TRANSGENOSIS WITH THE PARTICIPATION OF PLASMID RP1: INDICATION OF THE PRESENCE OF A "COMPOSITE PLASMID" IN AN INTERGENERIC HYBRID OF *Escherichia coli*

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One of the transconjugants (1-7) previously obtained by the writers by conjugation of *Escherichia coli* J-62 with *Pseudomonas aeruginosa* 1822, in addition to plasmid RP1 had acquired the ability to grow without proline and tryptophan. A careful study showed that during conjugation of the transconjugant 1-7 with various strains of *E. coli* the plasmid RP1 and the chromosomal genes are transmitted together, whereas during transduction with the aid of bacteriophage P1 they are transmitted independently; fertility is found only in transductants carrying the plasmid RP1. The results of these experiments suggest that during intergeneric conjugation chromosomal genes can be transferred even without any firm link with the plasmid (as in the case of "composite plasmids"). Corresponding fragments of the chromosome of *Ps. aeruginosa* in cells of *E. coli* evidently form small nontransmissible replicons.

KEY WORDS: *transconjugant; transductant; plasmid; bacteriophage; chromosomal genes.*

During conjugation of *Pseudomonas aeruginosa* 1822 (RP1) with *E. coli* strain J-62, along with other transconjugants a transconjugant subsequently described as strain 1-7 was obtained [1]. This strain is interesting because, besides resistance to kanamycin (Km), tetracycline (Tc), and penicillin (Pn), it acquired independence from two amino acids: proline (Pro) and tryptophan (Trp). The writers postulated initially that strain 1-7 carries a replaced plasmid (R'protrp), like the plasmids R'pro and R'his, but no direct evidence could be obtained that this suggestion was correct.

Since much remains unexplained in the transmission of the R plasmids of chromosomal genes [2], in this investigation a careful genetic study was made of strain 1-7.

EXPERIMENTAL METHOD

The characteristics of the strains of *E. coli* used are given in Table 1. The methods of conjugation and transduction were described previously [1]. All that need be said here is that during conjugation nalidixic acid (100 μg/ml) was used for contraselection. The results of the conjugation experiments were expressed as the ratio of the number of transconjugants to the number of donor cells, and the results of the transduction experiments as the ratio of the number of transductants to the number of plaque-forming particles of bacteriophage P1.

EXPERIMENTAL RESULTS

Data on the frequency of transmission of both plasmid RP1 and of chromosomal markers pro^+ and trp^+ by conjugation are given in Table 2. It will be noted that plasmid R is trans-

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TABLE 1. Characteristics of Strains of *Escherichia coli*

Strain*	Phenotypic characteristics	Remarks
AB1157	$Thr^-Leu^-Pro^-Arg^-His^-Str^rRec^+$	Obtained from Prof. A. P. Pekhov (Patrice Lumumba Peoples' Friendship University)
AB2463	$Thr^-Leu^-Pro^-Arg^-His^-Str^rRec^-$	Obtained from the Museum of the Institute of General Genetics, Academy of Sciences of the USSR
PA6021	$Thr^-Leu^-Pro^-Trp^-Arg^-Pur^-Thi^-Str^r$	Obtained from the Museum of the Institute of General Genetics, Academy of Sciences of the USSR
J-62	$Pro^-His^-Trp^-Str^r$	Obtained from Prof. D. G. Kudlai (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR)
1-7	$His^-Str^rPn^rKm^rTc^r$	Obtained in the writers' laboratory by conjugation of *Ps. aeruginosa* 1822 (RP1) with *E. coli* J-62 [1]

*Nal^r mutants of strains AB1157, AB2463, PA6021, and J-62 were obtained by the present authors.

TABLE 2. Transmission of Genetic Markers in Crosses of Strain 1-7 with Different Recipients

Cross	Selective marker	Frequency of transmission
1-7×PA6021 Nal^r	Km^r	4×10^{-4}
	Pro+	1×10^{-5}
	Trp+	7×10^{-5}
1-7×AB1157 Nal^r	Km^r	2×10^{-4}
	Pro+	2×10^{-5}
1-7×AB2463Nal^r	Km^r	1×10^{-4}
	Pro+	0

TABLE 3. Transmission of Genetic Markers by Phage P1 Propagated on Strain 1-7

Selective marker	Frequency of transduction in case of recipient	
	E. coli J-62	E. coli PA6021
Tc^r	$3\cdot10^{-7}$	$1\cdot10^{-7}$
Km	$1\cdot10^{-7}$	$1\cdot10^{-7}$
Pn^r	$2\cdot10^{-6}$	$1\cdot10^{-6}$
Pro+	$1.2\cdot10^{-6}$	3×10^{-6}
Trp+	$3.8\cdot10^{-6}$	$2.5\cdot10^{-6}$

mitted with equal frequency to all recipients including Rec^- strains, whereas the chromosomal marker pro^+ is transmitted only to Rec^+ recipients. Transmission of the chromosomal marker trp^+ could be judged only from experiments with strain PA6021, for no tryptophan-dependent mutant with defective recombination ability was available.

Analysis of the transconjugants selected in 1-7 × PA6021 crosses for inheritance of nonselective markers gave the following results. If selection was carried out on minimal medium without tryptophan, all the Trp^+ transconjugants carried the R plasmid and half of them had the Pro^+ phenotype. If selection was carried out on medium without proline, all the Pro^+ transconjugants were Trp^+ and R^+.

Table 3 contains the results of a study of transfer of the corresponding genetic material from strain 1-7 to two recipient strains with the aid of bacteriophage P1. It shows that the frequency of transduction of each of the genetic markers was the same for both recipients. Investigation of transductants "cleared" on appropriate selective media showed that all three markers are transmitted by phage P1 independently; none of the 400 transductants acquired two or, still less, three markers simultaneously.

Transductants selected for resistance to Km or Tc were resistant to two other antibiotics also: Pn and Tc or Km and Pn, respectively (indicating the presence of a "complete" R plasmid. Meanwhile, about two-thirds of the transductants from medium with Pn carried the Pn^r determinant only. The latter was evidently connected with dissociation of the RP1 factor, with the formation of a smaller plasmid resembling RP1-1, carrying only one determinant, namely Pn^r [5, 7]. The possibility cannot be ruled out that this was also responsible for the higher frequency of transduction for the penicillin marker ($1\text{-}2\cdot10^{-6}$) observed in these experiments (with selection on media with Km and Tc the yield of transductants was an order

of magnitude smaller). The relatively higher frequency of transmission of chromosomal markers than of the RP1 plasmid will also be noted.

Some of the transductants were tested for their ability to act as donors in conjugation with strain J-62 Nal^r. The results showed that transductants carrying the RP1 plasmid but not pro^+ or trp^+ markers possessed this ability. Like the latter, the "shortened" R plasmid also proved to be nontransmissible.

It can be concluded from the results of these experiments that in the cells of strain 1-7 the RP1 factor and fragments of the chromosome of *Ps. aeruginosa* with pro^+ and trp^+ markers were not physically linked but exist independently in an autonomous state; together they form what Clowes [4] describes as a "composite plasmid." Incidentally, the possibility that *E. coli* may also contain a "composite R factor," like ΔA or ΔS [3], has already been reported [8]. One of the components of our own compoiste plasmid (RP1) is transmissible, the other two are nontransmissible. Unfortunately, the question of how they are transmitted together cannot yet be answered; nevertheless, it likewise cannot be answered in the case of other composite plasmids [2, 4].

As regards the absence of fertility in transductants with the Pn^r determinant only the following alternative can be proposed: Either it is a nontransmissible plasmid arising during propagation of phage P1 on strain 1-7 or a plasmid of type RP1-1 becomes incorporated into the chromosome of the transductant, a characteristic feature of plasmids RP1 and RP1-1 [6, 7], which thereby lose some of their own functions [5].

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