}$. Both *P. putida* AC20 and *P. fluorescens* were shown to donate their acquired resistance properties back to PL1. No transfer of RPL11 was detected from PL11 to *E. coli* K12 (PL353), to *E. coli* C (PL358), or to *Proteus mirabilis* PM17 at the level of $10^{-8}$. These properties of host range and transfer frequency are similar to those of R factors of incompatibility group P-2 as described by Bryan (2, 3) and Jacoby (9).

**Efficiency of transfer and incompatibility studies.** *P. aeruginosa* variants harboring R factors of different incompatibility groups were constructed as described above. In transfer efficiency studies, RPL11 was mated from PL11 into the recipients shown in Table 2. RPL11 transferred with similar high frequency to PL1 and to PL1 containing either of the factors RP1, S-a or R64 (factors of incompatibility groups P, W, and C, respectively). Frequency of transfer of RPL11 to PL1 (pMG1), or to *280 met* (R931), representative R factors of group P-2, was lower by at least 100-fold.

Since all antibiotic markers carried by R931 or by pMG1 are also carried by RPL11, it was impossible to examine compatibility by using maintenance studies of RPL11 in the presence of these factors. The markers on RP1, S-a, or R64 permit selection of PL1 containing either of these factors with the simultaneous selection of RPL11. Strains containing each pair of factors were purified and were then incubated in antibiotic-free tryptic soy broth for 30 generations. PL1 retains RPL11 through this procedure (Table 3); the other three R factors (S-a, R64, RP1) were equally maintained singly by PL1 (latter data not shown). Strain PL1 also retains

markers of both RPL11 and S-a, and of both RPL11 and R64 at high frequencies. PL1 maintains markers of both RPL11 and RPl at a frequency of 60%. From these data, RPL11 is not a member of the R factor incompatibility groups W or C and shows only a marginal compatibility with a representative of group P. On the basis of surface exclusion, indicated earlier in Table 2, RPL11 is probably a member of incompatibility group P-2.

**Pyocin type and immunotype are independent of RPL11.** The effect of RPL11 upon host pyocin type was examined with the typing set of Jones et al. (10). PU21 is altered to be nearly nontypable after acquisition of pMGl (Table 4). In contrast, the pyocin type of PU21 (RPL11) is nearly unchanged from that of PU21. p MG1 or RPL11 had little or no effect on the pyocin type of PL1.

RPL11 did not confer or alter immunotype among a variety of strains. Beginning with PL11 (type 7) as donor and PL1 (nontypable) as recipient, the transcipient PLi (RPL11) remained nontypable. Similarly, strains PU21 and PU21 (RPL11) both share the immunotype pattern 3 + 7. Strain PU21 (RPL11) was used in turn in mating experiments with each of the seven antigenic index strains. All seven strains acquired resistance traits without any change in their immunotype.

**Presence of RPL11 is correlated with satellite DNA.** Evidence for the nature of RPL11 was sought by the analysis of crude cell lysates on CsCl equilibrium gradients. Crude extracts of PL11, PU21, PL1, PU21 (RPL11), and PL1 (RPL11) were examined (Fig. 1). No evidence of satellite DNA appeared for either of the antibiotic-sensitive strains PU21 and PL1. All three strains showing transmissible resistance show satellite DNA with a buoyant density of 1.717 g/cm$^3$.

## DISCUSSION

Factor RPL11 was chosen for study as a representative of a large population of factors determining resistance to both gentamicin and carbenicillin in *P. aeruginosa* (13). Our initial efforts have examined properties of RPL11 which are of potential use in characterizing the development and spread of this population of R factors in the local hospital.

RPL11 is readily transmissible to other strains of *P. aeruginosa*, and to other

TABLE 2. *Transfer frequency of RPL11 from PL11*

| Recipient strain | R factor incompatibility group | RPL11 property selected[a] | Transfer frequency[b] |
|---|---|---|---|
| PL1 | | Cb or Sm | $8 \times 10^{-1}$ |
| PL1 (RP1) | P | Sm | $5 \times 10^{-1}$ |
| PL1 (S-a) | W | Cb | $4 \times 10^{-1}$ |
| PL1 (R64) | C | Sm | $8 \times 10^{-1}$ |
| PL1 (pMG1) | P-2 | Cb | $2 \times 10^{-4}$ |
| 280 *met*(R931) | P-2 | Cb | $3 \times 10^{-3}$ |

[a] Symbols indicate resistance to antibiotics in the plate: Cb, carbenicillin (50 μg/ml); and Sm, streptomycin (100 μg/ml).

[b] Frequencies based on number of RPL11 transcipients per donor as titered at the end of the mating.

TABLE 3. *Stability of R factors in PL1*

| R factors | Markers scored[a] | Maintenance[b] (%) |
|---|---|---|
| RPL11 | Cb, Sm, Gm, Tc | 100 |
| RPL11; S-a | Cb + Km | 100 |
| RPL11; R64 | Sm + Tb | 97 |
| RPL11; RP1 | Sm + Km | 60 |

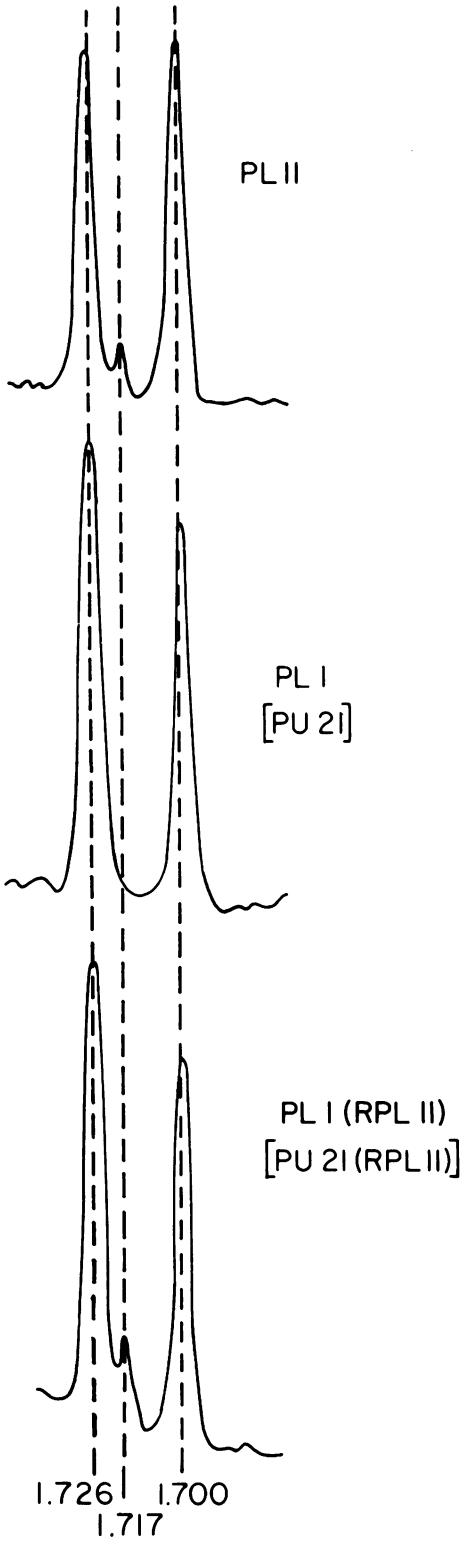
[a] Symbols refer to markers scroed based upon antibiotics in the plate: Cb, carbenicillin (50 μg/ml); Sm, streptomycin (100 μg/ml); Gm, gentamicin (1 μg/ml); Tc, tetracycline (100 μg/ml); Km, kanamycin (50 μg/ml); and Tb, tobramycin (10 μg/ml).

[b] Co-maintenance values based upon titers on tryptic soy agar and on selection plates with pooled antibiotics after culture for 30 generations in nonselective media.

TABLE 4. *Effect of R factors pMG1 and RPL11 on pyocin type of PU21 and PL1*

| Strain | ALA pyocin indicator strains[a] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| PU21 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + |
| PU21 (pMG1) | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − |
| PU21 (RPL11) | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | − | + | + |
| PL1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PL1 (pMG1) | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PL1 (RPL11) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

[a] The ALA pyocin indicator strains are those described by Jones et al. (10). Growth inhibition of pyocin indicator strains is scored as (+).




*Pseudomonas* species at reduced frequencies. No transfer was effected to *Escherichia* or *Proteus* species. This host range and the incompatibility properties described here for RPL11 are consistent with R factors of incompatibility group P-2 (2, 3, 9). The influence of group P-2 R factors upon host pyocin typing has been noted by others (2, 3, 9). Jacoby (9) showed the group P-2 R factor pMG1 greatly interfered with pyocin production when present in strain PU21. Bryan et al. (3), using other P-2 factors, reported no effect upon pyocin type of strain 280 even though these same R factors did alter the pyocin type of other strains. Both these observations are generally supported by our data, with a different set of pyocin indicator strains. Factor pMG1 has little effect upon pyocin production in PL1, a derivative of 280, while greatly restricting pyocin production in PU21. Interestingly, RPL11 has little or no effect upon pyocin type in either PU21 or PL1. Clearly, the fidelity of pyocin typing is dependent upon characteristics both of the strain and of particular R factors which may be present.

For a number of strains, presence of RPL11 has no influence upon strain immunotype, using antisera to the seven major types.

The presence of resistance conferred by RPL11 is correlated with the presence of extrachromosomal DNA with a buoyant density of 1.717 g/cm$^3$. Further analyses of this satellite DNA are in progress to determine if it is the DNA of RPL11.

Bryan et al. have recently shown a number of their collection of P-2 factors to transfer gentamicin acetyltransferase I capacity (3). This enzyme does not effectively inactivate tobramycin (3). As this property is consistent with the tobramycin-sensitive nature of RPL11, gentamicin resistance with RPL11 may be due to a similar acetylase gene.

Isolation of R factors transmitting resistance for both gentamicin and carbenicillin resistance is a recent development. Kabins et al. have described some of the enzymatic resistance mechanisms of a multiply resistant *P. aeruginosa* isolated from human urine, strain POW (11). As this manuscript was completed,

FIG. 1. *Densitometer tracings of ultraviolet photographs of crude lysates centrifuged to equilibrium in CsCl. Proteus mirabilis PM17 DNA was included as density marker in each cell. Density determinations are based upon published values of 1.726 g/cm$^3$ for Pseudomonas aeruginosa (2) and 1.700 g/cm$^3$ for P. mirabilis (12). Data for PU21 and PU21 (RPL11) was indistinguishable from that seen for PL1 and PL1 (RPL11), respectively.*

Bryan et al. published a further characterization of POW, which they term POW 151. This strain is shown to transfer resistance to gentamicin and carbenicillin, together with resistance to tobramycin, streptomycin, and sulfonamide (3). These authors have identified the R factor, R151, as a member of incompatibility group C (P-3). Our studies of RPL11 indicate members of incompatibility group P-2 can also co-transfer determinants for resistance to gentamicin and carbenicillin.

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