ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1974, p. 239-252

Vol. 6, No. 3
Printed in U.S.A.

Properties of R Plasmids Determining Gentamicin Resistance by Acetylation in *Pseudomonas aeruginosa*

GEORGE A. JACOBY

Massachusetts General Hospital, Boston, Massachusetts 02114

Received for publication 15 April 1974

Two clinical isolates of *Pseudomonas aeruginosa*, one a pyocin type 5 strain from Atlanta, could transfer gentamicin resistance by conjugation. Donor and recipient strains inactivated gentamicin by acetylation. The R plasmids, pMG1 and pMG2, also determined resistance to sisomicin, another substrate of gentamicin acetyltransferase I, sulfonamides, and streptomycin, but not resistance to kanamycin, neomycin, tobramycin, butirosin, or BB-K 8. They were transmissible to many strains of *P. aeruginosa*, including a Rec$^-$ strain, but not to *Escherichia coli* or other enterobacteriaceae. These R plasmids were compatible with R plasmids transmissible to *P. aeruginosa* from *E. coli*, including members of C, N, P, and W incompatibility groups. From a strain carrying pMG1 and a compatible plasmid, pMG1 was transferred independently but transfer of the second plasmid often resulted in cotransfer of pMG1. In contrast, pMG1 and pMG2 were incompatible with pseudomonas R plasmids R931 and R3108, and with R931 they readily formed recombinant plasmids. The four plasmids in this incompatibility group determine additional biological properties, including resistance to inorganic and organic mercury compounds, to ultraviolet light, and to certain deoxyribonucleic acid phages. pMG1 and pMG2 also phenotypically inhibited pyocin production. Consequently such R plasmids alter the phage and pyocin types of their host strains.

Pseudomonas aeruginosa is well known to be resistant to many antibiotics. Most clinical isolates are susceptible to gentamicin, which consequently has been widely and successfully used in treating serious *P. aeruginosa* infections. Exceptional strains, however, have been reported to be gentamicin resistant, for example, strains from burn units at the Grady Memorial Hospital in Atlanta and the Red Cross Hospital in Capetown, South Africa (46, 52). These strains inactivate the antibiotic by acetylation (11, 39). This report concerns the properties of R plasmids in these strains which are responsible for transmissible gentamicin resistance. A preliminary account was presented at the Annual Meeting of the American Society for Microbiology, 6-11 May 1973, Miami Beach, Fla. Recently Bryan et al. have also described R plasmids determining gentamicin resistance in similar strains (9).

R plasmids in *P. aeruginosa* can be broadly divided into two types: those transmissible only from one pseudomonas strain to another, and those transmissible to *Escherichia coli* as well. Pseudomonas R plasmids of the latter type belong to two of the growing number of fi$^-$ incompatibility groups, groups P (16) and C (15). Members of other established incompatibility groups have not been transferred to *P. aeruginosa* by conjugation (14). The R plasmids responsible for gentamicin acetylation are not transmissible to *E. coli* or other enterobacteriaceae. They belong to the same incompatibility group as plasmids R931 and R3108 of Bryan et al. (9). They have been found compatible in *P. aeruginosa* with R plasmids of groups C and P and also with plasmids of groups W and N, here reported to be transmissible to pseudomonas.

In addition, these plasmids determine a number of other properties not previously known to be associated with pseudomonas R factors, such as resistance to ultraviolet (UV) light, to Hg^{2+}, and to organic mercurials. They interfere with pyocin production and the propagation of certain deoxyribonucleic acid (DNA) phages. Since phage susceptibility and pyocin production are widely used for typing *P. aeruginosa*, these effects of R plasmids have important implications for epidemiological studies.

MATERIALS AND METHODS

Bacterial strains. Table 1 lists the pertinent characteristics and source of the strains used.

TABLE 1. *Bacterial strains*

Strain	Pertinent characteristics[a]	Source
Pseudomonas aeruginosa		
Capetown no. 18	pMG1 (Gm Sm Su)	C. G. Drube (52)
Stone no. 130	pMG2 (Gm Sm Su)	C. G. Drube (52)
Ps. 931	R931 (Sm Tc)	L. E. Bryan (10)
280 *met*$^-$ (R3108)	*met* R3108 (Sm Su Tc)	L. E. Bryan (9)
Ps. 1822	RP1 (Cb Km Tc)	E. J. L. Lowbury (37)
PU1 (OT15)	FP2$^+$	J. S. Loutit (35)
PU10 (OT651)	FP$^-$	J. S. Loutit (33)
PU21 (OT47 rif^r)	FP$^-$ *ilvB112 leu-1 str^r-1 rif^r*	J. S. Loutit (35)
PAO303	FP$^-$ *arg-18*	A. J. Clark (42)
JC9010	FP$^-$ *arg-18 rec les*	A. J. Clark
PAO307	FP$^-$ *arg-54*	B. W. Holloway (42)
PAT904 rif^r	FP* *his-404 str^r rif^r*	R. H. Olsen (40)
Pseudomonas fluorescens		
PFO 15.4	*met str^r*	R. H. Olsen (40)
Enterobacteriaceae		
Escherichia coli C		S. E. Luria
E. coli K-12 AB1932-1	F$^-$ *arg met xyl gal lac tsx nal*	D. H. Smith (25)
E. Coli K-12 J53 (R7K)	F$^-$ *met pro* R7K (Cb Sm)	N. Datta (12)
E. coli K-12 J53 (R46)	F$^-$ *met pro* R46 (Cb Sm Su Tc)	N. Datta (13)
E. coli K-12 J5(R64)	F$^-$ *met pro* R64 (Cb Cm Gm Km Su)	Y. A. Chabbert (54)
Proteus mirabilis F67	*nic*	M. N. Swartz (44)
Serratia 7840		L. J. Kunz
Klebsiella 6910		L. J. Kunz
Enterobacter 7507		L. J. Kunz
Herellea 7580		L. J. Kunz

[a] The nomenclature for plasmid markers used here is that presented by R. P. Novick, R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow, *Uniform nomenclature for bacterial plasmids: a proposal*, at the American Society for Microbiology Conference on Extrachromosomal Elements in Bacteria, 10–11 January 1974. Resistance phenotypes are Cb, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Su, sulfonamide; and Tc, tetracycline. FP2 is the sex factor originally detected in pseudomonas strain 2 (27).

PU21, the *P. aeruginosa* strain used as recipient or donor in conjugations, is a *rif^r* derivative of OT47, and FP$^-$ *ilvB112 leu-1 str^r-1* strain derived by Loutit et al. from the PAO line of Holloway (35). It has several desirable properties: a low frequency of spontaneous antibiotic-resistant mutants, good recipient ability for chromosomal gene transfer (35), susceptibility to well-characterized DNA and ribonucleic acid (RNA) phages, and a close relationship to other useful strains such as prototrophic PU1 (OT15), which carries the pseudomonas sex factor FP2, and strain PU10 (OT651), a mercury-susceptible derivative of PU1 cured of FP2 (33).

Pyocin indicator strains 1 to 8 and A to E of Gillies and Govan (22, 23) were obtained from L. J. Morse (Bacteriol. Proc., M33, 1970), and indicator strains NIH 1 to 27 were obtained from J. J. Farmer III (19). Host strains for propagating typing phage came from M. T. Parker and E. A. Asheshov (45).

Media. Selection plates contained minimal medium A (17), 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-histidine, 30; L-leucine, 80; L-isoleucine, 70; L-methionine, 30; nicotinic acid, 0.12; and L-valine, 117. For *P. aeruginosa*, antibiotics were added at the following concentrations (in micrograms per milliliter): carbenicillin, 1,000; gentamicin, 20; rifampin, 100; streptomycin, 100; sulfadiazine, 5,000; tetracycline, 100; and tobramycin, 10. For *E. coli* and other enterobacteriaceae, lower concentrations were used (in micrograms per milliliter): gentamicin, 10; streptomycin, 25; and sulfadiazine, 100. For selecting or scoring Hg^{2+} resistance, 0.1 mM $HgCl_2$ was added to appropriately supplemented minimal plates.

For liquid cultures, pseudomonas strains were grown in nutrient broth (Difco) containing 4 mg of potassium nitrate (NNB) per ml. Nitrate was added since it can be used by *P. aeruginosa* as a terminal electron acceptor, permitting more uniform growth throughout the medium, and has been reported to enhance conjugal recombinant formation (35). *E. coli* and other enterobacteriaceae were grown in L broth (32) with 2 mg of glucose per ml.

Mating. In most experiments, exponentially growing cultures of donor and recipient were mixed at 10^8 cells/ml in a volume of 2 ml of NNB and incubated

without shaking in a 125-ml flask at 37 C. Control flasks contained donor or recipient alone. Mating was terminated by centrifugation and vigorous agitation as the cell pellet was suspended in medium A without ammonium sulfate (A-N). Dilutions in A-N were spread on minimal plates containing antibiotic or $HgCl_2$, and, when PU21 was used as recipient, rifampin was used for counterselection. When PU21 was used as donor, counterselection was by omission of isoleucine, leucine, and valine. Frequency of transfer was calculated with respect to the number of donor cells at the end of mating. For matings with *P. fluorescens*, experiments were conducted at 30 C.

For the kinetics of R plasmid transfer (Fig. 1), donor and recipient cultures were mixed at 3×10^8 cells/ml, incubated for 5 min at 37 C, and diluted gently 100-fold into prewarmed NNB. Periodically, samples of 0.1 ml were removed to tubes containing 3 ml of 0.75% agar at 45 C and agitated in a mechanical shaking device (36) to interrupt mating, and the contents were then poured onto selection plates. For delayed selection with gentamicin, plates were incubated for 4 h at 37 C, and an amount of gentamicin to give a final concentration of 20 μg/ml was introduced beneath the agar along the bottom of the petri dish. Plates were kept overnight at 4 C to allow diffusion of the antibiotic and then reincubated at 37 C.

Curing. Cells carrying pMG1 or pMG2 were treated with mutagens or with agents reported to eliminate plasmids from *E. coli* (1, 55) or *P. aeruginosa* (33, 43). Survivors were plated on L plates (32) and then replicated for antibiotic-susceptible colonies, which were retested by spotting.

Cells were grown overnight in NNB containing 100 or 200 μg of acriflavin per ml starting with an inoculum of 10^5 organisms/ml. An inoculum of 10^8 cells/ml was incubated in NNB containing 10, 20, or 30 μg of mitomycin C per ml for from 4 h to 3 days. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (nitrosoguanidine) treatment at 500 μg/ml for 30 min followed the procedure of Adelberg et al. (2). Sodium dodecyl sulfate was used at 100 mg/ml in NNB and also in Penassay broth (Difco) adjusted to pH 7.6 (1). An inoculum of 10^4 to 10^5 cells/ml was sampled after overnight growth and also after several days. UV treatment was to 1% survval.

Resistance to antibiotics. Agar dilution minimal inhibitory concentration values were determined by spotting 10^4 to 10^5 organisms from an overnight culture in NNB on Mueller-Hinton medium (Difco) plates containing serial dilutions of antibiotics. Drugs not available commercially were obtained from H. R. Black (Lilly, tobramycin), C. G. Drube (Schering, gentamicin B or Sch 14342, sisomicin), F. Kobayashi (Kowa, lividomycin A), H. E. Machamer (Parke-Davis, butirosin, paromomycin), and K. E. Price (Bristol, BB-K 8).

Resistance to metal ions and organic mercury compounds. Metal ion resistance was tested on brain heart infusion (Difco) agar plates containing graded concentrations (5×10^{-6} to 10^{-2} M) of $AgNO_3$, $CdSO_4$, $CoCl_2$, $CuSO_4$, $HgCl_2$, $NiSO_4$, or $Pb(C_2H_3O_2)_2$, applying 10^4 to 10^5 cells per spot test. Resistance to mercurochrome, phenylmercuric nitrate, and thimerosal was tested in the same manner.

Resistance to UV irradiation. Late log-phase cells grown in NNB were centrifuged and resuspended in medium A-N at about 10^9 cells/ml. Five milliliters of cell suspension in a 10-cm diameter dish was gently agitated 50 cm beneath a Sylvania germicidal lamp delivering 9.2 ergs per mm^2 per s at this distance. Samples were removed at intervals and plated on L plates for survivors with precautions to minimize photoreactivation.

Resistance to alkylating agents. Cells were grown in NNB to late log phase, centrifuged, and resuspended at about 10^9 cells/ml in medium A-N containing either 20 mg of methylmethane sulfonate per ml or 500 μg of nitrosoguanidine per ml. Each mixture was incubated at 20 C without shaking and sampled at 0, 15, 30, and 60 min for survivors.

Resistance to bacteriophage. Phages B3, D3, E79, F116, and G101 were provided by B. W. Holloway (26), phages C5, M6, PB1, PO4, PP7, and 7S by D. E. Bradley (6-8), and 22 typing phages by M. T. Parker and E. A. Asheshov (45). Phage was propagated in ZC agar overlays on Z Agar plates and harvested in Z broth (53). Typing phages were grown at 32 C on their homologous propagating strains, and the other phages were grown at 37 C on PU21, or, where indicated, on PU21 (pMG2). Lysates were sterilized with chloroform except for chloroform-susceptible C5, F116, and Col 18, which were passed through 0.45-μm pore size membrane filters (Millipore Corp.). For quantitative tests, phage was diluted in Z broth and titered on PU21 or its R plasmid derivatives by using ZC top agar and Z plates.

Qualitative susceptibility tests with typing phage were performed at a dilution which just failed to give confluent lysis on the propagating strain (routine test dilution). With few exceptions the lytic spectrum for the typing phages on the entire set of propagating strains corresponded to that supplied with them. Test strains were grown overnight in Trypticase soy broth (BBL) containing 10 mg of potassium nitrate per ml (NTSB). Two milliliters of a 1:10 dilution was applied to the surface of a Trypticase soy agar (BBL) plate, the excess was removed, and the plate was allowed to dry before spotting with a multiple spotting device. Once phage had absorbed to the medium, plates were incubated at 32 C for 12 to 14 h. Ten plaques per spot or less was considered a negative typing reaction.

Phage adsorption was studied as described by Bradley (7). Bacteria at 10^9 to 2×10^9 cells/ml NNB were mixed with an equal volume of phage at 4×10^7 plaque-forming units/ml and shaken at 37 C. Samples were removed at 0 and 20 min, centrifuged to remove bacteria, and titered on PU21 to determine the percentage of phage adsorbed.

Pyocin typing. Pyocin typing by the cross-streaking technique was performed on Trypticase soy agar plates containing 5% defibrinated horse blood (BBL) as described by Gillies and Govan (22, 23). For the pyocin production method of Farmer and Herman (19), strains were grown in NTSB (29) and induced

with 1 μg of mitomycin C per ml. Lysates were tested on indicator strains grown in NTSB and applied to Trypticase soy agar plates.

Plasmid compatibility. By using donor strains listed in Table 1, plasmids RP1 and R3108 were transmitted to PU21 directly, and plasmids R7K, R46, R64, and R931 were transferred to strains PAT904 *rif*^{r}, PA0303, or PA0307 as intermediate hosts and then to PU21. In each case 10^8 cells of donor and recipient were incubated overnight in 2 ml of NNB, and growth of the donor on selection plates was prevented by rifampin or omission of a nutritional requirement. Selection was made for resistance to carbenicillin (RP1), gentamicin (R64), streptomycin (R7K, R46), or tetracycline (R3108). Details will be reported more fully elsewhere (G. A. Jacoby, manuscript in preparation), but for each plasmid it was established that resistance to the expected non-selected antibiotic markers was acquired concomitantly and that resistance was serially transmissible to PU1.

To test compatibility, PU21 (pMG1) at 10^8 cells/ml was mixed with PU1, PU1 (RP1), PU1 (R7K), PU1 (R46), PU1 (R64), PU1 (R931), or PU1 (R3108) at 5×10^8 cells/ml and incubated at 37 C for 2 h. Transfer of pMG1 was selected on minimal plates containing gentamicin or streptomycin in the case of PU1 (R64). The frequency of transfer was calculated with respect to the number of donor cells determined at the end of each mating. Compatibility was tested in two ways. Firstly, pMG1 transconjugants were spotted on antibiotic media, which allowed the presence of each R plasmid to be scored separately. As a control, the stability of the resident plasmid in the recipient was similarly scored. Secondly, a purified colony of PU1 carrying pMG1 and the second R plasmid was used as donor selecting for markers unique to each plasmid, and these transconjugants were spotted on antibiotic media to determine cotransfer or hybrid formation between pMG1 and the second R plasmid.

Gentamicin acetyltransferase activity. Gentamicin acetyltransferase was assayed spectrophotometrically as described by Benveniste and Davies (4) in sonic extracts of cells grown overnight in L media by using gentamicin free of interfering bisulfite supplied by the Schering Corp.

RESULTS

Transferable gentamicin resistance. Gentamicin-resistant clinical isolates Capetown no. 18 and Stone no. 130 were each mated overnight in NNB broth with *P. aeruginosa* recipient strain PU21, and selection for transfer of resistance was made on plates containing rifampin to kill the donor cells. Gentamicin-resistant transconjugants were obtained at a frequency of 10^{-4} to 10^{-6} per donor (Table 2). These gentamicin-resistant PU21 derivatives could transfer resistance to *P. aeruginosa* strain PU10 at a frequency of 10^{-2} to 10^{-3} in a 2-h mating. Along with gentamicin resistance, PU10 acquired resistance to streptomycin, sulfonamide, and Hg^{2+}. The kinetics of transfer are shown in Fig. 1. Streptomycin, sulfonamide, and Hg^{2+} resistance were transferred simultaneously between 70 and 80 min after mating was initiated. Gentamicin resistance appeared to be transferred 20 to 30 min later if the mating mixture was applied directly to gentamicin plates. If antibiotic was added to plates after 4 h of incubation, then gentamicin resistance entered between 80 and 90 min. Even this 10-min delay represented a lag in phenotypic expression since sulfonamide- or Hg^{2+}-resistant colonies selected at 70 or 80 min (before the apparent entry of the gentamicin resistance marker) on further testing proved to be uniformly gentamicin resistant as well. No matter which agent was used for selection, transconjugants were resistant to all three antibiotics and Hg^{2+}. The R plasmid determining this phenotype from Capetown no. 18 will be termed pMG1 and from Stone no. 130, pMG2.

Host range of pMG1 and pMG2. pMG1 and pMG2 were transmissible from PU21 to a variety of other *P. aeruginosa* strains. For example, pMG1 transferred to strain PAO 303 at a frequency of 2×10^{-6} after overnight mating and to JC9010, a *rec* derivative of PA0303, at 8×10^{-4}. Since JC9010 shows a several hundredfold decrease in the efficiency of recombination for chromosomal genes (A. J. Miller and R. V. Clark, personal communication), the observation that it is at least as efficient as PA0303 in accepting antibiotic resistance is consistent with transfer of an extrachromosomal plasmid.

pMG1 and pMG2 could be transferred to *P. fluorescens* strain PF015.4 at a frequency of 10^{-8}. Transfer could not be detected (frequency less than 10^{-8}) to *E. coli* K strain AB1932-1, restriction-less *E. coli* C, *Proteus mirabilis* strain F67, or to clinical isolates of serratia, klebsiella, enterobacter, or herellea. In experiments with *E. coli*, transfer of resistance to streptomycin, sulfonamide, and Hg^{2+} as well as to gentamicin was sought with consistently negative results. Under comparable conditions, an R factor with broad host range like RP1 transferred from PU21 to *E. coli* at a frequency of 4×10^{-3}. Thus pMG1 and pMG2 belong to the class of pseudomonas R plasmids not transmissible to enterobacteriaceae.

Stability of pMG1 and pMG2. Spontaneous loss of pMG1 or pMG2 was not observed, nor could curing be obtained after exposure to acriflavin, sodium dodecyl sulfate, mitomycin C, or UV. After treatment of PU21 (pMG1) with nitrosoguanidine, a nonreverting gentamicin-susceptible derivative, PU21 (pMG3), was obtained. PU21 (pMG3) is also sulfonamide and

Hg^{2+} susceptible but retains transmissible streptomycin resistance. No agent has been found to promote loss of the entire plasmid.

Biochemical basis of gentamicin resistance. By spectrophotometric assay (4), the presence of gentamicin acetyltransferase in sonic extracts of Capetown no. 18 and Stone no. 130 was confirmed (11, 39). R-plasmid-negative PU21 has no such activity, but PU21 (pMG1) and PU21 (pMG2) had gentamicin acetyltransferase. Furthermore, this activity was lost in gentamicin-sensitive PU21 (pMG3), supporting enzymatic inactivation as the mechanism of gentamicin resistance.

Spectrum of aminoglycoside resistance. Gentamicin acetyltransferase I has high specificity for gentamicin C components and for sisomicin, with very slight activity toward kanamycin B and tobramycin (11) and none for other aminoglycosides (5). The spectrum of aminoglycoside resistance determined by pMG1 and pMG2 is shown in Table 3, which gives agar dilution minimal inhibitory concentrations toward various drugs for PU1, PU1 (pMG1), and PU1 (pMG2). These R plasmids determine gentamicin and sisomicin resistance but do not enhance resistance to kanamycin, neomycin, paromomycin, lividomycin A, tobramycin, gentamicin B (Sch 14342), butirosin, or BB-K 8, as expected from the enzymatic specificity of gentamicin acetyltransferase I.

Other resistance determinants on pMG1 and pMG2. In addition to antibiotic resistance, some *E. coli* R plasmids are known to provide resistance to heavy metal ions (47), UV light (18), and bacteriophage (3). Mercury ion resistance is a property of the pseudomonas sex factor $FP2^{+}$ (34), but resistance to agents other than antibiotics has not yet been reported for pseudomonas R plasmids.

As indicated previously, pMG1 and pMG2 provide Hg^{2+} resistance. PU21, which is FP^{-}, was inhibited by 5×10^{-5} M $HgCl_2$ in brain heart infusion plates (34). PU21 (pMG1) and PU21 (pMG2) were resistant to 10^{-3} M $HgCl_2$ in this medium. Enhanced resistance was not detected to Ag^{+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, or Pb^{2+}.

The plasmid-containing strains were also tested for resistance to organic mercury compounds used as antiseptics. PU21 (pMG1) and PU21 (pMG2) were as susceptible as PU21 to phenylmercuric nitrate and thimerosal but were resistant to a 100-fold higher concentration of mercurochrome than was their plasmid-negative parent.

PU21, like other *P. aeruginosa* strains (26), was highly susceptible to UV radiation. Plasmids pMG1 and pMG2 increased the resistance of PU21 to UV light (Fig. 2). Cells carrying one of these plasmids showed 10^{-2} survivors after a dose of more than 700 ergs/mm^2, whereas a comparable degree of killing was produced in PU1 after a dose of about 180 ergs/mm^2. The mechanism of this UV radiation protection is not yet known. The plasmids were not able to correct enhanced UV light lethality in Rec^{-} strain JC9010. Quantitative measurements also failed to show enhanced survival of PU21 (pMG1) or PU21 (pMG2) compared with PU21 after exposure to such alkylating agents as methylmethane sulfonate or nitrosoguanidine.

Typing by phage susceptibility is widely used to characterize *P. aeruginosa* strains for epidemiological investigation. Table 4 shows the

TABLE 2. *Origin of strains transferring gentamicin resistance*

Strain	Origin	Pyocin type	Frequency of transfer of gentamicin resistance to PU21
Capetown no. 18	Red Cross Hospital Burn Unit, Capetown, South Africa	29	4×10^{-6}
Stone no. 130	Grady Memorial Hospital, Atlanta, Ga.	5	3×10^{-4}

TABLE 3. *Spectrum of aminoglycoside resistance*

Strain	MIC (μg/ml)[a]										
	strep	kana	neo	paro	livA	gent	sis	tobr	genB	but	BB-K 8
PU1	50	200	50	500	50	2.5	5	2.0	100	25	5
PU1 (pMG1)	5000	100	100	1000	50	100	100	1.0	50	10	5
PU1 (pMG2)	5000	100	100	1000	50	100	100	1.0	50	15	5

[a] Minimal inhibitory concentration (MIC) values were determined by agar dilution. Antibiotic concentrations are given in terms of the free base. Abbreviations: strep, streptomycin; kana, kanamycin; neo, neomycin; paro, paromomycin; livA, lividomycin A; gent, gentamicin complex; sis, sisomicin; tobr, tobramycin; genB, gentamicin B or Sch 14342; but, butirosin.

response of PU21, PU21 (pMG1), and PU21 (pMG2) to 22 phages in a standard typing set. Plasmid-containing strains were not lysed by phages 16, F8, or M6 so that the phage type has been altered.

This effect was investigated further by utilizing phages that have been well characterized regarding their chemical composition and mode

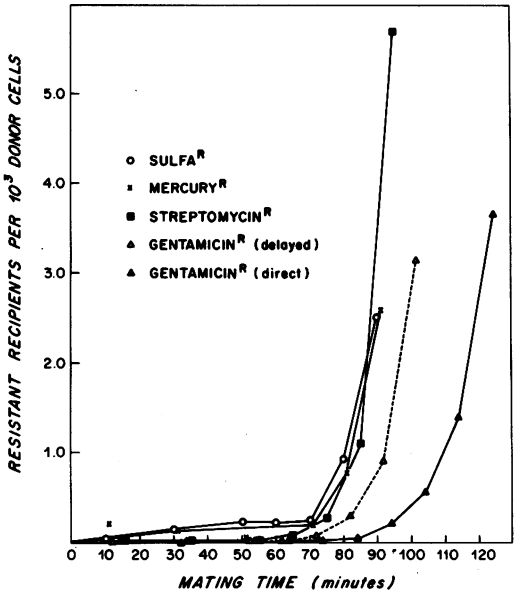

FIG. 1. *Kinetics of pMG1 transfer. PU21 (pMG1) and PU10 at 3 × 10^8 cells/ml were mixed, diluted 1:100 at 5 min, and sampled periodically for the indicated transconjugants.*

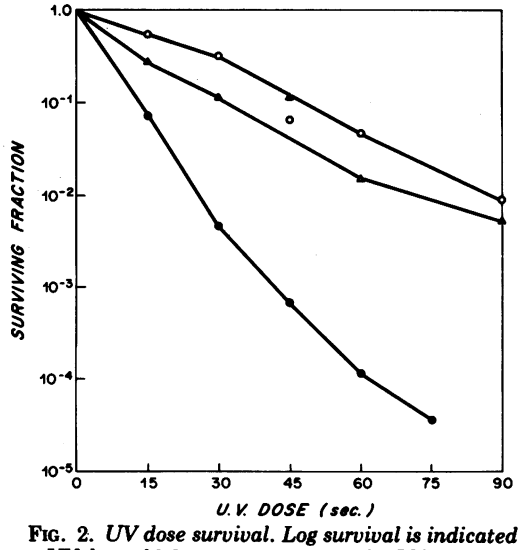

FIG. 2. *UV dose survival. Log survival is indicated at a UV dose of 9.2 ergs per mm² per s for PU1, ●; PU1 (pMG1), Δ; and PU1 (pMG2), ○.*

TABLE 4. *Effect of pMG1 and pMG2 on phage type*

Strain	Typing phage[a]																			
	2	7	16	21	24	31	44	68	73	F7	F8	F10	109	119x	352	1214	M4	M6	Col 11	Col 18	Col 21	188
PU21	+	+	+	+	0	0	+	+	0	+	+	0	+	+	0	+	0	+	0	0	0	+
PU21 (pMG1)	+	+	0	+	0	0	+	+	0	+	0	0	+	+	0	+	0	0	0	0	0	+
PU21 (pMG2)	+	+	0	+	0	0	+	+	0	+	0	0	+	+	0	+	0	0	0	0	0	+

[a] Typing phages were grown on their homologous strains and spot tested at routine test dilution on a lawn of PU21 or its R plasmid derivatives.

of attachment to the cell (Table 5). Phages B3, D3, E79, F116, G101, PB1, and PO4 contain DNA, PP7 and 7S contain RNA, whereas the composition of C5 and M6 remains to be determined (6, 7, 8, 26). Some of these phages utilize polar pili for adsorption, whereas others either are not known to be pilus dependent or have been shown to attach nonselectively to the bacterial wall and hence have been listed as cell wall phages (Table 5) (7, 42, D. E. Bradley and T. L. Pitt, J. Gen. Virol., in press). Each phage was propagated on PU21 and then titered on PU21, PU21 (pMG1), or PU21 (pMG2) to determine the efficiency of plating. Plasmid-containing strains had only a slight reduction in the efficiency of plating pilus phages C5, F116, PO4, PP7, and 7S, but did not propagate pilus phage M6 or cell wall phages B3, D3, E79, G101, or PB1 (Table 5). Actually, with undiluted lysates of phage B3 minute plaques were observed, and with undiluted phage D3 there was partial lysis, but propagation did not occur in either case and host-modified phage could not be detected. Interference with propagation occurred at a stage subsequent to phage adsorption since adsorption of B3, D3, E79, G101, PB1, and M6 by PU21 and PU21 (pMG2) was equivalent (Table 6).

Effect of pMG1 and pMG2 on pyocin type. Another commonly used technique for characterizing *P. aeruginosa* isolates is pyocin typing. PU21, like most *P. aeruginosa* (26), is pyocinogenic, and by the cross-streaking plate technique of Gillies and Govan (22) it inhibited seven of eight primary indicator strains and four of five in an additional subset (Table 7). Remarkably, PU21 (pMG1) and PU21 (pMG2) had no pyocin activity against these indicators and thus became pyocin nontypable.

Farmer and Herman have developed another procedure for pyocin typing that utilizes mitomycin C in broth culture to induce pyocin formation. Lysates are then spot tested against 27 indicator strains (19). By this technique, PU21 had pyocin activity for 20 of 27 indicators (Table 8). Again the R plasmids interfered with pyocin production. Lysates of PU21 (pMG1) and PU21 (pMG2) were active against only one indicator strain. This remaining activity was shown to be due to pyocin and not phage since on serial dilution inhibition was lost without the appearance of phage plaques.

Incompatibility testing with pMG1 and pMG2. Unlike pMG1 and pMG2, some R plasmids discovered in *P. aeruginosa* are transmissible to *E. coli* and have been assigned to incompatibility groups, specifically group P for RP1 (24) and group C for R64 (15). Members of most other *E. coli* incompatibility groups are not transmissible to *P. aeruginosa*, exceptions being R plasmids of group W, such as R7K, and of group N, such as R46 (Jacoby, manuscript in preparation).

To test compatibility of pMG1 with these R plasmids, RP1, R64, R7K, and R46 were transferred to *P. aeruginosa* strain PU1. These PU1 derivatives were then used as recipients for pMG1 (Table 9). Plasmid pMG1 was transferred to strains carrying RP1, R64, or R7K at the same frequency as to R plasmid negative PU1. A slight (less than 10-fold) decrease in transfer frequency was observed with PU1 (R46) as recipient, but subsequent tests showed that this effect probably resulted from a variable decrease in the expression of gentamicin resistance in a strain carrying both pMG1 and R46. These pMG1 transconjugants were then tested for retention of the plasmid originally resident

TABLE 6. *Adsorption of phage to PU21 and PU21 (pMG2)*

Strain	% phage adsorbed[a]					
	B3	D3	E79	G101	PB1	M6
PU21	14	99.9	99.8	96.3	68	31
PU21 (pMG2)	13	99.8	99.8	96.1	69	35

[a] Phage at 4×10^7 plaque-forming units/ml was mixed with 10^8 to 2×10^8 bacterial cells/ml, shaken at 37 C, and sampled to determine phage counts in the supernatant after cells had been removed by centrifugation. The percentage of phage adsorbed was calculated from the ratio of counts at 20 min to counts at 0 time immediately after mixing.

TABLE 5. *Effect of pMG1 and pMG2 on phage propagation*[a]

Strain	Cell wall phage					Pilus phage					
	B3	D3	E79	G101	PB1	C5	F116	M6	PO4	PP7	7S
PU21	10^{12}	6×10^{10}	4×10^{10}	6×10^{9}	4×10^{9}	8×10^{9}	2×10^{11}	5×10^{10}	7×10^{8}	10^{12}	10^{11}
PU21 (pMG1)	$<10^{-11}$	$<10^{-9}$	$<10^{-9}$	$<10^{-8}$	$<10^{-8}$	0.7	0.4	$<10^{-9}$	0.3	0.3	0.3
PU21 (pMG2)	$<10^{-11}$	$<10^{-9}$	$<10^{-9}$	$<10^{-8}$	$<10^{-8}$	0.8	0.3	$<10^{-9}$	0.3	0.5	0.4

[a] Phage were grown on PU21 and titered on PU21 or its R plasmid derivatives. Efficiency of plating (EOP) was calculated from the ratio of plaque counts. For PU21, titer values are given; for PU21 (pMG1) and PU21 (pMG2), efficiency of plating values are given.

TABLE 7. *Effect of pMG1 and pMG2 on pyocin type*

Strain	Inhibition of Gillies and Govan indicator strain[a]												
	1	2	3	4	5	6	7	8	A	B	C	D	E
PU21	+	+	+	+	+	−	+	+	+	−	+	+	+
PU21 (pMG1)	−	−	−	−	−	−	−	−	−	−	−	−	−
PU21 (pMG2)	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] +, Inhibition; −, no inhibition.

TABLE 8. *Effect of pMG1 and pMG2 on pyocin type*

Strain	Inhibition of Farmer and Herman indicator strain[a]																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
PU21	+	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+	+	+	+	+	+	−	+
PU21 (pMG1)	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
PU21 (pMG2)	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] +, Inhibition; −, no inhibition.

TABLE 9. *Compatibility of pMG1 with other plasmids*

Recipient[a]	R plasmid incompatibility group	pMG1 transfer frequency[b]	$R^+pMG1^+/pMG1^+$ [c]	pMG1 transfer frequency from R^+pMG1^+ [d]	$R^+pMG1^+/pMG1^+$ in next recipient[e]	R^+ transfer frequency from R^+ $pMG1^+$[f]	$pMG1^+$ R^+/R^+ in next recipient[g]
PU1		5×10^{-3}					
PU1 (RP1)	P	10^{-2}	20/20	3×10^{-2}	0/60	10^{-2}	99/100
PU1 (R7K)	W	4×10^{-3}	17/20	2×10^{-3}	0/20	6×10^{-4}	17/20
PU1 (R64)	C	7×10^{-3}	20/20	2×10^{-2}	0/20	3×10^{-4}	1/19
PU1 (R46)	N	7×10^{-4}	38/40	7×10^{-3}	0/40	2×10^{-7}	2/2

[a] PU21 (pMG1) was mated with the recipients listed in this column for 2 h.

[b] Transfer of pMG1 was selected with gentamicin except with PU1 (R64) where streptomycin was used. This column gives the pMG1 transfer frequency for each recipient.

[c] This column shows the number of transconjugants carrying both pMG1 and the R plasmid resident in the recipient per number tested. Retention of RP1 and R7K was scored with carbenicillin, R64 with tobramycin, and R46 with tetracycline.

[d] Transconjugants from the first mating that carried both pMG1 and the second R plasmid were mated a second time with a rifampin-resistant derivative of PU1. This column gives the transfer frequency of pMG1.

[e] pMG1 transconjugants from the second mating were again scored for the presence of both R plasmids on media similar to that used after the first cross.

[f] This column gives the transfer frequency of the other R plasmid in the second cross.

[g] The number of transconjugants carrying both pMG1 and the second R plasmid is again indicated.

in the recipient by spotting on antibiotic media which allowed the presence of each R plasmid to be scored independently. When RP1 or R64 was the resident R plasmid, all pMG1 transconjugants tested carried both plasmids (Table 9). Three of 20 PU1 (R7K) recipients had lost the resident plasmid, but R7K was somewhat unstable in *P. aeruginosa* and showed a comparable frequency of loss spontaneously from PU1 (R7K) in control experiments. On testing PU1 (R46) (pMG1) derivatives picked directly from selection plates, 40 of 40 colonies carried both plasmids, whereas with further purification, two of these clones lost R46, a higher frequency than seen in control experiments, although 95% of the clones were stable.

To show that each R plasmid was independently maintained in these double-plasmid-containing strains, they were used as donors to a further recipient selecting for transfer of a marker unique to each plasmid. These transconjugants were then scored for the presence of the second R plasmid. pMG1 was transmissible at a frequency of about 10^{-2} from each double R plasmid combination (Table 9). On spot testing, these second-step pMG1 transconjugants carried pMG1 alone, indicating that it was independently transferrable from the double-R

plasmid donors. Results on selecting for transfer of the other R plasmid from pMG1$^+$ R$^+$ strains were more complicated. The frequency of transfer of the other plasmid was similar to that observed in control experiments from a strain carrying it alone, i.e., high for RP1, intermediate for R7K and R64, and low for R46. On spot testing, the majority of second-step transconjugants receiving RP1, R7K, and R46 also received pMG1. Nonetheless, in a third round of transfer (data not shown) such strains, like the original double pMG1$^+$ R$^+$ strains, could still donate pMG1 independently, indicating that whatever the mechanism of this pMG1 cotransfer, it does not involve a permanent physical association of the two R plasmids. Thus, these transfer experiments and the stability of pMG1 together with RP1, R7K, R64, and R46 indicate that pMG1 does not belong to the P, W, C, or N incompatibility group.

Like pMG1 and pMG2, R plasmids R931 and R3108, which were discovered by Bryan et al. in clinical *P. aeruginosa* strains, are not transmissible to *E. coli* (9). To test compatibility of these plasmids with pMG1, they were transferred to strain PU1, and these derivatives were again used as recipients for pMG1. The frequency of transfer of pMG1 was reduced 10- to 100-fold to PU1 (R931) and PU1 (R3108) (Table 10). In addition, pMG1 eliminated plasmid R3108 from all transconjugants tested. With PU1 (R931) as recipient, on further testing 18 of 60 transconjugants carried markers from each plasmid. Several of these colonies were purified and used as donors to a new recipient. Whether selection was made for a marker originally carried by pMG1 or by R931, all transconjugants on this round of mating continued to carry markers from both parental plasmids, and the same result was obtained (data not shown) on another round of mating, suggesting that composite plasmids had been formed by recombination. In a reciprocal cross with R931 as the entering and pMG1 as the resident plasmid (data not shown), 18 of 20 transconjugants tested carried markers from both parents, and on further testing as donors, these colonies also behaved as recombinant plasmids. Similar results were obtained with pMG2 in identical experiments. Thus pMG1, pMG2, R931, and R3108 belong to the same incompatibility group.

Table 11 shows additional similarities between pMG1, pMG2, R931, and R3108. All mediate resistance to streptomycin and to

TABLE 10. *Incompatibility of pMG1 with R931 and R3108*

Recipient[a]	pMG1 transfer frequency[b]	R$^+$pMG1$^+$/pMG1$^+$[c]	pMG1 transfer frequency from R$^+$pMG1$^+$[d]	R$^+$pMG1$^+$/pMG1$^+$ in next recipient[e]	R$^+$ transfer frequency from R$^+$pMG1$^+$[f]	pMG1$^+$R$^+$/R$^+$ in next recipient[g]
PU1	4×10^{-3}					
PU1 (R931)	10^{-4}	18/60	10^{-2}	70/70	10^{-2}	70/70
PU1 (R3108)	4×10^{-5}	0/20				

[a] PU21 (pMG1) was mated with the recipients listed in this column for 2 h.
[b] Transfer of pMG1 was selected on gentamicin.
[c] Transconjugants were spot tested on tetracycline for retention of R931 or R3108. The number of colonies resistant to both tetracycline and to gentamicin per number tested is indicated.
[d] Seven tetracycline- and gentamicin-resistant transconjugants from PU21 (pMG1) × PU1 (R931) were purified and back-crossed to PU21. This column gives the average transfer frequency for gentamicin resistance.
[e] Ten gentamicin-resistant transconjugants from each of these crosses were tested for tetracycline resistance.
[f] This column gives the average transfer frequency for tetracycline resistance in the back-cross to PU21.
[g] Ten tetracycline-resistant transconjugants from each of seven crosses were tested for gentamicin resistance.

TABLE 11. *Properties of pMG1, pMG2, R931, and R3108*

Plasmid	Antibiotic resistance[a]	Inhibition of pyocin production	Resistance to:[b]										
			UV	Hg^{2+}	Mercurochrome	Phenylmercuric nitrate	Thimerosal	Phages					
								B3	D3	E79	G101	PB1	M6
pMG1	Gm Sm Su	Yes	r	r	r	s	s	r	r	r	r	r	r
pMG2	Gm Sm Su	Yes	r	r	r	s	s	r	r	r	r	r	r
R931	Sm Tc	No	r	r	r	s	s	r	r	r	r	r	r
R3108	Sm Su Tc	No	s	r	r	r	r	r	r	s	r	s	s

[a] Abbreviations: Gm, Gentamicin; Su, sulfonamide; Sm, streptomycin; Tc, tetracycline.
[b] Abbreviations: r, Resistant; s, susceptible.

Hg^{2+}. R931 provides UV-light resistance and gives the same pattern of phage resistance as pMG1 and pMG2. Strains carrying R3108, in contrast, are resistant to phages B3, D3, and G101 but susceptible to E79, PB1 and M6. In addition to Hg^{2+} resistance, R3108 also provides resistance to phenylmercuric nitrate and thimerosal.

These properties of R3108 allow it to be used to show that UV-light resistance and inhibition of phage propagation and pyocin production are characteristics intrinsic to pMG1. PU21 (R3108) was mated with PU1 (pMG1) selecting for tetracycline resistance. Transconjugants were obtained at a low frequency. As expected from the fact that pMG1 and R3108 belong to the same incompatibility group, all had lost gentamicin resistance as pMG1 was eliminated. Concomitantly they became susceptible to UV radiation (Fig. 3), fully susceptible to phages E79, PB1 and M6, and pyocin typable, indicating that the effect on these properties is indeed determined by pMG1.

DISCUSSION

Currently more than 95% of clinical *P. aeruginosa* isolates are susceptible to gentamicin. Although these pathogens are characteristically resistant to many other antibiotics, R-plasmid-mediated resistance appears to be uncommon (9, 30). The donor strains used here, Capetown no. 18 and Stone no. 130, were chosen as exceptionally gentamicin-resistant isolates. Both originated in burn units where *P. aeruginosa* is a common pathogen and where the clinical use of gentamicin favors the emergence of resistant strains.

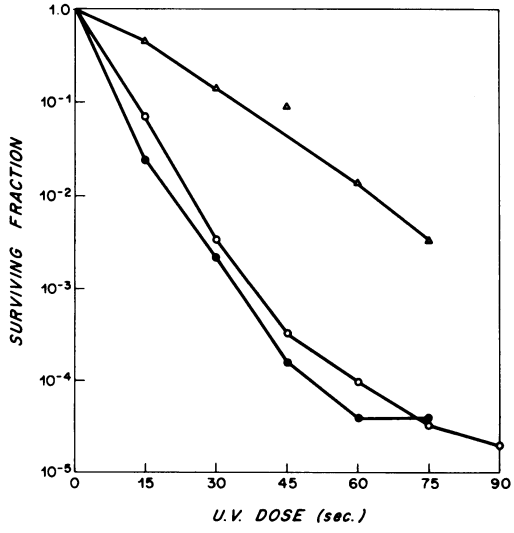

FIG. 3. *UV dose survival. Log survival is indicated at a UV dose of 9.2 ergs per mm² per s for PU1 (pMG1), Δ; PU1 (R3108), ●; and a gentamicin-susceptible transconjugant in a mating PU21 (R3108) × PU1 (pMG1) selected for tetracycline resistance,* ○.

Transfer of gentamicin resistance was detected by conjugation with a rifampin-resistant *P. aeruginosa* recipient, PU21, using rifampin for counterselection. Although commonly used curing agents did not eliminate gentamicin resistance from PU21, a plasmid must be involved since streptomycin and sulfonamide resistance were acquired concomitantly and resistance to all three antibiotics could be serially transmitted from PU21 to other pseudomonas strains, including Rec⁻ JC9010. The plasmids have been termed pMG1 and pMG2, and although they were detected in clinical isolates of different pyocin type and geographical origin, their properties have so far proven identical.

Because of its clinical importance, the biochemical basis of gentamicin resistance in *P. aeruginosa* has been intensively studied. N. Tanaka in 1970 reported that gentamicin-resistant strains from the Grady Memorial Hospital outbreak possessed ribosomes resistant to the action of gentamicin (50). However, Brzezinska et al. (11) found that such strains, including Stone no. 130 used here, inactivated gentamicin enzymatically by *N*-acetylation of the 2-deoxystreptamine moiety. Capetown no. 18, the other donor strain, has also been reported to have similar activity (39). Gentamicin acetyltransferase could be demonstrated in extracts of PU21 (pMG1) and PU21 (pMG2), but not in plasmid-negative PU21 or in PU21 (pMG3), a gentamicin-susceptible mutant of pMG1, thus supporting R-plasmid-determined gentamicin acetyltransferase as the biochemical basis of antibiotic resistance in these strains. Furthermore, the spectrum of aminoglycoside resistance produced by pMG1 and pMG2 (Table 3) is precisely that expected from the substrate specificity of gentamicin acetyltransferase I (5, 11).

Although transmissible at low frequency to *P. fluorescens*, transfer of pMG1 and pMG2 could not be detected to enterobacteriaceae including *E. coli* K or restriction-less *E. coli* C. This property distinguishes these plasmids from R plasmids of broad host range previously discovered in *P. aeruginosa* such as RP1 and R64, the latter a plasmid that also determines gentamicin resistance but by a different inactivating enzyme, gentamicin adenylyltransferase (54). In *E. coli* there is a close correlation between plasmid incompatibility group and host range (14). R plasmids of incompatibility groups P (RP1) and C (R64) can be transferred from *E. coli* to *P.*

aeruginosa, but members of most other incompatibility groups cannot be, exceptions being members of group W (R7K) and group N (R46) (Jacoby, manuscript in preparation). In *P. aeruginosa*, Bryan et al. described R plasmids such as R931 and R3108 that are restricted in host range and cannot be transmitted to *E. coli* (9). They also have reported transmissible gentamicin resistance from *P. aeruginosa* strains inactivating gentamicin by acetylation (9) and have shown that these plasmids were incompatible with R931 and R3108 but compatible with P-type plasmid RP4, which is now felt to be identical to RP1 (28).

Plasmids pMG1 and pMG2 have been found compatible in *P. aeruginosa* with P-type RP1, C-type R64, N-type R46, and W-type R7K but incompatible with R931 and R3108 (Tables 9 and 10). pMG1 and R931 or R3108 are unable to coexist stably in the same host cell. One plasmid is either eliminated by the other, or the two plasmids undergo recombination and become serially cotransmissible. This covalent linkage of incompatible plasmids must be distinguished from a more temporary association seen between some compatible plasmids that also results in cotransfer. Thus, from a strain carrying pMG1 and a second plasmid, such as RP1, selection for RP1 usually results in the concomitant transfer of pMG1, but transfer of pMG1 occurs independently (see Table 9). Such unilateral cotransfer can be a prelude to physical recombination. In an attempt to pass pMG1 to *E. coli* from a strain carrying both pMG1 and RP1, streptomycin-resistant transconjugants have been obtained at low frequency in which this pMG1 determinant has been linked to RP1 with retention of P-type specificity (unpublished data). Possibly such events are more common in *P. aeruginosa* than in *E. coli* and provide a mechanism for generating new assortments of plasmid genes.

The appropriate nomenclature for the incompatibility group of pMG1, pMG2, R931, and R3108 is problematic. These R plasmids clearly do not belong to established *E. coli* incompatibility groups C, N, P, or W. Bryan et al. (9) proposed incompatibility group P-2 as a designation for this class in pseudomonas with R plasmids like RP1 assigned to group P-1. C-, N-, or W-type plasmids, which are compatible in *P. aeruginosa* as in *E. coli* (unpublished data), would then be assigned to further P groups.

Such a dual system of nomenclature may be unavoidable at this stage of our knowledge of incompatibility groups in pseudomonas. Unfortunately, R plasmids of group P-2 cannot be transferred to *E. coli* to exclude the possibility that they belong to another already established incompatibility class. An R plasmid is known in *E. coli* that determines gentamicin inactivation by acetylation (51). However, this plasmid, R135, and other members of the M incompatibility group are not transmissible to *P. aeruginosa* (unpublished data).

pMG1 and related plasmids share a number of biological properties in addition to non-transmissibility to *E. coli* (Table 11), including resistance to inorganic and organic mercury compounds, to UV radiation, and to certain bacteriophages. pMG1 and pMG2 interfere with pyocin production as well. For Hg^{2+} resistance the mechanism has recently been established. Summers and Lewis (49) have shown that PU21 (pMG1) and PU21 (pMG2) (designated PU21/PS18 and PU21/Stone in their Table 2) determine an inducible system converting Hg^{2+} into a volatile form, probably Hg^{0}. The pseudomonas sex factor FP2 determines Hg^{2+} resistance by the same mechanism (49). Parenthetically, pMG1 and pMG2 have been tested in FP^{-} strain PU10 to see if they promote the transfer of chromosomal genes to PU21 with negative results. $FP2^{+}$ PU1 carrying pMG1 or pMG2 remains a good chromosome donor, so that FP2 is compatible with these R factors (unpublished data). A mechanism for resistance to organic mercurials is also known in pseudomonas. Tonomura and co-workers have characterized an inducible system that catalyzes the release and reduction of mercury from phenylmercuric acetate and other organic mercury compounds (20, 21). Strains carrying R3108, which promotes high level resistance to phenylmercuric nitrate, have similar enzymatic activity (S. Silver, personal communication). Differences among the plasmids in this group in resistance to mercurial compounds could reflect either differences in the specificity of the enzymes involved or in their inducibility.

The mechanism by which this group of plasmids protect against UV irradiation is not yet known. In *Salmonella typhimurium* an N-group R plasmid has been shown to protect against both UV light and the lethal effect of alkylating agents (38). pMG1 and pMG2 do not enhance survival after exposure to such alkylating agents as methylmethane sulfonate or nitrosoguanidine and might determine an enzyme specifically involved in excision repair of UV-induced lesions from DNA (38). If so, they might be expected to correct the phenotype of excision-deficient mutants of *P. aeruginosa*. They did not enhance survival of UV radiation hypersensitive Rec^{-} JC9010, but should be tested further in a larger set of radiation sensitive mutants of

P. aeruginosa, such as those recently described by Kung and Lee (31).

pMG1, pMG2, and R931 have been shown to effect the propagation of certain DNA phages (Tables 5 and 11), including one that is pilus dependent (M6) as well as several that attach to the cell wall (B3, D3, E79, G101, and PB1). R3108 produces a more selective inhibition of phage growth since strains carrying this plasmid are still lysed by E79, PB1, and M6. The mechanism of this alteration is not known. Phage adsorption (Table 6) is not changed. DNA restriction has not been excluded as the responsible mechanism but no host-modified phage have been detected. pMG1 and pMG2 also interfere with pyocin production by strain PU21. This effect could result from the elimination of a pyocinogenic plasmid, but in *P. aeruginosa* pyocin production is determined by chromosomal genes (27). Actually, it is possible to show that, whatever the mechanism, the interference with pyocin production is a phenotypic change since elimination of pMG1 by introduction of R3108 restores pyocin activity. Further studies are required to determine if the effect is specific for only certain pyocins and if pyocin susceptibility is altered as well.

The biological utility of plasmid-borne prevention of phage propagation and pyocin production is less obvious than of Hg^{2+} and UV light resistance. However, naturally occurring *P. aeruginosa* strains are almost invariably lysogenic and pyocinogenic (26). Prevention of phage propagation promotes R plasmid spread between otherwise incompatible mating pairs when the recipient is lysogenic for a phage to which the donor would otherwise be susceptible, and prevention of pyocin production similarly limits lysis of a potential recipient by pyocin otherwise produced by an R plasmid donor.

For epidemiological purposes it is important to be able to distinguish different *P. aeruginosa* strains. Pigment formation, biochemical capacity, and antibiotic resistance have not proven useful since the majority of strains are quite uniform in these respects. Reliance has been placed on typing by phage susceptibility, pyocin production or susceptibility, and serological reaction. Whatever method is used, it has been found that clinical isolates can be subdivided into many different groups. It has usually been assumed that differences between these groups are due to stable chromosomal genes. However, it seems increasingly likely that *P. aeruginosa* is rich in extrachromosomal DNA, and the influence of these plasmids on the techniques used to differentiate strains must be taken in account. The effect of R plasmids is particularly important since antibiotic usage in a hospital can substantially alter their prevalence.

The strain in which pMG2 originated came from the Grady Memorial Hospital outbreak where gentamicin-resistant pyocin type 5 strains largely replaced the previous mixture of gentamicin susceptible pyocin types under the selective pressure of gentamicin usage (46, 48). In this outbreak cross-infection is thought to have played the major role in the spread of pyocin type 5 strains (46, 48). However, the spread of R plasmid pMG2 through a population of gentamicin-susceptible strains of diverse pyocin types would also produce gentamicin-resistant isolates which were restricted in pyocin type or nontypable. Since pMG2 and related plasmids interfere with the propagation of certain typing phages as well, the phage type of plasmid recipients would also change. In the future, the influence of R plasmids on pyocin and phage typing must be appreciated in epidemiological investigations.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant no. AI 10237 from the National Institute of Allergy and Infectious Diseases.

Thanks are due to Lorraine Sutton for expert technical assistance and to the many donors of bacterial strains, phage, and antibiotics used in this work.

LITERATURE CITED

1. Adachi, H., M. Nakano, M. Inuzuka, and M. Tomoeda. 1972. Specific role of sex pili in the effective eliminatory action of sodium dodecyl sulfate on sex and drug resistance factors in *Escherichia coli*. J. Bacteriol. **109**:1114-1124.
2. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788-795.
3. Bannister, D., and S. W. Glover. 1968. Restriction and modification of bacteriophages by R^+ strains of *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **30**:735-738.
4. Benveniste, R., and J. Davies. 1971. Enzymatic acetylation of aminoglycoside antibiotics by *Escherichia coli* carrying an R factor. Biochemistry **10**:1787-1796.
5. Benveniste, R., and J. Davies. 1973. Mechanisms of antibiotic resistance in bacteria. Annu. Rev. Biochem. **42**:471-506.
6. Bradley, D. E. 1966. The structure and infective process of a *Pseudomonas aeruginosa* bacteriophage containing ribonucleic acid. J. Gen. Microbiol. **45**:83-96.
7. Bradley, D. E. 1973. Basic characterization of a *Pseudomonas aeruginosa* pilus-dependent bacteriophage with a long non-contractile tail. J. Virol. **12**:1139-1148.
8. Bradley, D. E., and D. Robertson. 1968. The structure and infective process of a contractile *Pseudomonas aeruginosa* bacteriophage. J. Gen. Virol. **3**:247-254.
9. Bryan, L. E., S. D. Semaka, H. M. Van Den Elzen, J. E. Kinnear, and R. L. S. Whitehouse. 1973. Characteristics of R931 and other *Pseudomonas aeruginosa* R factors. Antimicrob. Ag. Chemother. **3**:625-637.
10. Bryan, L. E., H. M. Van Den Elzen, and J. T. Tseng.

1972. Transferable drug resistance in *Pseudomonas aeruginosa*. Antimicrob. Ag. Chemother. **1**:22-29.
11. Brzezinska, M., R. Benveniste, J. Davies, P. J. L. Daniels, and J. Weinstein. 1972. Gentamicin resistance in strains of *Pseudomonas aeruginosa* mediated by enzymatic N-acetylation of the deoxystreptamine moiety. Biochemistry **11**:761-766.
12. Coetzee, J. N., N. Datta, and R. W. Hedges. 1972. R factors from *Proteus rettgeri*. J. Gen. Microbiol. **72**:543-552.
13. Datta, N., and R. W. Hedges. 1971. Compatibility groups among *fi*⁻ R factors. Nature (London) **234**:222-223.
14. Datta, N., and R. W. Hedges. 1972. Host range of R factors. J. Gen. Microbiol. **70**:453-460.
15. Datta, N., and R. W. Hedges. 1972. R factors identified in Paris, some conferring gentamicin resistance, constitute a new compatibility group. Ann. Inst. Pasteur **123**:849-852.
16. Datta, N., R. W. Hedges, E. J. Shaw, R. B. Sykes, and M. H. Richmond. 1971. Properties of an R factor from *Pseudomonas aeruginosa*. J. Bacteriol. **108**:1244-1249.
17. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
18. Drabble, W. T., and B. A. D. Stocker. 1968. R (Transmissible drug-resistance) factors in *Salmonella typhimurium:* pattern of transduction by phage P22 and ultraviolet-protective effect. J. Gen. Microbiol. **53**:109-123.
19. Farmer, J. J. III, and L. G. Herman. 1969. Epidemiological fingerprinting of *Pseudomonas aeruginosa* by the production of and sensitivity to pyocin and bacteriophage. Appl. Microbiol. **18**:760-765.
20. Furukawa, K., and K. Tonomura. 1972. Metallic mercury-releasing enzyme in mercury-resistant *Pseudomonas*. Arg. Biol. Chem. (Tokyo) **36**:217-226.
21. Furukawa, K., and K. Tonomura. 1972. Induction of metallic mercury-releasing enzyme in mercury-resistant *Pseudomonas*. Arg. Biol. Chem. (Tokyo) **36**:2441-2448.
22. Gillies, R. R., and J. R. W. Govan. 1966. Typing of *Pseudomonas pyocyanea* by pyocine production. J. Pathol. Bacteriol. **91**:339-345.
23. Govan, J. R. W., and R. R. Gillies. 1969. Further studies in the pyocine typing of *Pseudomonas pyocyanea*. J. Med. Microbiol. **2**:17-25.
24. Grinsted, J., J. R. Saunders, L. C. Ingram, R. B. Sykes, and M. H. Richmond. 1972. Properties of an R factor which originated in *Pseudomonas aeruginosa* 1822. J. Bacteriol. **110**:529-537.
25. Harwood, J. H., and D. H. Smith. 1969. Phenotypic suppression by streptomycin-adenylate and bluensomycin-adenylate. Genet. Res. **14**:259-273.
26. Holloway, B. W. 1969. Genetics of *Pseudomonas*. Bacteriol. Rev. **33**:419-443.
27. Holloway, B. W., V. Krishnapillai, and V. Stanisich. 1971. *Pseudomonas* genetics. Annu. Rev. Genet. **5**:425-446.
28. Holloway, B. W., and M. H. Richmond. 1973. R-factors used for genetic studies in strains of *Pseudomonas aeruginosa* and their origin. Genet. Res. **21**:103-105.
29. Jones, L. F., B. V. Pinto, E. T. Thomas, and J. J. Farmer III. 1973. Simplified method for producing pyocins from *Pseudomonas aeruginosa*. Appl. Microbiol. **26**:120-121.
30. Kawakami, Y., F. Mikoshiba, S. Nagasaki, H. Matsumoto, and T. Tazaki. 1972. Prevalence of *Pseudomonas aeruginosa* strains possessing R factor in a hospital. J. Antibiot. (Tokyo) **25**:607-609.
31. Kung, A. H. C., and B. T. O. Lee. 1973. The isolation and survival characterization of radiation and chemical-mutagen sensitive mutants of *Pseudomonas aeruginosa*. Mutat. Res. **20**:175-190.
32. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190-206.
33. Loutit, J. S. 1969. Investigation of the mating system of *Pseudomonas aeruginosa* strain 1. V. The effect of N-methyl-N'-nitro-N-nitrosoguanidine on a donor strain. Genet. Res. **14**:103-109.
34. Loutit, J. S. 1970. Investigation of the mating system of *Pseudomonas aeruginosa* strain 1. VI. Mercury resistance associated with the sex factor (FP). Genet. Res. **16**:179-184.
35. Loutit, J. S., L. E. Pearce, and M. G. Marinus. 1968. Investigation of the mating system of *Pseudomonas aeruginosa* strain 1. I. Kinetic studies. Genet. Res. **12**:29-36.
36. Low, B., and T. H. Wood. 1965. A quick and efficient method for interruption of bacterial conjugation. Genet. Res. **6**:300-303.
37. Lowbury, E. J. L., A. Kidson, H. A. Lilly, G. A. J. Ayliffe, and R. J. Jones. 1969. Sensitivity of *Pseudomonas aeruginosa* to antibiotics: emergence of strains highly resistant to carbenicillin. Lancet **2**:448-452.
38. MacPhee, D. G. 1972. Effect of an R factor on resistance of *Salmonella typhimurium* to radiation and chemical treatment. Mutat. Res. **14**:450-453.
39. Mitsuhashi, S., F. Kobayashi, and M. Yamaguchi. 1971. Enzymatic inactivation of gentamicin C components by cell-free extracts from *Pseudomonas aeruginosa*. J. Antibiot. (Tokyo) **24**:400-401.
40. Olsen, R. H., and P. Shipley. 1973. Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. J. Bacteriol. **113**:772-780.
41. Pemberton, J. M. 1973. F116: a DNA bacteriophage specific for the pili of *Pseudomonas aeruginosa* strain PAO. Virology **55**:558-560.
42. Pemberton, J. M., and B. W. Holloway. 1972. Chromosome mapping in *Pseudomonas aeruginosa*. Genet. Res. **19**:251-260.
43. Rheinwald, J. G., A. M. Chakrabarty, and I. C. Gunsalus. 1973. A transmissible plasmid controlling camphor oxidation in *Pseudomonas putida*. Proc. Nat. Acad. Sci. U.S.A. **70**:885-889.
44. Rownd, R., R. Nakaya, and A. Nakamura. 1966. Molecular nature of the drug-resistance factors of the enterobacteriaceae. J. Mol. Biol. **17**:376-393.
45. Shooter, R. A., K. A. Walker, V. R. Williams, G. M. Horgan, M. T. Parker, E. H. Asheshov, and J. F. Bullimore. 1966. Faecal carriage of *Pseudomonas aeruginosa* in hospital patients. Lancet **ii**:1331-1334.
46. Shulman, J. A., P. M. Terry, and C. E. Hough. 1971. Colonization with gentamicin-resistant *Pseudomonas aeruginosa*, pyocine type 5, in a burn unit. J. Infect. Dis. **124** (Suppl.):S18-S23.
47. Smith, D. H. 1967. R factors mediate resistance to mercury, nickel, and cobalt. Science **156**:1114-1116.
48. Stone, H. H., and L. D. Kolb. 1971. The evolution and spread of gentamicin resistant pseudomonads. J. Trauma **11**:586-589.
49. Summers, A. O., and E. Lewis, 1973. Volatilization of mercuric chloride by mercury-resistant plasmid-bearing strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. J. Bacteriol. **113**:1070-1072.
50. Tanaka, N. 1970. Biochemical studies on gentamicin resistance. J. Antibiot. (Tokyo) **23**:469-471.
51. Umezawa, H., M. Yagishawa, Y. Matsuhashi, H. Naganawa, H. Yamamoto, S. Kondo, T. Takeuchi, and Y. A. Chabbert. 1973. Gentamicin acetyltransferase in *Escherichia coli* carrying R factor. J. Antibiot. (Tokyo) **26**:612-613.
52. Weinstein, M. J., C. G. Drube, E. L. Moss, Jr., and J. A.

Waitz. 1971. Microbiologic studies related to bacterial resistance to gentamicin. J. Infect. Dis. **124** (Suppl.):S11–S17.

53. Weppelman, R. M., and C. G. Brinton, Jr. 1970. Infection of *Pseudomonas aeruginosa* spheroplasts by RNA from a pilus phage. Virology **41**:116–134.
54. Witchitz, J. L., and Y. A. Chabbert. 1971. Resistance transférable à la gentamicine. I. Expression du caractère de résistance. Ann. Inst. Pasteur **121**:733–742.
55. Yoshikawa, M. 1971. Selective enrichment of R^- segregants as the main mechanism of 'curing' of the R factor by acridine dyes. Genet. Res. **17**:9–16.