ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1982, p. 525-526
0066-4804/82/090525-02$02.00/0

Vol. 22, No. 3

Cloning the Gentamicin Resistance Gene from a *Pseudomonas aeruginosa* Plasmid in *Escherichia coli* Enhances Detection of Aminoglycoside Modification

ALICE S. PRINCE†* AND GEORGE A. JACOBY

Infectious Disease Unit, Massachusetts General Hospital, Boston, Massachusetts 02114

Received 9 April 1982/Accepted 15 June 1982

Cloning the gene for gentamicin resistance from *Pseudomonas aeruginosa* plasmid pMG35 on the high-copy-number *Escherichia coli* cloning vehicle pMK20 allowed detection of 6′-*N*-acetyltransferase activity that was not readily detected when the parent plasmid was present either in *P. aeruginosa* or *E. coli*.

Plasmid-determined resistance to aminoglycoside antibiotics in *Pseudomonas aeruginosa* is usually the result of aminoglycoside-modifying enzymes, but plasmid-determined resistance to streptomycin or kanamycin has been reported that seems not to involve detectable phosphorylating, acetylating, or adenylylating activity (6, 7). During a study of the mechanism of gentamicin resistance in a group of clinical *P. aeruginosa* isolates, several gentamicin resistance plasmids were detected that appeared not to code for aminoglycoside-modifying activity (4). To characterize the resistance mechanism further, the gentamicin resistance gene from one such plasmid has been cloned in *Escherichia coli* on a high-copy-number vector.

Plasmid pMG35 is a 28-kilobase, transfer-deficient plasmid conferring resistance to carbenicillin (Cb), gentamicin (Gm), kanamycin (Km), streptomycin (Sm), spectinomycin, sulfonamide (Su), tobramycin (Tm), and mercuric ion that was mobilized from a clinical isolate of *P. aeruginosa* into *P. aeruginosa* PAO38 (Rif^r) (9). Although strain PAO38(pMG35) required a high minimal inhibitory concentration of gentamicin, no gentamicin adenylyltransferase and negligible gentamicin acetyltransferase activity could be detected with a phosphocellulose paper binding assay (Table 1). In the same extracts adenylylation with streptomycin as the substrate was readily evident. When pMG35 was introduced into *E. coli* MV10, a C600 *trpΔE5* K-12 derivative (1), by transformation (8), gentamicin acetyltransferase activity was still only barely detectable.

† Present address: Department of Medicine, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

Plasmid pMG35 replicates in strains PAO38 and MV10 at a relatively low copy number as estimated from plasmid DNA yields obtained by standard Sarkosyl (2) or Triton X-100 (5) lysis procedures. To increase the expression of the gene products conferring gentamicin resistance, a *Pst*I fragment from pMG35 was cloned into the *Pst*I site of the *E. coli* cloning vehicle pMK20 (5) (Fig. 1). Plasmid pMK20 is a ColE1 derivative

TABLE 1. Level of resistance and aminoglycoside-modifying activity of plasmid pMG35 derivatives

Strain	Gentamicin MIC[a] (μg/ml)	Gentamicin acetyltransferase activity[b] (cpm)
P. aeruginosa		
PAO38	2	106
PAO38(pMG35)	80	210
PAO38(pMG59)	50	2,314
E. coli		
MV10	0.4	116
MV10(pMG35)	5	267
MV10(pMK20)	0.4	117
MV10(pASP11)	10	533

[a] Minimum inhibitory concentration (MIC) was determined in Mueller-Hinton (Difco) broth by tube dilution technique with an inoculum of 10^5 cells per ml.

[b] Gentamicin acetyltransferase activity was determined as described by Haas and Dowding (3). Cells were grown in L medium and disrupted by sonication, and an extract was prepared by centrifugation at 8,000 rpm. Portions of the supernatant were incubated at pH 7.8 with [^{14}C]acetyl coenzyme A (New England Nuclear Corp.) or [*U*-^{14}C]ATP (data not shown) and gentamicin (0.1 μg/ml), and the absorption of modified gentamicin to phosphocellulose paper was measured in a scintillation counter. Assays were performed in duplicate. For comparison, the activity of a known 6′-*N*-acetyltransferase producing strain, PAO38(pMG59), is indicated.

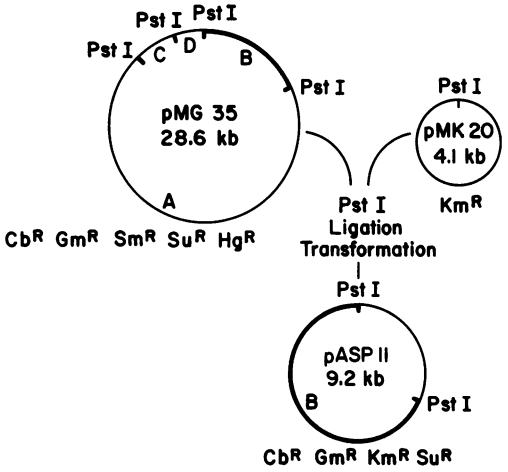

FIG. 1. Construction of plasmid pASP11.

that determines kanamycin resistance via aminoglycoside 3′-*O*-phosphotransferase and has a copy number of 70 per chromosome (5). Plasmid pMG35 DNA was cleaved with restriction endonuclease *Pst*I and ligated to pMK20 digested with *Pst*I by using T4 DNA ligase under standard conditions. The ligated DNA was transformed (8) into *E. coli* MV10, and kanamycin- and carbenicillin-resistant clones were selected and screened for gentamicin resistance. Plasmid DNA from gentamicin-resistant clones was isolated by using a Triton X-100 lysis procedure (5) and examined by agarose gel electrophoresis. One of these clones contained a hybrid plasmid designated pASP11 (Fig. 1) (map of pMG35 obtained from A. Prince, unpublished data) that consisted of a 5.1-kilobase *Pst*I fragment of pMG35 ligated to pMK20. As estimated from plasmid DNA yield by using techniques comparable for pMG35, pASP11 had a high copy number consistent with the pMK20 replicon and can be amplified in the presence of chloramphenicol. It carried the Cb Gm Km Su Tm resistance phenotype from pMG35.

An extract of MV10(pASP11) possessed gentamicin acetyltransferase activity clearly greater than that in strain MV10 or MV10(pMK20) and threefold higher than that in strain MV10(pMG35) (Table 1). When assayed for acetyltransferase activity with different kanamycins, strain MV10(pASP11) modified kanamycin A and kanamycin B, but not kanamycin C, which lacks a 6′-amino group, indicating that it produces an aminoglycoside 6′-*N*-acetyltransferase.

R-plasmid gene dosage is known to affect both the level of antibiotic resistance and the amount of antibiotic-modifying enzyme that is produced (10). In *P. aeruginosa* or *E. coli* the gentamicin resistance plasmid pMG35 produced such a low level of 6′-*N*-acetyltransferase that it was unclear whether resistance could be attributed to this mechanism. By amplifying the number of gene copies by cloning the gene for gentamicin resistance on a high-copy-number replicon, the level of resistance was enhanced and the gene product could be reliably detected. Thus, the technique of cloning genes responsible for antibiotic resistance onto high-copy-number vehicles can be useful in elucidating mechanisms of antibiotic resistance.

We thank David Figurski for plasmid pMK20 and many helpful discussions.

A.S.P. was supported by a fellowship from the Cystic Fibrosis Foundation, and G.A.J. was supported by grant PCM-7912052 from the National Science Foundation.

LITERATURE CITED

1. **Figurski, D. H., and D. R. Helinski.** 1979. Replication of an origin containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc. Natl. Acad. Sci. U.S.A. **76**:1648–1652.
2. **Figurski, D., R. Meyer, D. S. Miller, and D. R. Helinski.** 1976. Generation in vitro of deletions in the broad host range plasmid RK2 using phage Mu insertions and a restriction endonuclease. Gene **1**:107–119.
3. **Haas, M. J., and J. E. Dowding.** 1975. Aminoglycoside-modifying enzymes. Methods Enzymol. **43**:611–628.
4. **Jacoby, G. A.** 1980. Plasmid determined resistance to carbenicillin and gentamicin in *Pseudomonas aeruginosa*, p. 83–96. *In* C. Stuttard and K. R. Rozee (ed.), Plasmids and transposons. Environmental effects and maintenance mechanisms. Academic Press, Inc., New York.
5. **Kahn, M., R. Kolter, C. Thomas, D. Figurski, R. Meyer, E. Remaut, and D. R. Helinski.** 1979. Plasmid cloning vehicles derived from plasmids ColE1, F, R6K, and RK2. Methods Enzymol. **68**:268–280.
6. **Kono, M., and K. O'Hara.** 1976. Mechanisms of streptomycin (SM)-resistance of highly SM-resistant *Pseudomonas aeruginosa* strains. J. Antibiot. **29**:169–175.
7. **Kono, M., and K. O'Hara.** 1977. Kanamycin-resistance mechanism of *Pseudomonas aeruginosa* governed by an R-plasmid independently of inactivating enzymes. J. Antibiot. **30**:688–690.
8. **Kushner, S. R.** 1978. An improved method for transformation of *E. coli* with ColE1 derived plasmids, p. 17–23. *In* H. W. Boyer (ed.), Genetic engineering. Elsevier/North Holland Biomedical Press, New York.
9. **Pemberton, J. M., and B. W. Holloway.** 1972. Chromosome mapping in *Pseudomonas aeruginosa*. Genet. Res. **19**:251–260.
10. **Uhlin, B. E., and K. Nordström.** 1977. R plasmid gene dosage effects in *Escherichia coli* K-12: copy mutants of the R plasmid R1*drd-19*. Plasmid **1**:1–7.