Uncommon Properties of pLD105 Conjugative Plasmid

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Plasmid pLD105 isolated from a clinical strain of *E. coli* determines nitrofuran resistance due to inactivation of low-molecular-weight nitrofuran reductase subunit. pLD105 plasmid belongs to IncF. It is a conjugative plasmid and mobilizes chromosome markers, but is not transmitted to strains containing other plasmids. However, the presence of pLD105 plasmid in the recipient strain does not prevent incorporation of other plasmids, including nonconjugative ones. Transfer of nonconjugative plasmids from the donor to a recipient strain carrying pLD105 was denoted as "reverse donation".

Key Words: *plasmids; conjugation; conduction; reverse donation; nitrofurans*

Nitrofuran (Nif) resistance of some clinical strains of enteric microflora is determined by genes of pLD105 conjugative plasmid encoding a small polypeptide inhibiting the low-molecular-weight subunit of nitrofuran reductase [1]. This differentiates pLD105 from R-Utrecht [7], R46 [9], pKM101 [10], pEB017 [12], and R648 [13] plasmids encoding a protein similar to RecA and triggering SOS reactions, which determine Nif resistance [11].

Combination of kanamycin resistance plasmid gene and Nif resistance chromosome mutation in the same cell leads to abnormal increase in kanamycin resistance [2]. We demonstrated the same effect for pLD105 plasmid: the presence of this plasmid 8-10-fold increased kanamycin resistance of the strain with RP1 plasmid. However, attempts at construction of polyplasmid strains led to an uncommon phenomenon: pLD105 plasmid was never transferred into strains containing another plasmid, but if pLD105 was introduced first into the strain, it did not prevent incorporation of other plasmids. The presence of pLD105 in the recipient strain promoted transfer of nonconjugative plasmids from the donor. We analyze these facts in this paper.

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MATERIALS AND METHODS

Culturing was carried out in Luria and minimum Spizaisen media. Thiamine (10 μg/ml) and amino acids (50 μg/ml each) were added to Spizaisen medium, if necessary.

Transformation of *E. coli* cells was carried out as described previously [6]. Conjugation of *E. coli* strains and evaluation of sensitivity to "sex-dependent" phages were carried out by a previously described method [8].

Phage adsorption on *E. coli* cells was visualized as described previously [3]. The strains, plasmids, and phages are listed in Table 1.

RESULTS

According to electrophoretic analysis, the molecular weight of pLD105 plasmid is 66-67 kDa.

All "male" phages used in the study, except M13, formed plaques on K12(pLD105) strain culture, and adsorption of MS2 phage on the respective pili was confirmed by electron microscopy. Hence, we referred pLD105 plasmid to F-like ones.

Plasmid pLD105 can mobilize chromosome markers with 10^{-7}-10^{-6} frequency (Fig. 1, *a*). On the other hand, we observed an uncommon phenomenon: pLD105 plasmid was not transferred into strains con-

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TABLE 1. Strains and Plasmids Used in the Study

Strain, plasmid, phage	Characteristics
E. coli strains	
C	Prototroph
K-12	Prototroph
J62-Rif^r	*pro*, *his*, *trp*, *str*, *rif*
327/74	*thi*, *thr*, *leu*
AB1157-Nal^r	*thi*, *pro*, *his*, *leu*, *arg*, *thr*, *str*, *nal*
Nonconjugative plasmids lacking *tra* operon	
pLD404 (obtained by Tn5 insertion in pLD403 plasmid)	Km^r, Sm^r, Ap^r, Tra$^-$
pEBgT2	Tc^r, Cm^r, Sm^r, Tra$^-$
Conjugated plasmids (Tra$^+$)	
pLD105	Nif^r, Tra$^+$
F'lac	LacZ, Tra$^+$
RP1	Tc^r, Ap^r, Km^r, Tra$^+$
R124	Tc^r, Tra$^+$
"Sex-dependent" phages recognizing strains carrying conjugative plasmids	
M13, f1	From group F (according to Bradley), containing single-stranded DNA
MS2, f2	From group E (according to Bradley), containing single-stranded RNA

taining other plasmids during conjugation with *E. coli* strain. In order to clear out the cause of this phenomenon, we used nonconjugative pLD404 plasmid, which was inserted into the cells of appropriate strains (Table 2) by transformation. Numerous transconjugates were obtained in the conjugation system of three strains: C(pLD404), J62-Rif^r, and C(F'lac); this indicated the presence of *mob* gene in pLD404.

Then we replaced strain C(F'lac) with strains AB1157-Nal^r(pLD105) and K12(pLD105) and conjugated each of them with strains C or 327/74 containing pLD404. We obtained transconjugates containing both plasmids, but all transconjugates had markers of only the strains initially containing the pLD105 plasmid. Hence, it was not pLD105 that was transferred to another strain, but vice versa, pLD105 mobilized pLD404 transfer "to itself" (Fig. 1, *b*; Table 2).

Similar results were obtained during conjugation of J62-Rif^r(pLD404) strain with AB1157-Nal^r(pLD105) strain, though selection was carried out in 3 media: Km+Nif, Km+Nif+Nal, and KM+Nif+Rif. In the latter case no transconjugates were detected, while in two other cases we detected about 10^3 colonies per dish. The presence of Rif^r and Nal^r markers in the donor or recipient was not essential for the conjugative properties of pLD105. Hence, we can speak about pLD404 transfer into the strain containing pLD105.

A similar phenomenon was also observed with such conjugative and nonconjugative plasmids as RP1, R124, and pEBgT2 (if the recipient strain contained these plasmids, it did not incorporate pLD105), and pLD105 had always to be incorporated the first for the construction of polyplasmid strains. As all these plasmids belong to different incompatibility groups, it seems that this phenomenon does not depend on surface exclusion [4].

Mobilization of nonconjugative plasmids is usually realized by two modes: (1) mobilized plasmid forms a co-integrate with the mobilizing plasmid and the recipient strain thus receives both plasmids ("conduction") or (2) no physical contact is formed between the mobilizing and mobilized plasmids; mobilized plasmid makes use of the conjugative bridge for autotransfer ("donation") [5]. We observed the following phenomenon: the mobilized plasmid DNA was transferred in the opposite direction — into the strain containing pLD105. As far as we know, it has not been heretofore shown that DNA can be transferred through pili in both directions. As pLD105 did not migrate from one cell into another, this phenomenon cannot be

TABLE 2. Characteristics of Transconjugates Obtained by Conjugation of Strains with pLD105 and pLD404 Plasmids

Strain containing pLD404 plasmid	Number of *Km^r*, *Nif^r* transconjugates with markers of			Strain containing pLD105 plasmid
	strain with pLD404	strain with pLD105	other	
C (prototroph)	0	19	0	AB1157-Nal^r (*thi*, *pro*, *his*, *leu*, *arg*, *thr*, *str*, *nal*)
J62-Rif^r (*pro*, *his*, *trp*, *str*, *rif*)	0	11	0	AB1157-Nal^r (*thi*, *pro*, *his*, *leu*, *arg*, *thr*, *str*, *nal*)
327/74 (*thi*, *thr*, *leu*)	0	14	0	K-12 (prototroph)

Note. Averaged data of three experiments are presented.

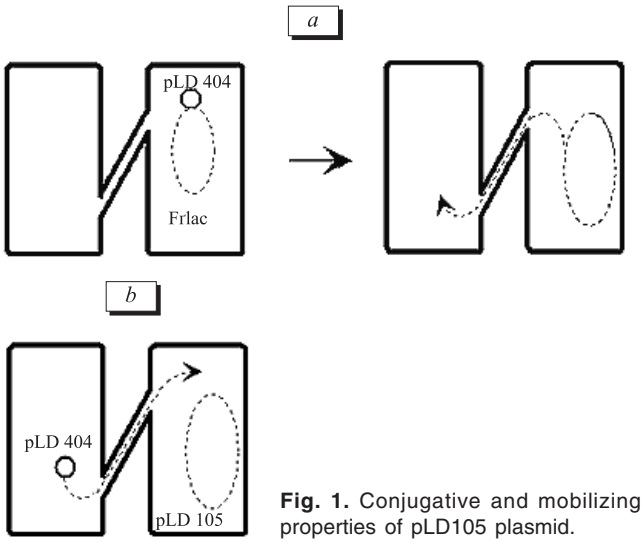

Fig. 1. Conjugative and mobilizing properties of pLD105 plasmid.

regarded as a variant of conduction. We called this mechanism "inverse donation". Further studies will be devoted to investigation of the mechanism of this phenomenon.

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