Mobilization of Non-conjugative Plasmids into *Rhodopseudomonas sphaeroides*

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The non-conjugative plasmids pAS9, RSF1010, pBR325 and pML21 were mobilized from *Escherichia coli* into *Rhodopseudomonas sphaeroides* by conjugative plasmids of the P-1 incompatibility group or their derivatives. The transfer of plasmids pAS9 and RSF1010 was *recA*-independent and both plasmids could be maintained in the autonomous state in *R. sphaeroides* cells. In contrast, the transfer of the plasmids pML21 and pBR325 based on the ColE1 replicon was dependent on the *recA* status of donor strains. Replication and maintenance of these plasmids occurred as a result of the production of cointegrates formed by recombination between the mobilized and mobilizing plasmids.

INTRODUCTION

The Gram-negative purple bacterium *Rhodopseudomonas sphaeroides* is an attractive model for investigating bacterial photosynthesis and nitrogen fixation. An efficient gene transfer system is essential for the genetic study of these processes in *R. sphaeroides*. One of the tools with most potential for the development of methods for complementation analysis and gene cloning is manipulation with plasmids which can be expressed in *R. sphaeroides* cells. A number of workers have reported that IncP-1 (Olsen & Shipley, 1973; Siström, 1977; Tucker & Pemberton, 1979) and IncW (Tucker & Pemberton, 1979) group R plasmids are stably maintained in *R. sphaeroides*. Recently, the mobilization of chromosomal genes at high frequency promoted by plasmids RP1::Tn501 (Pemberton & Bowen, 1981) and pAS8-121rep::Tn7 (Kamenewa *et al.*, 1982) have been demonstrated. Tucker & Pemberton (1980) have reported the transformation of *R. sphaeroides* with DNA of the temperate bacteriophage ϕ 6P. The successful development of a transformation method for *R. sphaeroides* by plasmid DNA has been described by Fornary & Kaplan (1982).

In this paper we describe studies of the mobilization of various non-conjugative plasmids, which are, or may be, used as recombinant DNA cloning vectors, from *E. coli* into *R. sphaeroides*.

METHODS

Bacterial strains and plasmids. The bacterial strains used were *R. sphaeroides* strain 2R (wild-type) and *E. coli* K12 strains C600 (*thr leu thi lac tonA supE*) and HB101 (*leu pro lac gal rpsL thi hsdR hsdM recA56*). All the strains were obtained from the collection of the Department of Genetics, Moscow State University. The plasmids utilized in this study are listed in Table 1.

Media. The media used were LB and minimal agar A (Miller, 1972) supplemented with 1 mg nicotinic acid, 1 mg biotin and 1 mg thiamin per litre.

For the selection of antibiotic resistance a minimal medium was supplemented with various antibiotics at the following concentrations ($\mu\text{g ml}^{-1}$): kanamycin (Km, 100); tetracycline (Tc, 10); streptomycin (Sm, 100); and trimethoprim (Tp, 50).

Drug resistance. Minimal inhibitory concentrations (MICs) of antibiotics were assayed from the curves of bacterial colony-forming ability on LB agar supplemented with different concentrations of antibiotics. MICs were determined as the minimal concentrations of antibiotics which decreased colony-forming ability by 50% after 48 h incubation.

Table 1. *Bacterial plasmids used*

Plasmid	Relevant properties*	Source or reference
pML21	Ice1 Km	Hershfeld <i>et al.</i> (1974)
pBR325	Ap Tc Cm	Bolivar (1978)
RSF1010	IncQ Sm Su	Guerry <i>et al.</i> (1974)
S-a	IncW Km Su Sm Cm Tra	Datta (1975)
RP1	IncP-1 Ap Km Tc Tra	A. I. Stepanov
RP1-6Rep(ts12)::Tn7	IncP-1 Km Tc Sm Tp Rep _{ts} Tra	G. B. Smirnov
RP1-6Rep(ts12)::Tn9	IncP-1 Km Tc Cm Rep _{ts} Tra	G. B. Smirnov
pAS8-101	Ice1 Ap Sm Tp Tra (derivative of RP4-ColE1 hybrid with insertion of Tn7 into Km determinant)	A. I. Stepanov
pAS9	Ice1 Ap Tc (Deletion mutant of RP4-ColE1 hybrid)	Stepanov <i>et al.</i> (1976)

* Ap, ampicillin resistance; Km, kanamycin resistance; Tc, tetracycline resistance; Sm, streptomycin resistance; Su, resistance to sulphonamides; Cm, chloramphenicol resistance; Tp, trimethoprim resistance; Ice1, immunity to colicin E1; Tra, self-mediated conjugation; Rep_{ts}, temperature-sensitive replication; Inc, incompatibility group.

Table 2. *MICs of antibiotics for R⁻ and R⁺ strains of R. sphaeroides*

Strain	MIC ($\mu\text{g ml}^{-1}$)					
	Km	Cm	Sm	Tp	Tc	Tc*
Wild-type	3	1	4	2	0.01	ND
RP1	400	ND	5	2	1-1.5	10-15
RP1-6Rep(ts12)::Tn7	400	ND	150	50	1-1.5	10-15
RP1-6Rep(ts12)::Tn9	400	1	ND	ND	1-1.5	10-15
S-a	50	1	25	ND	ND	ND

ND, Not determined.

* The cells were previously incubated on medium supplemented with 0.3 $\mu\text{g tetracycline ml}^{-1}$ for 3 h.

Mating procedure. For mobilization of non-conjugative plasmids triparental matings were used. Two RecA⁺ or RecA⁻ strains of *E. coli* were used as the donors: one of them contained the conjugative plasmid and the other the non-conjugative plasmid. Plate matings were performed by mixing 10⁹ cells each of overnight cultures of donor and recipient onto LB agar plates. The plates were incubated for 2.5-3 h at 35 °C. The cells were then washed from the agar surface and appropriate dilutions were plated onto selective medium to determine the viable count of the recipient population and the number of transconjugants formed. The transconjugant clones of *R. sphaeroides* were selected on minimal medium A supplemented with appropriate antibiotics. The colonies of *R. sphaeroides* were counted and analysed in 48-72 h. To induce resistance to tetracycline, *R. sphaeroides* cells or the conjugation mixtures were incubated in medium supplemented with 0.3 $\mu\text{g tetracycline ml}^{-1}$ for 3 h.

RESULTS

Expression of plasmid drug resistance genes in R. sphaeroides

It has been shown previously that the majority of plasmid drug resistance genes are expressed in *R. sphaeroides* cells, the exception being the genes for resistance to the β -lactam antibiotics (Sistrom, 1977; Tucker & Pemberton, 1979). In order to quantify the expression of plasmid drug resistance in *R. sphaeroides* 2R transconjugants, the MICs of several antibiotics were measured for the wild-type and for the strains carrying the plasmids S-a, RP1 and derivatives of RP1 carrying transposons Tn7 and Tn9. The results of these experiments are shown in Table 2. It was demonstrated that the resistance to chloramphenicol which is encoded by determinants of plasmid S-a and transposon Tn9 was not expressed in *R. sphaeroides*. The absence of resistance

Table 3. Mobilization of non-conjugative plasmids in triparental matings from *E. coli* into *R. sphaeroides* 2R

Donor*		Selection	Mobilization frequency
Strains	Plasmids		
RecA ⁺	RP1	Km	3.5×10^{-3}
	RSF1010	Sm	3×10^{-3}
RecA ⁻	RP1	Km	2×10^{-3}
	RSF1010	Sm	2×10^{-3}
RecA ⁺	pAS8-101	Sm	2×10^{-3}
	pAS9	Tc	3×10^{-5}
RecA ⁻	pAS8-101	Sm	1×10^{-3}
	pAS9	Tc	2.5×10^{-5}
RecA ⁺	pAS8-101	Sm	2×10^{-3}
	pBR325	Tc	1×10^{-7}
RecA ⁻	pAS8-101	Sm	1×10^{-3}
	pBR325	Tc	$< 10^{-10}$
RecA ⁺	pAS8-101	Sm	2×10^{-3}
	pML21	Km	2.5×10^{-6}
RecA ⁻	pAS8-101	Sm	1×10^{-3}
	pML21	Km	$< 10^{-10}$

* Each plasmid was in *E. coli* C600 or HB101 donor strain.

to this antibiotic is not due to plasmid segregation since all plasmids used in our experiments were stably maintained in *R. sphaeroides* under non-selective conditions and transferred all their markers in backcrosses with *E. coli*. The resistance to tetracycline encoded by RP1 was inducible (Table 2).

Mobilization of the plasmids RSF1010 and pAS9

Non-conjugative plasmids RSF1010 and pAS9 were transmitted at high frequencies independently of the *recA* status of the donor strains (Table 3). The presence of these plasmids in *R. sphaeroides* transconjugants was confirmed by agarose gel electrophoresis of DNA extracts (data not shown). Previously Fornary & Kaplan (1982) showed that RSF1010 is capable of stable maintenance in *R. sphaeroides* cells. Plasmid pAS9 is a deletion derivative of pAS8 (RP4-ColE1 hybrid) and can replicate in *E. coli* cells via RP4 or ColE1 replication systems (Sakanyan *et al.*, 1977). The coordinates of deletion are 17.3–50.4 kb according to the RP4 map (Sakanyan *et al.*, 1977) and pAS9, therefore, does not have *oriT* from RP4, which is located at 48 kb (Thomas *et al.*, 1979). The mobilization of pAS9 is therefore *recA*-independent and apparently initiated at the *bom/ni*c site of ColE1, which is included in pAS9. According to Warren *et al.* (1978) mobilization of ColE1 by conjugative plasmids is initiated at the *bom/ni*c site.

Mobilization of the plasmids pBR325 and pML21

The ColE1-like plasmid pBR325 and the mini-ColE1 derivative plasmid pML21 are well known cloning vectors for *E. coli*. Although the ColE1 replicon is not maintained in *R. sphaeroides* cells (Kameneva *et al.*, 1982), the inheritance of these plasmids may be obtained by cointegration with a mobilizing plasmid. Under our experimental conditions the frequencies of mobilization of pML21 and pBR325 from *E. coli* RecA⁺ strains were respectively three and four orders of magnitude lower than the transfer frequencies of conjugative mobilizing plasmids (Table 3). All transconjugants selected for markers of non-conjugative plasmids also carried all the markers from the mobilizing plasmids which are expressed in *R. sphaeroides*. When both donor strains were RecA⁻ the mobilization of pML21 and pBR325 was not observed (Table 3). Agarose gel electrophoresis of plasmid DNA from transconjugants showed the presence of additional plasmid DNA bands with sizes greater than those corresponding to mobilizing plasmids (data not shown). Transconjugants in which pML21 or pBR325 existed in the autonomous state were never obtained (about 20 transconjugants were tested in each case).

DISCUSSION

These results show that non-conjugative plasmids can be mobilized from *E. coli* into *R. sphaeroides*. This, as well as transformation, may be used to transfer plasmid vectors for cloning genes in *R. sphaeroides* cells. However, the absence of expression of genes determining resistance to chloramphenicol and β -lactam antibiotics in *R. sphaeroides* cells must be considered when choosing suitable cloning vectors. Mobilization allows the transfer of plasmids which are not able to exist in the autonomous state in *R. sphaeroides* through cointegration with a mobilizing plasmid. These cointegrates are formed by *recA*-dependent recombination (unpublished results) and the frequencies of mobilization of non-conjugative plasmids probably reflect the extent of homology of these plasmids with a mobilizing plasmid.

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