

## Transfer of IncN Plasmids to *Pseudomonas aeruginosa*

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Three of four N plasmids tested were found to be conjugatively transferable from *Escherichia coli* to *Pseudomonas aeruginosa*. The plasmids in the *Pseudomonas* transconjugants differed from the plasmids in the donor *E. coli* with respect to molecular weight, transfer ability, phenotype conferred, and stability. In some cases, the antibiotic and UV resistance genes appeared to integrate into the *P. aeruginosa* chromosome.

Plasmids belonging to incompatibility (Inc) group N have not yet been found in clinical isolates of *Pseudomonas aeruginosa* (8). In laboratory experiments, the transfer of N plasmids from *Escherichia coli* to *P. aeruginosa* has been analyzed genetically and found to occur at low frequency with some plasmids (e.g., R46) but not at all with others (R15, N3). Once transferred into *P. aeruginosa*, the N plasmids have been unstable and not retransferable back to *E. coli* (7, 8, 10). In the present investigation, we have analyzed both the physical and genetic characteristics of N plasmids in *P. aeruginosa*. Unlike previous investigators, we used a solid medium to effect the maximum frequency of conjugational transfer, based on the finding of Dennison and Baumberg (4).

Four N plasmids were studied, N3, pCF290, R45, and R46 (Table 1). *P. aeruginosa* strains, PAO642 and GT1 (formerly strain 280 *met*<sup>-</sup> Rif<sup>r</sup> [9]), were used as recipients; both strains were naturally resistant to 30 µg of nalidixic acid per ml. The *E. coli* donor strains were nalidixic acid-sensitive. Plasmid transfers were done on nonselective solid medium (DST agar) at 37°C overnight, and the transconjugants were selected on MacConkey agar containing nalidixic acid (30 µg/ml) and either streptomycin (Sm, 50 µg/ml for GT1 and 300 µg/ml for PAO642) or carbenicillin (Cb, 100 µg/ml for GT1 and 300 µg/ml for PAO642). Tetracycline (Tc; 16 µg/ml for GT1, 48 µg/ml for PAO642) and sulfamethoxazole (Su, 4 mg/ml) were used in some experiments.

We did not detect transfer of plasmid N3 to either *P. aeruginosa* strain. pCF290 transferred at low frequencies ( $10^{-7}$  to  $10^{-8}$  per recipient cell) to GT1 but not at all to PAO642. R46 transferred to GT1 ( $10^{-6}$  to  $10^{-8}$ ) and to PAO642 ( $10^{-4}$  to  $10^{-6}$ ), as did R45. The transfer frequencies of R45 to both recipients were highly variable ( $10^{-1}$  to  $10^{-6}$ ); the reason for this variability is still not understood, but temperature (30 to

39°C), concentration of calcium ions in the medium, and choice of the selecting antibiotic were found not to contribute to the variability. All four N plasmids transferred at high frequencies ( $10^{-1}$  to  $10^{-2}$ ) between *E. coli* strains and were stable in this species.

The antibiotic resistances and UV protection mediated by the N plasmids (Table 1) were expressed in both *P. aeruginosa* strains; however, neither GT1 nor PAO642 showed sensitivity to plasmid-specific phages IKE and PRD1. R45 and R46 transconjugants of PAO642 expressed resistance to arsenate, but those of GT1 did not. In contrast, we have found GT1(RP1) to be sensitive to phage PRD1 and GT1(R40a) to be resistant to arsenate. (RP1 is an IncP plasmid and R40a is an IncC plasmid.)

With R46, three types of *P. aeruginosa* transconjugants were obtained (Fig. 1A). Type 1 transconjugants contained a major species of plasmid DNA which migrated more slowly than did R46 DNA from *E. coli*, as if it had a molecular weight about  $2 \times 10^6$  greater than that of the *E. coli* plasmid. (This apparent increase in molecular weight did not occur with RP1 or R40a.) Two minor species of plasmid DNA were often seen in type 1 preparations (Fig. 1A, lane 3); assuming they were supercoiled molecules, their molecular weights were  $60 \times 10^6$  and  $72 \times 10^6$  (Md). Type 1 transconjugants usually occurred as a minority of the total. They could transfer their N plasmids to *E. coli* but not to other *P. aeruginosa* strains. Upon transfer to *E. coli*, the plasmid from a type 1 *P. aeruginosa* donor reassumed the mobility in agarose gels of the original R46 plasmid from *E. coli*, as though a segment of about 2 Md had been excised before or during transfer; it also reexpressed in *E. coli* the phenotypes of PRD1 and IKE sensitivity, arsenate resistance, and stable maintenance. R46 plasmids in type 1 and type 2 *P. aeruginosa* transconjugants were unstable, so

TABLE 1. N plasmids used in this study<sup>a</sup>

Plasmid	Phenotype in <i>E. coli</i>	Mol wt ( $\times 10^{-6}$ )
N3	Sm Su Tc Ike <sup>s</sup> PRD1 <sup>s</sup> Tra <sup>+</sup>	36
pCF290	Cb Sm Ike <sup>s</sup> PRD1 <sup>s</sup> Tra <sup>+</sup>	36
R45	Cb Su Tc As UV Ike <sup>s</sup> PRD1 <sup>s</sup> Tra <sup>+</sup>	28
R46	Cb Sm Su Tc As UV Ike <sup>s</sup> PRD1 <sup>s</sup> Tra <sup>+</sup>	32

<sup>a</sup> Symbols: Cb, Sm, Su, Tc, As, and UV denote resistance to carbenicillin (and ampicillin), streptomycin, sulfonamides, tetracycline, arsenate, and UV light, respectively; Ike<sup>s</sup> and PRD1<sup>s</sup> indicate susceptibility to the respective phages; and Tra<sup>+</sup> stands for transfer-proficiency. Plasmids N3, R45, and R46 have been described (except for the molecular weight of R45) (1, 6). Plasmid pCF290 has been characterized in this laboratory. All molecular weights have been determined by agarose gel electrophoresis with three or more reference plasmids: RP4 (36 Md), R26 (52 Md), R100 (70 Md), and R40a (96 Md) (6).

that less than 1% of the cells in a culture grown nonselectively for 20 generations retained the plasmid.

Most *P. aeruginosa* R46<sup>+</sup> transconjugant clones were classifiable as type 2. These clones exhibited two major plasmid species in agarose gels (Fig. 1A, lane 2); one migrated more slowly than R46 DNA from *E. coli* to a position expected for a supercoiled molecule of about 35 Md; the other major band appeared at a position lower than *E. coli* R46 DNA and corresponded to a mixture of supercoiled molecules with molecular weights from 27 to 32 Md. Two larger, minor species were often seen at positions expected for supercoiled molecules of 57 and 81 Md. Plasmids of type 2 transconjugants were not transferable by conjugation to *E. coli* or other *P. aeruginosa* strains. Many of the type 2 transconjugants appeared to have lost one or another of the unselected resistance determinants, usually Sm or Tc.

A third type of R46 transconjugant, designated type 3, was obtained in some experiments. The type 3 clones were stable with respect to the plasmid-mediated antibiotic resistances (Cb Sm Su Tc) and UV protection did not contain plasmid DNA demonstrable in gels (Fig. 1A, lane 1) and were not capable of retransfer of plasmid characters. It seems likely that the type 3 transconjugants resulted from the integration of a portion of N plasmid DNA into the *P. aeruginosa* chromosome.

With plasmid R45, transconjugants of type 2 and type 3 were obtained, and they had the same properties as those from R46; however, type 1 R45 transconjugants were not encountered. Plasmid pCF290 was unique among the N plas-

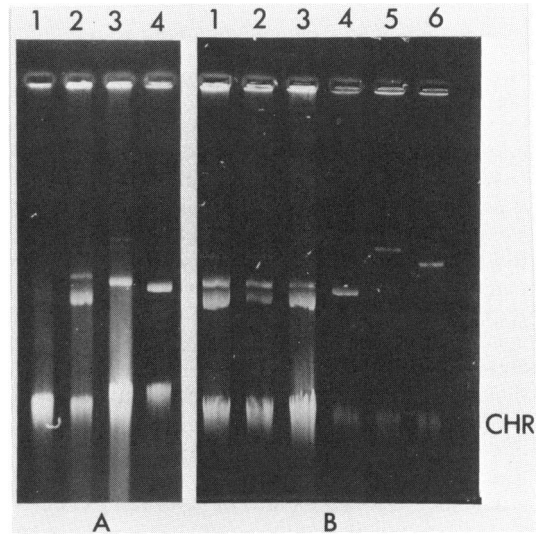


FIG. 1. Agarose gel electrophoresis of N plasmid DNA. Plasmid DNA was isolated by the method of Portnoy and White described by Crosa and Falkow (3). Electrophoresis was done in a horizontal gel apparatus with 0.6% agarose at 150 V for 5.5 h. A: (lane 1) strain GT408 (=GT1[R46 type 3]); (lane 2) GT409 (=GT1[R46 type 2]); (lane 3) GT410 (=GT1[R46 type 1]); and (lane 4) *E. coli* RG2172 (=RG173[R46]). B: (lane 1) *E. coli* RG2376 (=RG176[R46 type 2]) transformed with DNA from *P. aeruginosa* GT409); (lane 2) *E. coli* RG2377 (=RG486[R46 type 2]), transduced with phage P1 grown on *E. coli* RG2376); (lane 3) GT409; and (lanes 4 to 6) reference plasmids R46, R100, and R26, respectively. RG173 is a rifampicin-resistant derivative of *E. coli* K-12 strain RG11 (5), RG176 is an *E. coli* C strain (5), and RG486 is a lactose-nonfermenting derivative of RG176. CHR denotes chromosomal DNA.

mids studied. Rare pCF290<sup>+</sup> transconjugants obtained with strain GT1 (three studied) were stable with respect to the plasmid markers. In addition, they were capable of retransfer of the plasmid to *E. coli*, and plasmid DNA from pCF290<sup>+</sup> GT1 isolates exhibited in gels the same kind of apparent acquisition of about 2 Md of DNA as the type 1 R46<sup>+</sup> transconjugants, with a return to "normal" size upon retransfer to *E. coli*.

The exact nature of the plasmid DNA from R46<sup>+</sup> and other N<sup>+</sup> *P. aeruginosa* transconjugants remains to be determined in future experiments. Physical analysis will be facilitated by the fact that a restriction map of R46 has recently been published (2). Some information has already been obtained by transforming and transducing *E. coli* with *P. aeruginosa*-derived type 2 R46 DNA. In Fig. 1B, it may be seen that type 2 R46 plasmid DNA from an *E. coli* transformant and subsequent transductant has the

same complexity and mobility as the plasmid DNA derived from the parent *P. aeruginosa* strain GT409. Both transformant and transducant had the same phenotype as GT409, except for stability; the plasmids were stably maintained in *E. coli* but not in *P. aeruginosa*.

We have not observed any interconversion of type 1 and type 2 transconjugants, but type 3 isolates have been obtained from primary type 1 transconjugants. The mechanism of type 3 transconjugant formation may involve transposition. It has been reported that the R46 resistance genes for ampicillin, streptomycin, and sulfonamides are carried on a transposon (11). Some of the type 3 transconjugants we have studied also exhibit the plasmid mediated UV protection phenotype, a function not yet associated with a transposon.

The fact that N plasmids in laboratory experiments can transfer antibiotic resistance genes to *P. aeruginosa* and that these genes can become stably associated with the *P. aeruginosa* genome (type 3 transconjugants) indicates that the pool of resistance determinants in enterobacteria may be more accessible to *P. aeruginosa* than previously believed.

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