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TRANSMISSIVITY RANGE OF GENETIC TRANSFER FACTORS pAP38, pAP39, pAP41, and pAP42

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KEY WORDS: plasmid; genetic transfer factor; mobilization for transfer; transconjugant; transposon; cointegrate.

The F-like genetic transfer factors pAP38, pAP39, pAP41, and pAP42 were identified in cells of strains of *Escherichia coli* isolated from man and animals [3], and their molecular weights are 49.3×10^6, 42.6×10^6, 40.0×10^6, and 32.2×10^6 daltons, respectively. Whereas transfer factors pAP38 and pAP42 are representatives of FVII and FIX incompatibility groups, respectively, factors pAP39 and pAP41 have atypical incompatibility and belong simultaneously to incompatibility groups FI and FIV [1, 2].

The writers showed previously that these transfer factors can be transmitted into cells of untyped and typed strains of *E. coli* and also into bacteria of the genera *Erwinia* and *Hafnia*, in which they are also able to replicate.

The aim of the present investigation was to study the ability of all these genetic transfer factors to be transmitted into bacteria of more distant species and to express themselves in these bacteria.

EXPERIMENTAL METHOD

Genetic transfer factors pAP38, pAP39, pAP41, and pAP42, marked by transposon Tn9 (pAP38::Tn9, pAP39::Tn9, pAP41::Tn9, pAP42::Tn9) and contained in cells of *E. coli* AP115, mutant factor pAP38-2drd, marked with this same transposon (pAP38-2drd::Tn9) and contained in *E. coli* 132, and also conjugative plasmid RP4ApNmKmTc, contained in *E. coli* AE, were used in the experiments.

The transmissivity range of the transfer factors was studied by using cells of strains *Pseudomonas putida* BKMB-901, *Pseudomonas fluorescens*, *Rhizobium leguminosarum* BKMB-115, *Azospirillum lipoferum*, and *Agrobacterium tumefaciens* as recipients.

Donor cells of *E. coli* AP115 and AP132, containing one or the other transfer factor, were crossed with recipient cells of *Ps. putida*, *Ps. fluorescens*, and *A. tumefaciens* by standard methods in nutrient both followed by selection of transconjugants on nutrient agar containing chloramphenicol and streptomycin. Crosses in which *A. lipoferum* or *R. leguminosarum* cells served as receipients were carried out on solid potato medium or agarized synthetic medium, also with the addition of chloramphenicol and streptomycin. "Three-parent" crosses were carried out by the method described previously [3]. The sensitivity of bacteria containing plasmids pAP42 and RP4 to phages MS2 and PRD1 was determined by the agar layers method.

EXPERIMENTAL RESULTS

Despite many repetitions of the experiments, no transconjugants could be isolated from a single cross in which *E. coli* AP115 or AP132 cells containing one or the other genetic trans-

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TABLE 1. Donor Ability of Transconjugants of *Ps. putida* and *R. leguminosarum*

Crosses	Frequency of transmission of markers			
	Cm	Ap	Km	Km
P. putida (pAPP42)(RP4) ×E. coli	$2{,}0 \cdot 10^{-3}$	$2{,}0 \cdot 10^{-3}$	$1{,}2 \cdot 10^{-3}$	$1{,}8 \cdot 10^{-3}$
P. putida (pAP42)(RP4) × P. fluorescens	$3{,}0 \cdot 10^{-4}$	$4{,}4 \cdot 10^{-4}$	$2{,}2 \cdot 10^{-4}$	$2{,}5 \cdot 10^{-4}$
R. leguminosarum (pAP42)(RP4) × E. coli C-600	$2{,}3 \cdot 10^{3}$	$1{,}3 \cdot 10^{-3}$	$7{,}5 \cdot 10^{-4}$	$1{,}5 \cdot 10^{-3}$

fer factor were the donors and cells of *Ps. putida*, *Ps. fluorescens*, *R. leguminosarum*, *A. lipoferum*, and *A. tumefaciens* served as recipients. The only exceptions were crosses in which the donors were *E. coli* AP132 cells, containing the derepressed mutant of transfer factor pAP38-2drd, and the recipients were *A. lipoferum* cells. In these crosses the frequency of transmission of factor pAP38-2drd::Tn9 was 3.8×10^{-7}, but Cm transconjugants, selected on solid potato medium with chloramphenicol and streptomycin, did not give subsequent growth when reseeded on similar medium, indicating that the mutant factor cannot be maintained and replicate in bacteria of this species.

Since the genetic transfer factors studied could not be transmitted to bacteria of species more remote from *E. coli* then *Erwinia* and *Hafnia*, or if transmission was possible, as was the case with mutant factor pAP38-2drd::Tn9, the transfer factor could not be maintained in the new cell medium, it was decided to study whether these factors could be mobilized for transfer into bacteria of species remote from *E. coli* with the aid of plasmic FP4. This plasmid, as abundant evidence in the literature shows, easily crosses barriers of species, and is thus a "cosmopolitan" plasmid. However, to study this problem the only suitable test object was found to be transfer factor pAP42 which, unlike the other transfer factors studied, induces sensitivity of the *E. coli* cell which contains it to phage MS2, so that it can be reliably identified during subsequent crosses.

Consequently, in the next experiments "three-parent" crosses were carried out as follows: *E. coli* AE(RP4) × *E. coli* AP115(pAP42::Tn9) × *Ps. putida* BKMB-901 and *E. coli* AE(RP4) × *E. coli* AP115 (pAP42::TN9) × *R. leguminosarum* in order to mobilize the pAP42 factor with the aid of plasmid RP4 for transfer into *Ps. putida* or *R. leguminosarum*. These experiments were successful, for Cm recombinants were selected in crosses of both types with a frequency of 2×10^{-7} to 2.5×10^{-7}. A study of the genetic structure of Cm transconjugants selected from both types of crosses showed that most of them are resistant to both chloramphenicol and kanamycin simultaneously, i.e., most of these transconjugants contain both mobilized (pAP42) and mobilizing (RP4) plasmids. A study of the stability of the plasmids in the transconjugants showed that they are characterized by spontaneous elimination during prolonged subcultures of the transconjugants, and that both plasmids undergo elimination simultaneously.

To determine the nature of relations between plasmids pAP42 and RP4 in transconjugants of *Ps. putida* and *R. leguminosarum* the latter were crossed with *E. coli* and *Ps. fluorescens*, and transconjugants were selected separately for resistance to chloramphenicol (marker of factor pAP42) and resistance to ampicillin, kanamycin, and tetracycline (markers of plasmid RP4). The results of these experiments are given in Table 1.

As Table 1 shows, transmission of markers of plasmids pAP42 and RP4 from *Ps. putida* BKMB-901 to *E. coli* C-600 and *Ps. fluorescens* takes place with practically equal frequency; this means that both plasmids are transmitted with equal frequency in these crosses. Similarly both plasmids also are transmitted from *R. leguminosarum* BKMB-115 to *E. coli* C-600. The presence of both plasmids in transconjugants of *E. coli* C-600 from crosses of the two types mentioned above was confirmed by the discovery of sensitivity of these transconjugants simultaneously to phages MS2 and PRD1. In other words, plasmids pAP42 and RP4 were transmitted in these crosses as fused structures.

It can be concluded from these results that genetic transfer factors pAP38, pAP39, pAP41, like other F-like plasmids, are characterized by limited transmissivity and are incapable of independent transfer to bacteria of species too remote from *E. coli*. However, they are prob-

bably accessible for mobilization for transfer to these bacteria with the aid of other conjugative plasmids. This hypothesis is supported by data on mobilization of factor pAP42 for transfer with the aid of plasmid RP4. The results also suggest that in the course of mobilization of plasmid pAP42 by plasmid RP4, cointegrative structures are formed in which the two component plasmid parts (pAP42 and RP4) are capable of expression. However, the problem of how replication of these cointegrates takes place requires special study.

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EFFECT OF PHENOBARBITAL ON THE CYTOGENETIC ACTIVITY OF CYCLOPHOSPHAMIDE

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We now know that the late effects of some chemicals are determined by their active metabolites formed with the participation of mixed function oxygenases (MFO), enzymes located in membranes of the endoplasmic reticulum. Hence the need to study the influence of substances modifying MSO activity on the mutagenic effect of chemicals in experiments on mammals.

The modifying effect of phenobarbital (PB), a known inducer of the MFO system, on the frequency of chromosomal aberrations was studied in rat bone marrow cells under the influence of cyclophosphamide (CP). The cytogenetic activity of CP has been well studied in experiments on mammals, the results of which have shown that the active principle of CP consists of its metabolites formed through the participation of MFO [1, 2, 8].

EXPERIMENTAL METHOD

CP (from Jenapharm, East Germany) and PB sodium (from Farmakon, Czechoslovakia) were used and were dissolved in sterile distilled water. Experiments were carried out on noninbred male albino rats weighing 160-200 g. Six groups of animals were used, with 5-8 rats in each group. Animals of group 1 received a single intraperitoneal injection of CP in a dose of 25 mg/kg; animals of groups 2 and 3 received PB by three intraperitoneal injections in doses of 2 and 80 mg/kg, respectively, at intervals of 24 h; animals of groups 4 and 5 received PB by the same scheme, and also CP in a dose of 25 mg/kg 24 h after the last injection of PB. The animals of group 6 served as the control.

For cytogenetic analysis rats were killed 24 h after the last injection of the substances. Films of metaphase chromosomes of bone marrow cells were prepared by the standard method. In each animal 100 metaphases were analyzed. Cytogenetic analysis was carried out on numbered slides. Single and paired fragments and chromatid and chromosmal exchanges were counted. Cells with 10 chromosomal aberrations or more were classed as metaphases with multiple aberrations. Differences between frequencies of cells with aberrations in different groups were evaluated by the chi-square test.

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