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Properties of an R Plasmid in *Pseudomonas aeruginosa* Producing Amikacin (BB-K8), Butirosin, Kanamycin, Tobramycin, and Sisomicin Resistance

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Pseudomonas aeruginosa strain GN315, producing kanamycin acetyltransferase, can transmit a plasmid determining resistance to kanamycin, amikacin (BB-K8), butirosin, tobramycin, and sisomicin, but not gentamicin, to other pseudomonas strains. This plasmid, pMG5, belongs to the same incompatibility group as plasmids determining gentamicin resistance in *P. aeruginosa* via gentamicin acetyltransferase I. Like other members of this group, it also confers resistance to Hg^{2+}, organomercurials, and certain deoxyribonucleic acid phages. When pMG5 is introduced into a cell carrying a related R factor, some transconjugants can subsequently cotransfer new combinations of resistance determinants as though recombinant plasmids had been formed.

Most clinical isolates of *Pseudomonas aeruginosa* are susceptible to gentamicin and tobramycin, but strains have been found that are gentamicin resistant and tobramycin susceptible, gentamicin and tobramycin resistant, or, least commonly, gentamicin susceptible and tobramycin resistant. These phenotypes can be accounted for by the substrate specificities of known inactivating enzymes in *P. aeruginosa* (2). Gentamicin acetyltransferase I inactivates gentamicin but has little activity against tobramycin (4). Gentamicin adenylyltransferase is active against both drugs (9), and kanamycin acetyltransferase is active against tobramycin but not against all the components of the gentamicin complex (1).

In some *P. aeruginosa* strains these inactivating enzymes are determined by transmissible R plasmids. A *P. aeruginosa* strain inactivating gentamicin by adenylylation can transfer resistance to *Escherichia coli* (10) where the R factor involved, R64, belongs to incompatibility group C (5, 11). *P. aeruginosa* strains inactivating gentamicin by acetylation can transfer resistance to other pseudomonas strains but not to *E. coli* (3, 7). R factors of this type, such as pMG1, are compatible with C-, N-, P-, and W-type R plasmids in *P. aeruginosa* but not with certain other pseudomonas R plasmids that have been assigned to incompatibility group P-2 (3, 7). In addition to antibiotic resistance, members of the P-2 incompatibility group may confer resistance to mercuric ion, organomercurials, ultraviolet radiation, and certain deoxyribonucleic acid phages and may also inhibit pyocin production (7).

An R plasmid in *P. aeruginosa* determining kanamycin acetyltransferase has not yet been reported. This paper describes such an R factor, relates its properties to R plasmids determining other aminoglycoside-inactivating enzymes in pseudomonas, and suggests a way in which new combinations of R factor genes may evolve.

MATERIALS AND METHODS

Most of the strains, media, and techniques have been described previously (7). *P. aeruginosa* GN315 was provided by K. E. Price, Bristol Laboratories. Gentamicins C_1, C_{1a}, and C_2 came from G. H. Wagman, Schering Corp.

RESULTS

The donor strain *P. aeruginosa* GN315 was isolated from a patient in Japan by Mitsuhashi (12) and has been shown to produce kanamycin acetyltransferase (13). The initial recipient strain was *ilv leu rif*^{r} PU21 (7). Tobramycin-resistant transconjugants were selected by using rifampin for counterselection after overnight mating and appeared at a frequency of 10^{-2} per donor cell. Sulfonamide resistance was acquired concomitantly. The R plasmid involved, pMG5, could be transferred to other *P. aeruginosa* strains but not to *E. coli* strains K or C (frequency less than 10^{-8} per donor).

Classification of pMG5. Compatibility testing (Table 1) was carried out in *P. aeruginosa* against representatives of those incompatibility

groups that can be transferred from *E. coli* (7) and against pMG1 as an example of the P-2 incompatibility group (3, 7). Plasmid pMG5 transferred to recipient strains carrying P-, C-, W-, or N-type R factors at the same frequency as to R plasmid-negative PU1. Furthermore, these pMG5 transconjugants continued to carry resistance markers determined by the P-, C-, W-, or N-type plasmid resident in the recipients, indicating that pMG5 does not belong to any of these incompatibility groups.

In contrast, with PU1 (pMG1) as recipient, the frequency of pMG5 transfer was decreased 10^{-4}. The majority of these transconjugants had lost streptomycin and gentamicin resistance determined by pMG1, suggesting that pMG5 and pMG1 are not able to coexist in the same host and hence belong to the same incompatibility group.

TABLE 1. *Compatibility of pMG5 with other plasmids*

Recipient[a]	R plasmid incompatibility group	pMG5 transfer frequency[b]	R+ pMG5+/ pMG5+ [c]
PU1		3×10^{-1}	
PU1 (RP1)	P	5×10^{-1}	20/20
PU1 (R64)	C	5×10^{-2}	20/20
PU1 (R7K)	W	2×10^{-1}	20/20
PU1 (R46)	N	2×10^{-1}	20/20
PU1 (pMG1)	P-2	3×10^{-5}	8/20[d]

[a] PU1 is a prototrophic FP2+ *P. aeruginosa* strain (7). R+ derivatives of PU1 were constructed (7) from donor strains supplied by E. J. L. Lowbury (RP1), Y. A. Chabbert (R64), N. Datta (R7K, R46), and C. Drube (pMG1).

[b] Donor strain was PU21 (pMG5). Transconjugants were selected on minimal tobramycin plates after a 2-h mating except with PU1 (R64), where BB-K8 was used for selection. Transfer frequency is expressed per donor cell with recipients in excess.

[c] Number of pMG5 transconjugants retaining antibiotic resistance markers of the plasmid resident in the recipient/number tested.

[d] For further characterization, see Table 3.

Other properties of pMG5. pMG5 shares a number of other properties with pMG1. Both fail to transfer to *E. coli*, both produce Hg^{2+} resistance, and both inhibit the propagation of certain deoxyribonucleic acid phages (Table 2). pMG5 differs from pMG1 in not producing ultraviolet light resistance and not inhibiting pyocin production. Also, pMG5 confers resistance to phenylmercuric nitrate, as does another member of the P-2 incompatibility group but not pMG1 (7).

Phenotypes of pMG5 pMG1 recombinants. In the cross between PU21 (pMG5) and PU1 (pMG1), 8 of 20 transconjugants retained antibiotic resistance markers determined by pMG1 (Table 1). These 20 transconjugants were examined in greater detail for other properties that distinguish the two plasmids. Phenylmercuric nitrate resistance could not be tested since the FP2 sex factor in PU1 itself confers phenylmercuric nitrate resistance.

Six phenotypes could be distingushed from the patterns of antibiotic resistance and inhibition of pyocin production exhibited by these transconjugants (Table 3). Some retained both gentamicin and streptomycin resistance and some either streptomycin or gentamicin resistance, although most were susceptible to both antibiotics. None were ultraviolet light resistant but three failed to produce pyocin.

Representatives of each type were crossed to another recipient. In each case all properties were cotransferred regardless of which antibiotic (for those resistant to more than tobramycin) was used for selection. These genetic tests suggest that in the original pMG5 × pMG1 cross incompatibility was expressed either by loss of pMG1 or by the formation of recombinant plasmids carrying various combinations of parental genes.

TABLE 2. *Properties of pMG1 and pMG5*

Plasmid	Antibiotic resistance[a]	Transmissible to *E. coli*	Resistance to[b]				Inhibition of pyocin production[c]
			Hg^{2+}	Phenyl mercuric nitrate	UV	Phage	
pMG1	GM SM SU	No	R	S	R	R	Yes
pMG5	TB SU	No	R	R	S	R	No

[a] Abbreviations: GM, Gentamicin; SM, streptomycin; SU, sulfonamide; TB, tobramycin.

[b] Mercury resistance was tested with brain heart infusion agar plates containing 10^{-3} M $HgCl_2$ or 5×10^{-4} M phenylmercuric nitrate. Plasmid-negative PU21 is inhibited by a 5×10^{-5} M concentration of either compound. Ultraviolet light (UV) resistance was determined from survival curves at a UV dose of 9.2 ergs per mm^2 per s. Resistance to pseudomonas phages B3, D3, E79, G101, PB1, and M6 was tested by quantitative plate counts. PU21 is sensitive to each of these phases, but none propagate on these R+ derivatives.

[c] Pyocin production was determined by the Gillies and Govan technique (6).

TABLE 3. *Phenotypes of pMG5 × pMG1 transconjugants*[a]

GM	SM	UV	Pyocin production	No.
R	R	S	+	2
R	S	S	+	4
R	S	S	−	1
S	R	S	+	1
S	S	S	−	2
S	S	S	+	10
				20

[a] Tobramycin-resistant transconjugants in the cross PU21 (pMG5) × PU1 (pMG1) of Table 1 were purified and tested for the indicated properties. Abbreviations: GM, Gentamicin; SM, streptomycin; UV, ultraviolet light.

Spectrum of aminoglycoside resistance. The spectrum of aminoglycoside resistance produced by pMG5 is shown in Table 4. As expected from the substrate specificity of kanamycin acetyltransferase (1, 2), pMG5 conferred resistance to kanamycin, amikacin (BB-K8), butirosin, gentamicin B (Sch 14342), sisomicin, and tobramycin. It provided resistance to gentamicin C_{1a}, slight resistance to gentamicin C_2, but no resistance to gentamicin C_1 and hence did not significantly enhance resistance to the gentamicin complex. Data for pMG1 and R64 are indicated for comparison. Each of these plasmids conferred resistance to gentamicin C_1, C_{1a}, and C_2, but neither provided resistance to amikacin or butirosin.

DISCUSSION

The R plasmid described here, pMG5, originated in a strain known to produce kanamycin acetyltransferase (13) and confers on its host resistance to those aminoglycosides with a free 6′-amino group whose acetylation results in loss of antibiotic activity (1). Thus, in addition to kanamycin resistance, pMG5 confers resistance to amikacin, butirosin, gentamicin B (Sch 14342), sisomicin, and tobramycin (Table 4) as expected from the in vitro activity of extracts of its parent strain toward aminoglycoside antibiotics (8, 12, 13). At present clinical *P. aeruginosa* isolates with this resistance pattern are uncommon, but as these new aminoglycosides come into broader use, such R plasmids will provide one mechanism for the emergence of resistant strains.

pMG5, which was discovered in a *P. aeruginosa* strain from Japan, appears to belong to the same incompatibility group as R plasmids discovered in resistant pseudomonas strains from Atlanta, Capetown, and Canada (7), so that already plasmids of this type (P-2) are widely distributed geographically. In addition, cotransfer experiments suggest that plasmids of this group can recombine as well as segregate when two are introduced into the same host cell, even when specific selection for retention of the resident plasmid is not applied. Thus, among pMG5 transconjugants in a cross with pMG1 are cells retaining gentamicin resistance, streptomycin resistance, or inhibition of pyocin production, markers originally carried by pMG1 but subsequently transferable together with pMG5. Confirmation of recombinant plasmid formation by co-curing or cotransduction has not yet been possible, but this apparent tendency to form hybrid R factors may provide an important mechanism in *P. aeruginosa* for evolving new assortments of R plasmid genes.

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TABLE 4. *Spectrum of aminoglycoside resistance*

Strain	MIC (μg/ml)[a]													
	SM	KM	NM	PM	LV-A	GM	GM-C_1	GM-C_{1a}	GM-C_2	GM-B	SS	TB	BT	AK
PU1	50	100	50	1,000	100	5	15	5	5	100	10	1	50	5
PU1 (pMG5)	20	1,000	100	500	100	10	10	50	10	>100	50	100	>100	50
PU1 (pMG1)	10,000	100	50	1,000	100	200	>100	100	>100	50	100	5	50	10
PU1 (R64)	20	100	50	500	50	500	>100	>100	>100	>100	>100	100	25	2

[a] Minimal inhibitory concentration (MIC) values were determined by agar dilution in Mueller-Hinton medium. Antibiotic concentrations are indicated in terms of the free base. Abbreviations: SM, streptomycin; KM, kanamycin; NM, neomycin; PM, paromomycin; LV-A, lividomycin A; GM, gentamicin C complex; GM-C_1, GM-C_{1a}, GM-C_2, gentamicin C components; GM-B, gentamicin B or Sch 14342; SS, sisomicin; TB, tobramycin; BT, butirosin; AK, amikacin or BB-K8.

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